QUANTIFICATION OF ESCHERICHIA COLI AND ENTEROCOCCI LEVELS IN WET WEATHER AND DRY WEATHER FLOWS

by

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A THESIS

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DEDICATION

This thesis is dedicated to my parents and sister who always encouraged and supported me in fulfilling my ambitions. I also owe an undying debt of gratitude to Bawa, who encouraged me in this research despite the often encountered frustrations.

LIST OF ABBREVIATIONS AND SYMBOLS

ADEM	Alabama Department of Environmental Management
AHs	Aliphatic Hydrocarbons
ARDRA	Amplified Ribosomal DNA Restriction Analysis
ASTM	American Society for Testing and Materials
BEACH	Beaches Environmental Assessment and Coastal Health
BST	Bacterial Source Tracking
° C	Degrees of Celsius
^{137}Cs	Isotope of Carbon
C.L	Confidence Level
cfu	Colony Forming Units
COV	Coefficient of Variation
CSO	Combined Sewer Overflow
CWA	Clean Water Act
CWP	Center for Watershed Protection
DAS 1	4,4'-bis-[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)Amino]Stilbene-2,2'-disulfonate
DI	Deionized Water

DNA	Deoxyribonucleic Acid
DSBP	Distyryl Biphenyls
E. coli	*Escherichia coli*
EC	*Escherichia coli*
ELISA	Enzyme-linked Immunosorbent Assay
EPA	Environmental Protection Agency
ETA	Estimate in Mann-Whitney Test
^{0} F	Degrees of Fahrenheit
FC	Fecal Coliforms
FDA	Food and Drug Administration
FRNA	F-Specific Coliphage
FS	Fecal Streptococci
FWAs	Fluorescent Whitening Agents
FWPCA	Federal Water Pollution Control Administration
GC/MS	Gas Chromatograph / Mass Spectrophotometer Detector
GI	Gastrointestinal Illness
HCGI	Highly Credible Gastrointestinal Illness
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography

IDDE	Illicit Discharge Detection and Elimination
IDEXX	IDEXX Laboratories Inc.
in/hr	Inches/hour
ITS	Internal Transcribed Spacer
KP	Klebsiella Pneumoniae
LAB	Linear Alkylbenzenes
LAS	Linear Alkylbenzene Sulphonates
LDL	Lower Detection Limit
MAR	Multiple Antibiotic Resistance
MBAS	Methylene Blue Active Substance
µg/L	Micrograms Per Liter
mg/L	Milligrams Per Liter
min	Minute
MINITAB	MINITAB Software
mL	Milliliters
MPN	Most Probable Number
MUG	4-methyl-umbelliferyl- ß-D- glucuronide
NA	Not Available
NATC	National Technical Advisory Committee
NP	Not Prone to Urban Wildlife Use

NURP	National Urban Runoff Program
^{18}O	Isotope of Oxygen
ONPG	Ortho-nitrophenyl- ß-D-galactosidase
P	Prone to urban wildlife use
p	Probability
PA	Pseudomonas Aeruginosa
PAHs	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
pH	An expression for the concentration of H^+
QA	Quality Assurance
QC	Quality Control
RAPD-PCR	Randomly Amplified Polymorphic DNA -Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphisms
RNA	Ribonucleic Acid
TAWMSS	Toronto Area Watershed Management Strategy Study
UDL	Upper Detection Limit
UV	Ultra Violet
WWTP	Waste Water Treatment Plant

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The waters of the U.S are a valuable recreational resource. It is important to protect our waters so that beneficial uses like swimming, water rafting, surfing, kayaking, and bird watching can be safely enjoyed. The National Water Quality Inventory, 2000 report (EPA, 2000a), reported on the quality of assessed lakes, rivers and estuaries. 40% of rivers, 45% of lakes and 50% of estuaries that were assessed were not clean enough to support designated fishing and swimming uses. Pathogens were found to be one of the

leading causes of impairments in these waters. Urban runoff, and other wet-weather flow discharges, are a recognized leading source of these pathogens.

This research was initiated to quantify the levels of indicator bacteria, and their sources, in urban runoff. The main objective of this research was to identify possible sources, besides sanitary sewage contamination, of *E. coli* and enterococci bacteria in dry and wet-weather flows in storm drainage systems. The secondary objective of this study is to find how *E. coli* and enterococci could be effectively used for identifying sources of inappropriate discharges in storm drainage systems.

E. coli and enterococci levels larger than 2,400 and 24,000 MPN/100 mL, respectively, were observed in wet weather samples collected from various source areas, none of which was possibly contaminated with sanitary sewage. The levels of these indicator bacteria in the urban runoff source area samples exceeded the EPA 1986 water quality criteria very frequently. Since both the indicator organisms studied (*E. coli.* and enterococci) are only originate in intestines of warm-blooded animals, urban birds and other animals can be considered important sources of bacteria in stormwater. It was found during this research that the dry-weather outfall samples showing *E. coli* and enterococci levels higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL, respectively, are likely contaminated by sanitary sewage. Levels lower than these could possibly be caused by other sources, such as irrigation runoff, carwash water, laundry water, etc.

CHAPTER I

INTRODUCTION

1.1 Background

Earth can be called a Water Planet since two-thirds of the Earth's surface is covered with water. Water is an essential requirement for all human beings and animals and plays an important role in the working of the Earth's ecosystems. We use water for bathing and cleaning, drinking, cooking, gardening, fishing, aesthetic enjoyment, and relaxation. All aquatic life requires water for their habitat. (www.epa.gov/water/programs/owintro.html, Jan. 2003).

Millions of people depend on the freshwater in rivers, lakes, streams, and ground water supplies for their drinking water needs in the United States (www.epa.gov/water/programs/owintro.html, Jan. 2003). The waters of the U.S are also a valuable recreational resource. It is important to protect our waters so that activities like swimming, water rafting, surfing, kayaking, and bird watching can be safely enjoyed (EPA, 2002).

The US Environmental Protection Agency (EPA) is the leading agency involved in the nation's environmental science research, education, and assessment efforts. Most of the research work and setting of national standards for a variety of environmental issues is done by the EPA. The Clean Water Act (CWA), passed in 1972, is the cornerstone of surface water quality protection in the United States. The law authorizes the EPA to take

a variety of regulatory and nonregulatory measures to minimize direct pollutant discharges into waterways and manage polluted runoff (www.epa.gov/watertrain /cwa/, March, 2003). In the past 30 years since the passage of the CWA, tremendous advances has been made in the United States to clean up the aquatic environment by controlling point sources of pollution (such as from industries and sewage treatment plants). Unfortunately, there still are many rivers and lakes that are too contaminated to allow safe swimming and fishing, or that can be used for drinking water supplies. In *The National Water Quality Inventory: 2000 Report*, prepared under Section 305(b) of Clean Water Act (EPA, 2000a), states, tribes, territories, and interstate commissions, reported on the quality of assessed lakes, rivers and estuaries (Table 1.1). Of the 699,946 miles of rivers assessed, about 40% of were found polluted, i.e. not clean enough to support their designated beneficial uses. Similarly, 45% of lakes and 50% of estuaries that were assessed were not clean enough to support their designated beneficial uses (EPA, 2000a).

Table 1.1 Water Quality Summary of Assessed Rivers, Lakes, and Estuaries

Category of Water Body	**Total Size of Water Body**	**% of Total Assessed (Amount)**	**% Assessed Good**	**% Assessed Good but Threatened**	**% Assessed Polluted**
Rivers (miles)	3,692,830	19 % (699,946)	53%	8%	39%
Lakes (acres)	40,603,893	43% (17,339,080)	47 %	8%	45%
Estuaries (sq. miles)	87,369	36% (31,072)	45%	<4%	51%

Source: EPA, 2000a

Leading causes of impairments in assessed waters included bacteria, nutrients, metals (primarily mercury), and siltation (Table 1.2). *The National Water Quality Inventory: 2000 Report*, reported runoff from agricultural lands, Municipal point sources (sewage treatment plants), hydrologic modifications (such as channelization, flow

regulation, and dredging) and urban runoff/storm discharges as the primary source of current water quality impairments.

Table 1.2 Leading Causes and Sources* of Impairment in Assessed Rivers, Lakes, and Estuaries

Water Body Type	Leading Causes	Leading Sources
Rivers and Streams	Pathogens (Bacteria), Siltation (Sedimentation) Habitat Alterations	Agriculture, Hydrologic Modifications, Habitat Modifications
Lakes, Ponds and Reservoirs	Nutrients, Metals (Mainly Mercury) Siltation (Sedimentation)	Agriculture, Hydrologic Modifications, Urban Runoff/ Storm Sewers
Estuaries	Metals (Mainly Mercury), Pesticides Oxygen-Depleting Substances	Municipal Point sources Urban Runoff/ Storm Sewers, Industrial Discharges

Source: EPA, 2000a

* Excluding unknown, natural, and "other" sources.

Although considerable success has been achieved in cleaning up pollution from point sources, a lot still needs to be done to develop controls for nonpoint sources**,** such as polluted runoff from farms, urban areas, forestry, ranching, and mining operations (www.epa.gov/water/programs/owintro.html, Jan. 2003). This research was focused on degradation of water quality due to bacterial contamination from urban runoff only.

1.2 Definitions of Urban Runoff

Urban runoff is defined as any discharge from a storm drainage system. Urban runoff traditionally had been defined to include precipitation and washoff from lawns and other landscape areas, buildings, roadways and parking lots. But apart from that, it also includes water from many other sources that are not attributable to precipitation. These are termed "dry weather flows." During rain events, urban runoff mainly consists of

surface runoff from the urban drainage area. But during dry weather, flows enter the storm drainage system from infiltrating groundwater, permitted industrial wastewaters, separate sewer overflows or illicit/inappropriate entries to the storm drainage system (Pitt, 2001a). Therefore, urban runoff can be divided into two categories: wet weather flows and dry weather flows. Although levels of indicator bacteria were found in wet weather as well as dry weather flows during this project, more emphasis was given to sampling during wet weather.

1.2.1 Impact of Wet Weather and Dry Weather Flows on Receiving Water Quality

Initial urban runoff problems were restricted to concerns associated with flooding. Urbanization has led to increase in impervious areas, and hence increase in runoff volume and flow rates. This causes flooding and erosion, which continues to be a big concern associated with urban runoff. Recently, environmental scientists and engineers have shown concern over urban runoff's contribution to receiving water quality problems (Pitt, 2001a). Urban runoff has commonly been cited as the leading source of bacteria in national water quality inventory reports. High concentrations of pathogens and indicator organisms found in receiving waters are also a frequent cause of concern due to impacts on human health and restrictions on recreational use (Pitt *et al.,* undated). Detailed information concerning disease outbreaks and illnesses associated with poor quality is described in the next chapter.

It is difficult to isolate the effects of stormwater (wet weather) alone on receiving waters. The reason behind is that the receiving waters are also affected by other pollutant sources such as sewage and industrial point discharges and upstream agriculture non

point discharges. Even for those receiving waters that may not be obviously receiving any other discharges except stormwater, sanitary sewer overflows and other dry weather flows may be affecting the receiving waters. It is therefore a difficult task to identify specific cause and effect relationships associated with stormwater discharges alone because of the likely presence of more than one source (Pitt *et al*., undated).

1.3 Relationship between Pathogens and Indicator Organisms

Pathogens have been reported to be the one of the leading causes of impairment in rivers and streams, as previously shown in Table 1.2. Water borne pathogens are disease-causing microorganisms that actually generate the health risk, such as viruses, bacteria, and protozoa. These can cause acute respiratory illness, gastrointestinal problems, jaundice, and dehydration, inflammation of the brain, eye infections, and heart anomalies (EPA, 2000a). Hundreds of beaches are closed, or advisories posted, every year due to bacterial contamination. Historical data shows that during the period between 1992 to1997, about 600 to 1,300 beach closures, or advisories, took place annually in California alone (Schiffand and Kinney, 2000). Unfortunately, it not easy to detect and enumerate pathogens in receiving waters due to lack of technology, lack of expertise, and the high cost of detecting and enumerating the pathogens (Pitt *et al.,* undated). Therefore, indicator bacteria such as fecal coliforms, *E. coli*, enterococci, amongst others, have been used as surrogates for actual pathogens. Originally, indicators were selected that have the same origin as the pathogens (warm-blooded animal feces) and that are less susceptible to treatment or other removal mechanisms. If indicators are found, it was assumed that pathogens might be present. Therefore, the health risk associated with polluted water is

mostly determined from the presence of indicator bacteria. The microbiological water quality standards set by the states and the EPA are based on the presence and counts of indicator bacteria (Pitt *et al.,* undated). According to the EPA recommendations of 1976, water quality standards were based on fecal coliforms, replacing the use of total coliforms.

Epidemiological studies conducted by the EPA have more recently examined relationships between indicator organisms and illnesses. These studies have found that enterococci show better correlations with swimming-associated gastroenteritis than fecal coliforms. This was found to be true for both fresh water and marine water environments. Another organism, *E. coli*, was found to be a better indicator in fresh water because it showed high correlations with gastroenteritis in fresh water environments only. In 1986, the EPA therefore issued revised water quality criteria for swimming waters based upon enterococci and *E. coli* (EPA, 1986).

1.4 *E. coli* and Enterococci

Total coliforms, *E. coli* and enterococci were the three indicator organisms which were quantified in urban runoff (wet weather as well as dry weather flows) during this thesis research. *E. coli* is an abbreviation for *Escherichia coli* bacteria. *E. coli* is present in the in the intestines of animals and humans and is a type of fecal coliform bacteria. There are hundreds of strains of *E. coli* with most of the strains of *E. coli* being harmless. One strain, known as *E. coli* O157:H7 produces a powerful toxin and is an important cause of food borne and water borne illness (www.epa.gov/safewater/ecoli.html, Nov 2002). The combination of letters and numbers in the name of the bacterium refers to the

specific markers found on its surface and distinguishes it from other types of *E. coli.* The infection can be either food borne (eating inadequately cooked contaminated meat, drinking unpasteurized milk and juice, sprouts, lettuce, and salami) or waterborne (swimming-in sewage contaminated water or drinking inadequately chlorinated water) (www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli_g.htm, September, 2003). Like *E. coli*, enterococci are also found in the intestinal tract and feces of man and other animals. Apart from that, some species are also found in soil, food, water, and plants. Enterococci can survive and grow under extreme environmental conditions, including wide ranges of temperatures and salt concentrations, explaining why enterococci are commonly found in many areas (Andrew and Mitchell, 1997).

1.5 Project Objectives

It is clear from the above discussion that pathogens are the most significant cause of impairment in rivers and streams, and that urban runoff and other storm sewer discharges are a leading source of these pathogens. Until recently, not much research has been done to quantify the levels of indicator bacteria, and their sources, in urban runoff. The goal of this study is to help water quality managers in adopting suitable management practices to improve bacterial receiving water quality. The main objective of this research was to identify other possible sources of *E. coli* and enterococci bacteria in dry and wet weather flows in storm drainage systems, and how these sources compare to sanitary sewage contamination of receiving waters. The secondary objective of this study was to show how *E. coli* and enterococci could be effectively used for identifying sources of inappropriate discharges in storm drainage systems. Although a number of other

emerging techniques are available that are being used (as discussed in the literature review), the problem of developing low cost simple methodology still exists. The IDEXX Laboratories, Inc.'s analytical methods were used for this research, as they are simple, well accepted, and have a reasonable cost.

1.6 Thesis Outline

The research focused on analyzing stormwater runoff from four source areas: roof tops, open spaces, streets, and parking lots. The samples were analyzed for three indicator organisms: total coliforms, *E. coli* and enterococci. This thesis contains a literature review of bacterial contamination of receiving waters, along with a description of the sample collection and analysis procedures.

The second part of the research included bacterial analysis of dry weather samples taken from outfalls flowing into Cribbs Mill Creek, Tuscaloosa, Alabama. This was associated with an on-going project examining inappropriate storm drainage discharges, funded by the US EPA and conducted by the Department of Civil and Environmental Engineering at the University of Alabama in cooperation with the Center for Watershed Protection (CWP), Ellicott City, Maryland. A description of sample collection procedures and results of analysis of the dry weather samples is also presented.

In this dry weather project phase, local samples were collected from various source areas (termed "library" or reference samples) and analyzed for various chemical constituents, plus the bacterial indicators. These library samples were collected from the local Tuscaloosa sewage treatment plant, natural springs, irrigation runoff, city water taps, commercial car washes, and household and commercial laundry waters. Although

these samples were analyzed for a wide range of constituents, this thesis focused on the bacterial analysis for total coliforms, *E. coli* and enterococci.

Statistical tests were conducted using the laboratory results obtained from various phases of the research. Interpretation of the analytical results along with a summary and conclusions are also presented in the thesis.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

This chapter gives a general description about the public health risk and effects caused by pathogens and common indicator organisms found in urban runoff and receiving waters. The history of development of water quality criteria for bacteria for recreational waters is also discussed. Most of the research associated with pathogens and their effects in receiving waters can be divided into two broad categories:

(1) Epidemiology studies form one broad category. Recent epidemiology studies and their major findings are included in this chapter.

(2) The second category includes studies that focused on finding the sources of fecal contamination in either stormwater or receiving waters and their characteristics.

Urban receiving waters can have several beneficial uses, i.e. flood prevention, warm water fishery, biological uses, contact and non-contact recreation like swimming and boating, and even possibly water supply source (Burton and Pitt, 2002). It is unlikely to be able to achieve all these goals in urban receiving waters due to the very high costs associated with the necessary controls. However, it is important to take measures to achieve the basic beneficial uses of stormwater conveyance, aesthetics and some aspect of biological uses, although it is usually impractical to be able to achieve full biological integrity, or the maintenance of the natural eco-system. It may be possible to achieve

some of the higher goals of water contact recreation, consumptive fisheries, and water supplies near urban areas having large receiving waters and draining mostly undeveloped areas, with little urbanization (Burton and Pitt, 2002). As discussed in a previous chapter, despite considerable effort made by EPA and other environmental agencies, there are many instances where the attainment of designated beneficial uses has not been achieved. The *National Water Quality Inventory: 2000 Report* indicated that 40% of rivers, 45% of lakes and 50% of estuaries that were assessed were not clean enough to support fishing and swimming beneficial uses.

Various exposure pathways through which contaminated receiving water can cause potential human health problems include: 1) Exposure to contaminants present in recreational waters during contact and non-contact recreation, 2) Drinking of contaminated water, and 3) Consumption of fish and shell fish effected by pollutants (EPA, 2000a). There are two types of contaminants present in receiving waters that can directly affect public health. These are toxic chemicals and water borne disease causing pathogenic organisms like bacteria, viruses and protozoa. While toxic chemicals cause human birth defects, cancer, neurological disorders and kidney ailments, pathogens are responsible for causing acute respiratory illness, gastrointestinal illness, jaundice, dehydration, inflammation of the brain, eye and ear infections, and heart anomalies (EPA, 2000a). The *National Water Quality Inventory: 2000 Report*, reported pathogens as the leading cause of impairment in Rivers and streams.

The presence of pathogens in rivers and streams is a serious public health threat and urban runoff is one of the leading sources of pathogens. In an early review, Van Dozel *et al.,* (1967) reported that out of nearly 230 known water-borne disease outbreaks

in the United States, 29 were associated with stormwater runoff (many causes were not identified).

2.2 History of Bathing Beach Bacteriological Criteria Development

The development of water quality standards for bacteria followed a pattern characteristic of efforts to control pollution-associated health and ecological effects (EPA, 1983a). According to this pattern, the guidelines and standards are first developed on the basis of attainment with the best available control technology. In the formation of these standards, limited consideration is given to epidemiology and ecological evidence or data assessing the risk with respect to pollutant level in the environment. In the second step, modifications of these guidelines and standards are done on the basis of detectable risk using a limited amount of data that relates environmental health impacts with the levels of the pollutant. In the final stage, acceptable risk is used as the basis of development of guidelines. This requires a wide range of epidemiological or ecological data for mathematically modeling the relationship between the measure of water quality and the risk or degree of health impact (EPA, 1983a). Present water quality criteria or standards for recreational waters regarding health effects have already progressed through the final stage.

Pitt *et al.*, (undated) presented an overview of the history of US indicator bacteria standards for recreation waters. Most of the following discussion is based on this prior work. At the beginning of 20th century in the U.S., large amounts of untreated domestic sewage from urban areas were discharged to nearby fresh and marine waters through the sewerage systems. The fact that enteric disease-causing organisms are potentially present

in sewage was already established by that time. These pathogens resulted in the outbreak of water contact-associated illness, like the outbreak of typhoid fever in 1921 (EPA, 1983a).

The first step towards development of microbiological guidelines for the quality of recreational waters based on attainment was presented by W.J. Scott (EPA, 1983a). The classification scheme that he presented in 1934 was based on total coliforms indicator bacteria, the first generally accepted indicator for fecal pollution. Total coliform bacteria includes *Escherichia, Klebsiella,Citobacter, Enterobacter,* and a number of other bacteria (DHS, 1997). Total coliforms are present in the feces of warm-blooded animals, but they are also present in soil. Scott, on the basis of an extensive survey of the Connecticut shoreline, found that total coliform counts of <1,000 MPN/100 mL probably indicated water acceptable for swimming. Two other approaches supported this standard. One was adopted by California in 1943 (10 MPN/1mL , the same as 1,000 MPN/100mL) which they assumed related well with drinking water standards at that time. The second one was the analytical approach followed by Streeter (1951) (As presented by EPA 1983a) which was based on anticipated risks of Salmonellosis, or Salmonella typhosa, due to the presence of Salmonella and total coliforms in bathing waters. His conclusions also supported the 1,000 MPN/100 mL total coliform standard.

Studies conducted in the late 1940s by Stevenson (1953) measured the actual health risks associated with swimming in contaminated waters. These studies suggested that the above bacterial quality requirements (<1,000 MPN/100mL) might be relaxed without significant health impact on bathers. There results were based on one of the earliest bathing beach studies conducted at five locations, including Lake Michigan

(Chicago), Ohio River (KY), and three other locations. They found that when the average coliform count was 2,300 MPN/100 mL at one of the beaches in Chicago, a significant increase in gastrointestinal illness was seen among the swimmers. Similarly, at the Ohio River site, a positive correlation was found between exposure of swimmers to median total coliform densities of 2,700 MPN/100 mL and significant increases in gastrointestinal illness.

The problem with total coliforms as an indicator is that these bacteria also include many different genera, all of which may not have their origin from fecal wastes (e.g Citrobacter, Klebsiella, and Enterobacter) (Field *et al.,* 1993). Fecal coliforms (FC), a subgroup of total coliforms, were then used as a more sensitive indicator of fecal contamination. Fecal coliforms are found in the intestinal tract of humans and other warm blooded animals and were therefore used to distinguish between bacteria of animal origin from that of soil origin (DHS, 1997).

In 1968, the NATC (National Technical Advisory Committee) recommended guidelines related to detectable risk to the Federal Water Pollution Control Administration (FWPCA), the predecessor agency to the EPA. These guidelines recommended a geometric mean fecal coliform level of 200 MPN per 100 mL, based on the Stevenson (1953) study. The U.S. EPA adopted this level as the criterion for direct water contact recreation in 1976 (Cabelli *et al.,* 1979). Most of the states in the U.S also adopted this criterion by 1984.

The studies at the Great Lake (Michigan) and Ohio River (Ohio) sites, as published by Stevenson (1953), reported epidemiologically detectable health effect at 2,300-2,400 total coliforms per 100mL. This study also reported a fecal coliform/total

coliform ratio of 0.18 based on work in the Ohio River. From these data, it was concluded that detectable health effects occur at fecal coliform levels of 400 MPN/100 mL (18% of 2,300 = 414) (Pitt *et al.*, undated). Since the water quality standard should be better than the level that would cause a health effect, the NATC recommended a limit of 200 MPN/100mL for fecal coliforms (Dufour, 1984a). Although the EPA adopted the above criteria, the 1976 EPA report (described by Field *et al.,* 1993) admitted that more epidemiological evidence was needed to support the criterion, but concluded that fecal coliforms remained the best measure of microbiological water quality.

Many follow up studies were conducted since the mid 1970s to find indicators that have direct relationships to fecal contamination and that better correlate with health hazards (Pitt *et al.,* undated). Most of these studies examined the relationships between swimming and gastrointestinal disorders, along with indicator bacteria levels using fecal coliforms, *E. coli* and enterococci.

One of the two most important studies was conducted by the EPA at swimming beaches in New York, New Orleans, and Boston (Cabelli *et al.,* 1982). This study was conducted to address some of the contradictory issues from the previous studies. They found that a direct linear relationship exists between highly credible gastrointestinal illness (HCGI) and enterococci, as can be seen from the high correlation coefficients between the mean indicator density of enterococci and HCGI in Table 2.1 (Cabelli *et al.,* 1982).

Table 2.1 Correlation Coefficients between Disease and Indicator Organism Levels for Marine Water Exposures

Indicator Organism	**Number of Observations**	**HCGI Correlation Coefficient**	**GI Correlation Coefficient**
Enterococci	9	0.96	0.81
Escherichia coli	9	0.58	0.51
Klebsiella	11	0.61	0.47
Enterobacter-	13	0.64	0.54
Total coliforms	11	0.65	0.46
Clostridium	8	0.01	-0.36
Pseudomonas	11	0.59	0.35
Fecal coliforms	12	0.51	0.36
Aeromonas	11	0.60	0.27
Vibrio	7	0.42	0.05

Source: Cabelli *et al.,* 1982

These correlation coefficients were based on studies conducted at New York (marine water) beaches from 1970 to 1976. They found that correlation coefficients for fecal coliforms were small. This was an important finding because, at that time, most of the federal and state guidelines were based on fecal coliforms. The study concluded that in marine waters contaminated with municipal wastewater, there were good associations and consistencies at different locations over different years between enterococci levels and gastrointestinal symptoms.

Form 1979 to 1982, a series of freshwater swimming beach studies monitored enterococci, *E. coli* and fecal coliforms at Tulsa, OK, and Erie, PA (Dufour, 1984a). Table 2.2 shows correlation coefficients for GI and HCGI and three indicator organisms.

Table 2.2 Correlation Coefficients between Disease and Indicator Organism Levels for Freshwater Exposures.

	HCGI	**Total GI**	**Number of Study Units**
Enterococci	0.774	0.673	9
E. coli	0.804	0.528	9
Fecal coliforms	-0.081	0.249	7

Source: Dufour, 1984a

The freshwater exposure results showed higher correlations between both HCGI and total GI illnesses with *E. coli* and enterococci than for fecal coliforms. No important correlations were found between fecal coliform levels and GI or HCGI in this freshwater study.

From both of the above studies, the following conclusions were made (Dufour 1984b).

1) Enterococci could be used as a suitable indicator for both freshwater and marine waters.

2) *E. coli* could be used if only freshwaters were under study.

3) Fecal coliforms are not suitable indicators for monitoring the safety of bathing waters.

4) Bacteria and viruses have different die-off rates for different salinity conditions.

Dufour (1984b) therefore recommended that different numeric standards be used for marine waters and for freshwaters.

The other reasons *E. coli* was selected as the indicator of choice for freshwaters was its higher density than enterococci in both human feces and sanitary wastewater effluent, and its apparent hardiness in freshwater relative to that of enterococci (Field *et al.*, 1993).

2.2.1 EPA's 1986 Ambient Water Quality Criteria for Bacteria The U.S Environmental protection agency published *Ambient Water Quality Criteria for Bacteria* in 1986 (EPA 1986) based on the above two mentioned fresh and marine water studies. This document recommended water quality criteria for bacteria to protect bathers from gastrointestinal illness in recreational waters. The EPA recommended using criteria based on *E. coli* for fresh recreational waters and enterococci for both fresh and marine recreational waters. These two new indicators were recognized as being better predictors of acute gastrointestinal illness than fecal coliforms that were recommended by EPA prior to 1986. There are other health problems associated with swimming at contaminated beaches including skin, eye, and ear infections, but these indicators are related only to gastrointestinal illness. Skin and ear infections are usually caused by high concentrations of *Pseudomonas aeroginosa* and *Shigella* present in swimming waters (Pitt, 1983). The 1986 ambient water quality criteria were based on "acceptable" health risks involved in water's recreational use. The following are the regression equations used to calculate the geometric mean densities of indicator bacteria for water quality criteria (EPA, 2002):

Fresh Water

E. coli: log (geometric mean) = (0.1064 x illness rate) + 1.249
Enterococci: log (geometric mean) = (0.1064 x illness rate) + 0.668

Marine Water

Enterococci: log (geometric mean) = (0.0827 x illness rate) - 0.0164

The following equation was used to calculate single sample maximum values for both marine and freshwaters:

Log (SSMV*) = (Log (Geometric Mean Value)) + ((Confidence Level Factor) x (Log Standard Deviation))

* SSMV: Single sample maximum value.

Confidence level factors for different confidence levels:

75% = 0.68
82% = 0.94
90% = 1.28
95% = 1.65

Log standard deviation: Freshwater = 0.4

Marine Water = 0.7

Table 2.3 shows the recommended 1986 criteria for bacteria and the acceptable illness rates on which they were based.

Table 2.3 EPA's Ambient Water Quality Criteria for Bacteria – 1986

	Marine Waters	**Fresh Waters**
Acceptable gastroenteritis illness rate	19 per 1000 swimmers	8 per 1000 swimmers
Geometric mean density (MPN)	35 Enterococci/100 mL	33 Enterococci/100 mL or 126 *E. coli* /100 mL
Single sample maximum allowable density :		
Designated beach area (75 % C.L = Confidence level)	104 Enterococci/100 mL	61 Enterococci/100 mL or 235 *E. coli* /100 mL
Moderate full body contact recreation area. (82 % C.L = Confidence level)	124 Enterococci/100 mL	89 Enterococci/100 mL or 298 *E. coli* /100 mL
Lightly used full body contact recreation. (90 % C.L = Confidence level)	276 Enterococci/100 mL	108 Enterococci/100 mL or 406 *E. coli* /100 mL
Infrequently used full body contact recreation. (95 % C.L = Confidence level)	500 Enterococci/100 mL	151 Enterococci/100 mL or 576 *E. coli* /100 mL
Comparable fecal coliform exposure	200 fecal coliforms/100 mL	200 fecal coliforms/100mL

Source: EPA, 1986

The EPA issued draft implementation guidance for ambient water quality criteria for bacteria in May 2002 to provide guidance to the states and other authorities on

implementation of above criteria. In the draft, the agency strongly encouraged states and authorized tribes to adopt the above criteria or any other scientifically defensible water quality standard for their recreational waters to replace fecal or total coliform criteria. As reported in the EPA's May 2002 draft, only 18 states, 3 territories and 6 authorized tribes have adopted *E. coli* or enterococci based criteria for all or part of their waters designated for recreation. Major policy changes were recommended in the document regarding non-human fecal contamination. It was recommend that states and authorized tribes should not use broad exemptions from the bacteriological criteria for waters designated for primary contact recreation, assuming that water bodies that are impacted by non-human fecal contamination present no risk to human health. This was contrary to the EPA recommendations in its 1994 water quality standards handbook (EPA, 2002). The May 2002 draft recommended the use of a more conservative approach for waters that are heavily used for recreation, such as using criteria based upon lower illness rates, considering the use of the 75 % confidence level for single sample maximum values, more frequent monitoring etc. If a state opts to use the same illness rates for marine and recreational waters, then it should be no greater than 14 illnesses per 1000 swimmers, otherwise the freshwater criteria should be based upon a maximum of 14 illnesses per 1000 swimmers and marine water criteria maximum of 19 illnesses per 1000 swimmers.

Various epidemiology studies conducted since the EPA's 1984 epidemiology studies reaffirmed the scientific validity of the 1986 ambient water quality criteria for bacteria as described in the following discussion. Moreover, recently enacted BEACH (Beaches Environmental Assessment and Coastal Health) Act amendments require coastal and Great Lake states to adopt the 1986 water quality criteria for bacteria or some

other criteria that can prove to be as protective as EPA's for Great Lakes, marine and estuarine waters, by April 2004.

However, recent research has raised issues about the appropriateness of using *E. coli* and enterococci as indicators for assessing the risk of gastrointestinal illness in tropical recreational waters. Fujioka *et al.,* 1999; Fujioka and Byappanahalli, 1998; Lopez-Torres *et al.,* 1987 reported persistence of these indicators in soils and water bodies (as presented in EPA, 2002). Fujioka and Byappanahalli (1998) hypothesized that in cases where uniform tropical conditions persist, these indicators develop mechanisms to maintain viable cell populations for significant periods of time. The EPA recommended that at present there is not enough evidence to change the existing criteria for tropical recreational waters and plans to conduct further research into this issue.

2.3 Epidemiological Studies Conducted After 1984

Epidemiology is defined as the study of occurrence and causes of disease in human populations and the application of this knowledge to the prevention and control of health problems. Pathogens present in stormwater from separate storm drainage systems are likely as important a public health concern as are pathogens associated with sewage-contaminated water. As discussed in the previous chapter, it is difficult to identify the risks associated with stormwater alone because of the possibility of sewage contamination and the presence of other toxicants. Prior epidemiology studies have mostly focused on water contaminated by sanitary sewage, but some recent work has elaborated the increased health risk and problems associated with swimming in stormwater contaminated waters (Haile *et al.,* 1996). The levels of pathogens investigated

in past studies that have involved sanitary sewage contaminated waters only were generally within the range found in urban waters affected by stormwater only. Therefore, these studies are indicative of similar risks that may be involved in water contact recreational in waters affected by stormwater alone (Pitt *et al.,* undated).

Pruss (1998) presented an excellent review of all epidemiology studies on the health effects from exposure to recreational waters conducted since 1953. The nine marine studies and two freshwater studies that were conducted after 1984 (and the completion of the EPA "1986 bacteria criteria" studies) are presented in this section (Table 2.4). Most of these epidemiology studies examined relationships between swimming- associated gastroenteritis and indicator bacteria such as coliforms, *E. coli* and enterococci.

Table 2.4 Major Gastroenteritis Related Epidemiology Studies Conducted Since 1984

Name of Researcher	**Year of Study**	**Type of Water Body Studied**	**Microorganisms Evaluated**	**Relevant Findings**	**Location**
Fattal	1987	Marine	Fecal coliform Enterococci *E. coli*	Enterococci found the most predictive indicator for enteric disease symptoms	Israel
Cheung *et al.*	1990	Marine	Fecal coliforms *E. coli* *Klebsiella* spp. Enterococci Fecal streptococci Staphylococci *Pseudomonas aeruginosa* *Candida albicans* Total fungi	Best relationship between a microbial indicator density and swimming-associated health effects was between *E. coli* and highly credible gastrointestinal illness.	Hong Kong
Balarajan *et al.*	1991	Marine	Not known	The more the degree of exposure, the higher the risk of illness. Assuming risk rank 1 for non-exposed population, risk rank increased to 1.25 for waders, 1.31 for swimmers, and 1.81 for surfers and divers	United kingdom
Von Schirnding *et al.*	1992	Marine	Enterococci Fecal coliforms Coliphages Staphylococci F-male-specific Bacteriophages	Uncertainty in sources of fecal contamination may explain lack of statistically significant rates of illness between swimmers and non -swimmers.	Atlantic coast South Africa

Contd..

Corbett *et al*.	1993	Marine	Fecal coliforms Fecal streptococci	1) Gastrointestinal symptoms in swimmers did not increase with increasing counts of fecal bacteria. 2) Counts of fecal streptococci were worse predictors of swimming-associated illness than fecal coliforms.	Sydney , Australia
Kay *et al.*	1994	Marine	Total coliforms Fecal coliforms Fecal streptococci *Pseudomonas aeruginosa* Total staphylococci	1) Only fecal streptococci were associated with rates of gastroenteritis. 2) Risk of gastroenteritis did not increase until bathers were exposed to about 40 fecal streptococci per100 mL.	United Kingdom
Kueh *et al.*	1995	Marine	*E. coli* Fecal coliforms Staphylococci *Aeromonas* spp. *Clostridium perfringens* *Vibrio cholera* *Vibrio parahemolyticus* *Salmonella* spp. *Shigella* spp.	1) Also analysed stool specimens for rotavirus, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Aeromonas* spp.; throat swabs for Influenza A and B; Parainfluenza Virus types 1, 2, and 3; Respiratory Syncytial Virus; and Adenovirus. 2) Did not find a relationship between *E. coli* and swimming-associated illness [possibly due to low number of beaches sampled (only two)].	Hong Kong
McBride *et al*.	1998	Marine	Fecal coliforms *E. coli* Enterococci	1) Enterococci were most strongly and consistently associated with illness risk for the exposed groups. 2) Risk differences significantly greater between swimmers and non-swimmers if swimmers remained in water for more than 30 minutes.	New Zealand

Contd..

Haile *et al*.	1996	Marine	Total coliforms Fecal coliforms *E. coli* Enterococci	1) Results for enterococci indicate positive associations with fever, skin rash, nausea, diarrhea, stomach pain, Coughing, runny nose, and highly credible gastrointestinal illness. 2) Association of symptoms with both *E. coli* and fecal coliforms were very weak. 3) Total coliform to fecal coliform ratio very informative — below the cutpoint of 5.0, diarrhea and highly credible gastrointestinal illness were associated with a lower ratio regardless of the absolute level of fecal coliforms.	California, USA
Seyfried *et al*.	1985	Fresh	Fecal coliforms Fecal streptococci Heterotrophic bacteria *Pseudomonas aeruginosa* Total staphylococci	1) Small degree of correlation observed between streptococci and gastrointestinal illness. 2) Best correlation was between gastrointestinal illness and staphylococcus densities.	Canada
Ferley *et al*.	1989	Fresh	Fecal coliforms Fecal streptococci (Assumed similar to enterococci) *Pseudomonas aeruginosa*	1) Good relationship between swimming illness and fecal coliform and fecal streptococci concentrations. 2) Strongest relationship was between gastrointestinal disease and fecal streptococci densities.	France

Source: EPA, 2002

Seyfried *et al.,* (1985) found good correlations between total staphylococci and swimming associated total illness, plus ear, eye and skin illness. He found the correlation to be not as good between fecal streptococci and fecal coliform and total illness. Ferley, *et al.,* (1989) reported that skin ailments were more common for swimmers as compared to non swimmers and found good correlations between skin ailments and fecal coliforms, *Aeromonas Spp,* and *Pseudomonas aeruginosa*. Various other epidemiology studies related to the presence of pathogens in recreational water and their correlations with illness included work done by Fleisher 1991, Fleisher *et al.,* 1993, and Koenraad *et al.*, 1997.

Recent amendments to the Clean Water Act (BEACH Act) require EPA to evaluate potential human health risks that are associated with exposures to pathogens in costal recreational waters. The EPA is therefore planning to conduct more epidemiology studies in the future that may be used to revise and develop new water quality criteria for bacteria. The EPA is planning to study the range of indicator bacteria in marine as well as fresh recreational waters by the end of 2006.

2.4 Studies Investigating Sources of Indicator Bacteria in Wet Weather and Dry Weather Flows

2.4.1 Levels of Indicator Bacteria in Urban Runoff The level of indicator bacteria in urban stormwater has been found to vary during storms. A number of studies tried to find the effects of various factors such as rain intensity, flow, interevent period, etc., on bacterial concentrations in runoff. Some studies reported relationships between bacterial concentrations and observed factors, while others found no correlations.

Lager *et al.,* (1977) found that the two main factors that affected fecal coliform concentrations in urban stormwater were the interevent period and the intensity of rain. Seidler (1979) reported that there was a better correlation between bacteria concentrations during a storm event and the length of the dry interevent period before the storm than season or actual amount of rain. A number of other studies reported high bacterial concentrations during high urban runoff flows and lower concentrations otherwise (Evans and Owens 1972; Casserly and Davis 1979; Pontius 1977; Davis 1979; and Siedler 1979). However, many subsequent studies studied the relationships between bacterial concentrations in urban runoff and instantaneous flows and length of time since last rainfall (Oliveri *et al.,* 1977), intensity and amount of rain (Qureshi and Dutka, 1979), or flow only (Gupta *et al.,* 1981). These studies reported no effect of these factors on bacterial concentrations.

According to Pitt (undated) it is very hard to find consistent factors that influence the bacterial concentrations because of the variable nature of bacteria deposition, accumulation, and transport and die-off rates. He stated that the best approach in characterizing bacteria in urban runoff is to study as many storms as possible in the watershed of concern (as done in this research), instead of monitoring few storms many times (as was done during most of above studies). The National Urban Runoff Program (NURP) (EPA, 1983b), included 28 separately conducted projects throughout the U.S. that were conducted using similar methods. These projects collected stormwater data from 1979 through 1982 and more than 2300 separate storm events were studied, including fecal coliform analyses at many sites. The NURP projects found no correlations in fecal coliform concentrations based on land use, and reported high variability in

concentrations. The fecal coliform levels exceeded the water quality criteria for most events. The bacterial levels present in urban runoff during warmer periods of the year were found to be 20 times greater than those found during colder periods (EPA, 1999). The NURP program reported a fecal coliform range of 10 to 270,000 organisms/100 mL from 1600 observations. The average of the site means was 20,000 fecal coliforms/100 mL. Lawns, roads, leaky sanitary sewer lines, sanitary sewer cross-connections, animal wastes, and septic systems were expected to be the main sources of the bacteria (EPA, 1999). One of the important issues that emerged from the NURP studies was that of illicit discharges to storm drainage systems (dry weather flows). The executive summary of the NURP report states that dramatic improvements in the quality of urban stormwater discharges can be achieved by locating and eliminating these discharges (EPA, 1983b).

Leeming *et al.,* (1998) reported the following very high bacterial indicator densities (cfu/100mL) of *E. coli* and enterococci found from four sites along a stormwater drain in the Ripple side area of Geelong, Victoria, Canada (Table 2.5).

Table 2.5 Bacterial Indicator Densities along a Stormwater Drain in the Ripple Side

	Site 1		Site 2		Site 3		Site 4	
E. coli	1.1E+6	1.1E+4	1.1E+6	8.3E+3	3.0E+6	9.6E+3	2.5E+6	8.0E+3
Enterococci	3.9.E+4	1.6E+6	6.4E+4	3.8E+3	4.5E+5	4.2E+3	4.2E+5	5.3E+3

2.4.2 Sources A number of studies have been done to study potential sources of pathogens and indicator organisms in urban runoff. Pitt (undated) presented a review of various studies that investigated these sources. The following paragraphs are summarized from this earlier work. Most of the studies done in this field examined runoff at outfalls which can be possibly contaminated by inappropriate discharges in the drainage system, while some studies examined surface sheet flows which are unlikely to be contaminated

by sewage. The research conducted during this thesis investigated sheetflows during rains and sampled both outfalls and potential source area flows during dry weather.

The Toronto Area Watershed Management Strategy Study (TAWMSS) reported that sheetflows from sidewalks, roads, and bare ground (where dogs are likely 'walked') had high bacterial levels (Pitt and McLean, 1986). During the snow melt period, fecal coliform concentrations observed in sheetflows were mostly significantly lower than the outfall concentrations which indicated that sanitary sewage was entering the storm drainage system. Roof and freeway runoff showed low densities as compared to other source areas.

Gore and Storrie Ltd. /Proctor and Redfern Ltd.(1981) examined various sources in an attempt to explain the high dry weather coliform concentrations found in the Rideau River in Ottawa. They concluded that stormwater runoff was the likely source. They reported that the river has a slow travel time that makes it difficult to recover completely form one rainstorm before another begins.

A number of studies found that feces bacteria that had been deposited on the soil and on the surface of drainage areas serve as the most important sources of bacteria in runoff (Qureshi and Dutka 1979, Geldreich and Kenner 1969). Geldreich (1965) found that most of the bacteria on vegetation are of insect origin and don't contribute much to runoff. Geldreich *et al.,* (1980) reported recreational activities as a cause of increases in fecal coliform and fecal strep. concentrations and found that these organisms concentrate near the shore or areas of stratification. Gupta *et al.,* (1981) found high concentrations of fecal coliforms in runoff from an elevated bridge deck on a highway. They reported bird droppings and feces debris falling from livestock carrying vehicles as likely sources.

Field *et al.,* (1993) reported that stormwater runoff mainly contains indicator organisms that may not be necessarily of human fecal origin, i.e they may be originating from animal feces, vegetation and soils, etc. Moreover, many non-enteric pathogens also are present in stormwater and can cause respiratory illnesses and skin infections. Epidemiology studies show that the indicator bacteria such as *E. coli* and enterococci better predict the risk of gastrointestinal infections and not the risk of these other infections. Therefore, they concluded that receiving waters affected mainly by stormwater discharges, these indicators do not accurately predict the total illness producing capacity. They described a well-documented study that predicted the health risks associated with swimming in stormwater contaminated receiving waters (Calderon, *et al.,* 1991), which found that staphylococci (non-enteric pathogen) is better correlated with swimming associated illness than fecal indicators such as *E. coli*, fecal coliform etc. They concluded the great need for epidemiological investigations that can predict nonhuman and non-enteric pathogens related risks. Marsalek *et al.,* (1996) investigated microbiological pollution in the Canadian upper Great Lakes connecting channels. They concluded that the higher the density of sewer outfalls (storm sewers as well as CSOs), the more likely the extent of microbiological pollution. They also found dry weather sources as an important factor affecting the receiving water quality of a study area.

Schiffand and Kinney (2000) investigated sources of indicator bacteria in stormwater flows discharging to Mission Bay in San Diego. They found that long-term monitoring of Mission Bay indicated wet weather flows as the predominant source of bacterial contamination. Eighty-nine storm drains discharge to the bay. These storm drains either were not flowing during dry weather, or bacterial densities found in dry

weather flows were very low. Indicator bacteria studied included total coliforms, fecal coliforms and enterococcus. They reported that indicator bacteria densities found at the head of two watersheds draining into the bay were as high as they were at the mouth during storm events. This showed that high bacterial density found in stormwater were not caused by any point source, but by diffuse and widespread sources. Bacterial densities found in surface flows prior to entering the separate municipal storm sewer system from urban land uses were similar to in-stream densities.

Schiffand and Kinney (2000) also found no apparent relationship between flow and bacterial density. Indicator densities found at 20 monitoring stations on the bay showed higher bacterial densities during the wet months. They found bacterial densities in urban runoff from small catchments of 1-4 acres representative of urban land uses.

Mallin *et al.*, (2001) examined the effect of human use of coastal lands on microbial pollution of coastal waters in North Carolina. They concluded that in urbanizing coastal areas, decreasing the areas of impervious surfaces and increasing the areas of natural and constructed wetlands for passive stormwater runoff treatment can result in decreased bacteria abundance. They also suggested that in areas of animal husbandry, stormwater management practices designed to minimize sediment runoff and retention of natural wetlands can be effective in reducing pathogen loads entering into streams.

McLellan and Salmore (2003) examined the bacterial water quality of a swimming beach area on Lake Michigan. They found that *E. coli* concentrations were higher on shoreline regions than offshore regions (10-150 m from shore) during dry as well as wet weather. Water samples exceeded the 235 cfu/100mL *E. coli* in 5% of the

offshore samples (n=209) and 66% of the shoreline samples (n=675). They concluded that the source originated from the shoreline and localized impacts of bird feces and stormwater contributed to high bacterial densities.

2.4.3 Distinguishing Fecal Contamination Sources Some studies have been conducted to determine how to distinguish between various sources of fecal contamination. Geldreich (1965) reported that the ratio of fecal coliform to fecal strep. bacteria counts can be used to distinguish between human and animal sources of fecal contamination. He found this ratio to be 4 in case of human sources and less than 0.6 in case of animal fecal sources. However, further studies showed that this ratio is very sensitive and is valid only within 24 hours following discharge of the bacteria. Furthermore, water temperature, toxic metals, pH, etc., may alter the ratio (Geldreich and Kenner, 1969). An interesting study by Feachem (1975) found that if the FC/FS ratios are initially greater than 4, and then decrease with time, it indicates human sources. However, if the initial low ratio is less than 0.7, and it rise with time, it indicates non-human bacterial sources. The reason for this is the different die-off rates of both organisms. Pitt (1983) explained the sources of bacteria in the Rideau River, Ottawa, by finding this ratio in sheetflow water, Rideau River water and water samples collected at the swimming beaches (Table 2.6).

Table 2.6 Fecal Coliform to Fecal Strep. Bacteria Population Ratios in Study Area

Source Areas	**FC/FS Ratio**
Rooftop runoff	0.5
Vacant land sheetflow	0.3
Parking lot sheetflow	0.2
Gutter flows	0.2
Average of source area values	0.3
Rideau River Segment	
A	1.2

B	0.6
C	0.5
D	0.5
E	1.0
Average of river segment values	0.7
River Swimming Beaches	
Strathcona	2.8
Brantwood	2.3
Brighton	2.1
Mooney's Bay	1.7
Average of swimming beach values	2.2

Source : Pitt, 1983

Most recent pollution is found in sheetflow samples while the bacteria present in river and beach are older. As can be seen, the ratios change between less than 0.7 in the sheetflows, to 0.5 to 1.2 in river samples (because of older pollution as compared to sheetflows) and finally 1.7 to 2.8 in beach samples (shows older bacteria as compared to river water). Since the ratios start at less than 0.7 and increase with time, Pitt (1983) concluded that it is due to non-human sources based on the work by Feachem (1975). These results supported the conclusions of earlier studies on the Rideau River.

2.4.4 Emerging Tools for Identifying Sources of Discharges The following discussion is mostly summarized from a paper by Pitt (2001b).

Coprostanol and Other Fecal Sterol Compounds Utilized as Tracers of Contamination by Sanitary Sewage A more likely indicator of human wastes than fecal coliforms and other "indicator" bacteria may be the use of certain molecular markers, specifically the fecal sterols, such as coprostanol and epicoprostanol (Eaganhouse *et al.,* 1988). However, other carnivores also discharge these compounds in a drainage system (especially dogs). A number of research projects have used these compounds to investigate the presence of sanitary sewage contamination. The most

successful application may be associated with sediment analyses instead of water analyses. As an example, water analyses of coprostanol are difficult due to the typically very low concentrations found, although the concentrations in many types of sediment are quite high and much easier to quantify. Unfortunately, the long persistence of these compounds easily confuses recent contamination with historical, or intermittent contamination.

Particulates and sediments collected from coastal areas in Spain and Cuba receiving municipal sewage loads were analyzed by Grimalt *et al.*, (1990) to determine the utility of coprostanol as a chemical marker of sewage contamination. Coprostanol cannot by itself be attributed to fecal matter inputs. However, relative contributions of steroid components can be a useful indicator. When the relative concentrations of coprostanol and coprostanone are higher than their 5α epimers, or more realistically, other sterol components of background or natural occurrence, it can be useful.

Sediment cores from Santa Monica Basin, CA, and effluent from two local municipal wastewater discharges were analyzed by Venkatesan and Kaplan (1990) for coprostanol to determine the degree of sewage addition to sediment. Coprostanol was distributed throughout the basin sediments in association with fine particles. Some stations contained elevated levels, either due to their proximity to outfalls, or because of preferential advection of fine-grained sediments. A noted decline of coprostanol relative to total sterols from outfalls seaward indicated dilution of sewage by biogenic sterols. Other chemical compounds have been utilized for sewage tracer work. Saturated hydrocarbons with 16-18 carbons, and saturated hydrocarbons with 16-21 carbons, in addition to coprostanol, were chosen as markers for sewage in water, particulate, and

sediment samples near the Cocoa, FL, domestic wastewater treatment plant (Holm and Windsor, 1990). The concentrations of the markers were highest at points close to the outfall pipe and diminished with distance. However the concentrations of C16-C21 compounds were high at a site 800 m from the outfall indicating that these compounds were unsuitable markers for locating areas exposed to the sewage plume. The concentrations for the other markers were very low at this station.

The range of concentrations of coprostanol found in sediments and mussels of Venice, Italy, were reported by Sherwin *et al.,* (1993). Raw sewage is still discharged directly into the Venice lagoon. Coprostanol concentrations were determined in sediment and mussel samples from the lagoon using gas chromatography/mass spectroscopy (GC/MS). Samples were collected in interior canals and compared to open-bay concentrations. Sediment concentrations ranged from 0.2-41.0 μg/g (dry weight). Interior canal sediment samples averaged 16 μg/g compared to 2 μg/g found in open bay sediment samples. Total coprostanol concentrations in mussels ranged from 80 to 620 ng/g (wet weight). No mussels were found in the four most polluted interior canal sites.

Nichols *et al.,* (1996) also examined coprostanol in stormwater and the sea-surface microlayer to distinguish human versus nonhuman sources of contamination. Other steroid compounds in sewage effluent were investigated by Routledge *et al.,* (1998) and Desbrow *et al.,* (1998) who both examined estrogenic chemicals. The most common found were 17β-Estradiol and estrone which were detected at concentrations in the tens of nanograms per liter range. These were identified as estrogenic through a toxicity identification and evaluation approach, where sequential separations and analyses

identified the sample fractions causing estrogenic activity using a yeast-based estrogen screen. GC/MS was then used to identify the specific compounds.

Leeming *et al.,* (1996 and 1998) used a hybrid approach to distinguish sources of fecal pollution during a study at Port Phillip Bay, Australia. They found that ratios of sterol biomarkers (coprostanol and 24-ethylcoprostanol) to indicator bacteria (like themrotolerant coliforms, *E. coli* , faecal streptococci and enterococci) can be useful tools in distinguishing sources of fecal pollution. They found that the ratios of coprostanol to bacterial indicators were similar in water samples collected from nearby sewer mains and from wet weather outfalls. They therefore concluded that significant human fecal contamination was present in the wet weather flows at those sampling locations. Indicator bacteria densities found in dry weather flows implied significant fecal contamination, but the concentrations of fecal sterols were very low. This suggested that the source of contamination was not human or herbivore.

Jagals *et al.,* (1995) found that the sorbitol –fermenting bifidobacteria are very much specific to fecal pollution of human origin. However, these organisms were found to be short-lived in the environment (Resnic and Levin, 1981). They therefore concluded that the presence of sorbitol-fermenting bifidobacteria can serve as a good indicator of recent fecal pollution of human origin. They also found that highly resistant *R coprophilus* bacteria are specifically associated with fecal pollution of animal origin, which confirmed the findings of Oragui and Mara (1983).

Estimating Potential Sanitary Sewage Discharges into Storm Drainage and Receiving Waters using Detergent Tracer Compounds Detergent measurements (using

methylene blue active substance, MBAS, test methods) are the most successful individual tracer to indicate contaminated water in storm sewerage dry-weather flows (Pitt, *et al*. 1993 and current research). Unfortunately, the MBAS method uses hazardous chloroform or benzene for an extraction step. Different detergent components, especially linear alkylbenzene sulphonates (LAS) and linear alkylbenzenes (LAB), have also been tried to indicate sewage dispersal patterns in receiving waters. Boron, a major historical ingredient of laundry chemicals, can also potentially be used. Boron has the great advantage of being relatively easy to analyze using portable field test kits, while LAS requires chromatographic equipment. LAS can be measured using HPLC with fluorescent detection, after solid phase extraction, to very low levels. Fujita *et al.,* (1998) developed an efficient enzyme-linked immunosorbent assay (ELISA) for detecting LAS at levels from 20 to 500 μg/L.

LAS from synthetic surfactants (Terzic and Ahel, 1993), which degrade rapidly, as well as nonionic detergents (Terzic and Ahel, 1993), which do not degrade rapidly, have been utilized as sanitary sewage markers. LAS was quickly dispersed from wastewater outfalls except in areas where wind was calm. In these areas LAS concentrations increased in freshwater but were unaffected in saline water. After time, the lower alkyl groups were mostly found, possibly as a result of degradation or settling of longer alkyl chain compounds with sediments. Chung *et al.*, (1995) also describe the distribution and fate of LAS in an urban stream in Korea. They examined different LAS compounds having carbon ratios of C12 and C13 compared to C10 and C11, plus ratios of phosphates to MBAS and the internal to external isomer ratio (I/E) as part of their research. Gonález-Mazo *et al.,* (1998) examined LAS in the Bay of Cádiz off the

southwest of Spain. They found that LAS degrades rapidly (Fujita *et al.,* (1998) found that complete biodegradation of LAS requires several days), and is also strongly sorbed to particulates. In areas close to shore and near the untreated wastewater discharges, there as significant vertical stratification of LAS: the top 3 to 5 mm of water had LAS concentrations about 100 times greater than found at 0.5 m.

Zeng and Vista (1997) and Zeng *et al.,* (1997) describe a study off of San Diego where LAB was measured, along with polycyclic aromatic hydrocarbons (PAHs) and aliphatic hydrocarbons (AHs) to indicate the relative pollutant contributions of wastewater from sanitary sewage, nonpoint sources, and hydrocarbon combustion sources. They developed and tested several indicator ratios (alkyl homologue distributions and parent compound distributions) and examined the ratio of various PAHs (such as phenanthrene to anthracene, methylphenanthrene to phenanthrene, fluoranthene to pyrene, and benzo(a)anthracene to chrysene) as tools for distinguishing these sources. They concluded that LABs are useful tracers of domestic waste inputs to the environment due to their limited sources. They also describe the use of the internal to external isomer ratio (I/E) to indicate the amount of biodegradation that may have occurred to the LABs. They observed concentrations of total LABs in sewage effluent of about 3 μg/L, although previous researchers have seen concentrations of about 150 μg/L in sewage effluent from the same area.

The fluorescent properties of detergents have also been used as a tracer by investigating the fluorescent whitening agents (FWAs), as described by Poiger *et al.,* (1996) and Kramer *et al.,* (1996). HPLC with fluorescence detection was used in these studies to quantify very low concentrations of FWAs. The two most frequently used

FWAs in household detergents (DSBP and DAS 1) were found at 7 to 21 μg/L in primary sewage effluent and at 3 to 9 μg/L in secondary effluent. Raw sewage contains about 10 to 20 μg/L FWAs. The removal mechanisms in sewage treatment processes is by adsorption to activated sludge. The type of FWAs varies from laundry applications to textile finishing and paper production, making it possible to identify sewage sources. The FWAs were found in river water at 0.04 to 0.6 μg/L. The FWAs are not easily biodegradable but they are readily photodegraded. Photodegradation rates have been reported to be about 7% for DSBP and 71% for DAS 1 in river water exposed to natural sunlight, after one hour exposure. Subsequent photodegradation is quite slow.

Other Compounds Found in Sanitary Sewage that may be used for Identifying Contamination by Sewage Halling-Sørensen, *et al.,* (1998) detected numerous pharmaceutical substances in sewage effluents and in receiving waters. Their work addressed human health concerns of these low level compounds that can enter downstream drinking water supplies. However, the information can also be possibly used to help identify sewage contamination. Most of the research has focused on clofibric acid, a chemical used in cholesterol lowering drugs. It has been found in concentrations ranging from 10 to 165 ng/L in Berlin drinking water sampler. Other drugs commonly found include aspirin, caffeine, and ibuprofen. Current FDA guidance mandates that the maximum concentration of a substance or its active metabolites at the point of entry into the aquatic environment be less than 1 μg/L (Hun, 1998).

Caffeine has been used as an indicator of sewage contamination by several investigators (Shuman and Strand, 1996). The King County, WA, Water Quality Assessment Project is examining the impacts of CSOs on the Duwamish River and Elliott

Bay. They are using both caffeine (representing dissolved CSO constituents) and coprostanol (representing particulate bound CSO constituents), in conjunction with heavy metals and conventional analyses, to help determine the contribution of CSOs to the river. The caffeine is unique to sewage, while coprostanol is from both humans and carnivorous animals and is therefore also in stormwater. They sampled upstream of all CSOs, but with some stormwater influences, 100 m upstream of the primary CSO discharge (but downstream of other CSOs), within the primary CSO discharge line, and 100 m downriver of the CSO discharge location. The relationship between caffeine and coprostanol was fairly consistent for the four sites (coprostanol was about 0.5 to 1.5 μg/L higher than caffeine). Similar patterns were found between the three metals, chromium was always the lowest and zinc was the highest. King Co. is also using clean transported mussels placed in the Duwamish River to measure the bioconcentration potential of metal and organic toxicants and the effects of the CSOs on mussel growth rates (after 6 week exposure periods). Paired reference locations are available near the areas of deployment, but outside the areas of immediate CSO influence. *US Water News* (1998) also described a study in Boston Harbor that found caffeine at levels of about 7 μg/L in the harbor water. The caffeine content of regular coffee is about 700 mg/L, in contrast.

DNA Profiling to Measure Impacts on Receiving Water Organisms and to Identify Sources of Microorganisms in Stormwater This rapidly emerging technique seems to have great promise in addressing a number of nonpoint source water pollution issues. Kratch (1997) summarized several investigations on cataloging the DNA of *E. coli* to identify their source in water. This rapidly emerging technique seems to have great promise in addressing a number of nonpoint source water pollution issues. The

procedure, developed at the Virginia Polytechnic Institute and State University, has been used in Chesapeake Bay. In one example, it was possible to identify a large wild animal population as the source of fecal coliform contamination of a shellfish bed, instead of suspected failing septic tanks. DNA patterns in fecal coliforms vary among animals and birds, and it is relatively easy to distinguish between human and non-human sources of the bacteria. However, some wild animals have DNA patterns that are not easily distinguishable. Some researchers question the value of *E. coli* DNA fingerprinting believing that there is little direct relationship between *E. coli* and human pathogens. However, this method should be useful to identify the presence of sewage contamination in stormwater or in a receiving water.

One application of the technique, as described by Krane *et al.*, (1999) of Wright State University, used randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) generated profiles of naturally occurring crayfish. They found that changes in the underlying genetic diversity of these populations were significantly correlated with the extent to which they have been exposed to anthropogenic stressors. They concluded that this rapid and relatively simple technique can be used to develop a sensitive means of directly assessing the impact of stressors upon ecosystems. These Wright State University researchers have also used the RAPD-PCR techniques on populations of snails, pill bugs, violets, spiders, earthworms, herring, and some benthic macroinvertebrates, finding relatively few obstacles in its use for different organisms. As noted above, other researchers have used DNA profiling techniques to identify sources of *E. coli* bacteria found in coastal waterways. It is possible that these techniques can be expanded to enable

rapid detection of many different types of pathogens in receiving waters, and the most likely sources of these pathogens.

Stable Isotope Methods for Identifying Sources of Water Stable isotopes had been recommended as an efficient method to identify illicit connections to storm sewerage. A demonstration was conducted in Detroit as part of the Rouge River project to identify sources of dry weather flows in storm sewerage (Sangal *et al.,* 1996). Naturally occurring stable isotopes of oxygen and hydrogen can be used to identify waters originating from different geographical sources (especially along a north-south gradient). Ma and Spalding (1996) discuss this approach by using stable isotopes to investigate recharge of groundwaters by surface waters. During water vapor transport from equatorial source regions to higher latitudes, depletion of heavy isotopes occurs with rain. Deviation from a standard relationship between deuterium and ^{18}O for a specific area indicates that the water has undergone additional evaporation. The ratio is also affected by seasonal changes. As discussed by Ma and Spalding (1996), the Platte River water is normally derived in part from snowmelt from the Rocky Mountains, while the groundwater in parts of Nebraska is mainly contributed from the Gulf air stream. The origins of these waters are sufficiently different and allow good measurements of the recharge rate of the surface water to the groundwater. In Detroit, Sangal *et al.,* (1996) used differences in origin between the domestic water supply, local surface waters, and the local groundwater to identify potential sanitary sewage contributions to the separate storm sewerage. Rieley *et al.,* (1997) used stable isotopes of carbon in marine organisms to distinguish the primary source of carbon being consumed (sewage sludge vs. natural carbon sources) in two deep sea sewage sludge disposal areas.

Stable isotope analyses would not be able to distinguish between sanitary sewage, industrial discharges, washwaters, and domestic water, as they all have the same origin, nor would it be possible to distinguish sewage from local groundwaters if the domestic water supply was from the same local aquifer. This method works best for situations where the water supply is from a distant source and where separation of waters into separate flow components is not needed. It may be an excellent tool to study the effects of deep well injection of stormwater on deep aquifers having distant recharge sources (such as in the Phoenix area). Few laboratories can analyze for these stable isotopes, requiring shipping and a long wait for the analytical results. Sangal *et al.,* (1996) used Geochron Laboratories, in Cambridge, Massachusetts.

Dating of sediments using ^{137}Cs was described by Ma and Spalding (1996). Arsenic contaminated sediments in the Hylebos Waterway in Tacoma, WA, could have originated from numerous sources, including a pesticide manufacturing facility, a rock-wool plant, steel slags, powdered metal plant, shipbuilding facilities, marinas and arsenic boat paints, and the Tacoma Smelter. Dating the sediments, combined with knowing the history of potential discharges and conducting optical and electron microscopic studies of the sediments was found to be a powerful tool to differentiate between the different metal sources to the sediments.

Bacterial Source Tracking (BST) Techniques The entire bacterial source tracking techniques can be divided into three broad categories namely microbiological (molecular), biochemical and chemical. Of these, chemical methods are based on finding chemical compounds present in human wastewaters and can be used to determine if the source of pollution is human or not. These include the use of detergents/optical

brighteners, caffeine, coprostanol, etc. and were discussed in the above section. Biochemical methods are based on an effect of an organism's genes that actively produce a biochemical substance (www.maptech-inc.com, 2003). Molecular (DNA fingerprinting) techniques are based on the unique genetic make up of different strains of fecal bacteria and can be considered a subcategory of microbiological methods. This section gives short description and examples of studies that used microbiological and biochemical methods.

Traditionally, the fecal coliform to fecal streptococci ratio was used to distinguish human from non-human bacteria sources, which was discussed earlier in this section. The differences in fecal streptococcus group species composition among various types of animals has been observed during various studies. Different percentages and proportions of fecal streptococcus group species could be used to differentiate among various animals and to distinguish human sources from other animals This test involves moderate time, effort and cost. However, there are concerns about its validity (Sargeant, 1999). Various species-specific indicators could also be used to determine which specific species is the source. These are listed in Table 2.7. None of these could be used to quantify the source and more information is needed for their use in marine waters. Those having low survival rates represent recent fecal contamination and those having high survival rates may not represent recent fecal contamination.

Table 2.7 Species Specific Indicators and Sources Determined

Bacterial Species Or Indicator	**Source Determined**	**Survival Rate**	**Lab Costs**
Streptococcus bovis	Non-human, found in low numbers in humans	Low	Low to moderate
Clostridium perfringens	Point source of sewage pollution	High	Moderate, anaerobic laboratory procedures required
Bifidobacteria longum, B.adolescentis	Human source-point or non point	Low	High, analysis best with gene probe assays. More work needs to be done on lab methods
Bacteroides fragilis group	Recent point or non point human pollution	Low	High, analysis best with gene probe assays. More work needs to be done on lab methods
Rhodococcus coprophilus	Domestic grazing farm animals	Moderate	High, fairly complex test.

Source: Sargeant, 1999

Male specific RNA coliphages (FRNA phage), although not common in human and other animals, multiply in a sewage system and are also found to be a good sewage pollution indicator (Sargeant, 1999). The FRNA phage has been found to be source specific and can be divided into four groups. Group- I phages were detected in only domestic farm and feral zoo animals and Group II and III in humans (Kator and Rhodes, 1994). There still remain some issues to be resolved regarding the accuracy of these organisms.

Multiple Antibiotic Resistance (MAR) is a relatively new method that makes use of the fact that the bacteria from wildlife species generally lack in antibiotic resistance,

on the other hand, humans and domestic animals exhibit varying multiple MAR patterns. Resistance patterns for many different types and strengths of antibiotics are determined by analyzing either *E. coli* or fecal streptococci from different animal species (Sargeant, 1999). 82 % of the samples taken from areas impacted both by point and nonpoint sources were found resistant to one or more antibiotics. However, in areas impacted by nonpoint sources, the MAR index was 50 % lower (Parveen *et al.,* 1997). Although this method is found to be promising, it requires more time and the laboratory procedures are complicated and costly (Sargeant, 1999).

Another important technique uses a DNA-based approach. More recently, DNA-based approaches have been used for differentiating among the likely sources of pathogens. Table 2.8 lists the genotypic bacterial typing methods according to the ability to distinguish genus/species or subspecies/strains.

Table 2.8 Genotypic Bacterial Typing Methods

Methods Based on the Ability to Distinguish	
Genus/Species	**Subspecies/Strain**
Ribotyping/ Genetic fingerprinting tRNA- PCR ITS-PCR 16S rRNA sequencing	ARDRA Chromosomal RFLP ITS Sequencing Plasmid RFLP Pulsed-field gel electrophoresis (PFGE) Randomly amplified polymorphic DNA (RAPD) Rep-PCR

Source: Haejin Ha and Michael Stenstrom, undated.

ARDRA: Amplified ribosomal DNA restriction Analysis,

RFLP: Restriction fragment length polymorphisms

ITS: Internal Transcribed spacer

PCR: Polymerase chain reaction.

Genetic fingerprinting involves isolating pure cultures of *E. coli* from both the receiving water and suspected sources. Then DNA is isolated from these pure bacterial strains and compared. Several studies identified sources using this technique, but only a portion of the strains isolated from the receiving waters can be matched. In a study of Soos creek in Washington (Samadour and Chechowitz, 1995), 71% of the source matches belonged to 57 identified strains, while 29% were left unmatched (Sargeant, 1999). Samadpour conducted another study on San Diego beaches (CSDDEH, 1999), in which the maximum percentage of human isolates were matched during dry weather conditions and the maximum percentage of dog and bird isolates were matched during wet weather. None of human isolates were found in wet weather (Haejin Ha and Michael Stenstrom, undated). Although this method is excellent as far as determination of the sources is concerned, the quantification of the contribution from each source is not possible at this time. A large amount of fieldwork is required as numerous receiving water samples are necessary and fresh fecal samples from all possible sources must be collected and analyzed (Sargeant, 1999).

A brief description of the Rep-PCR method as summarized by Haejin Ha and Michael Stenstrom (Undated) is presented here. This method is called repetitive sequence-based polymerase chain reaction and yields DNA fingerprints comprised of multiple, differently-sized DNA amplicons. The main advantage of rep-PCR based chromosomal typing includes its speed, reproducibility, convenience, and modest resource requirements. Dombek *et al.,* (2000) in their study using this technique found out that 100% of the chicken and cow isolates, 83% of the human isolates and between 78% and 90% of the other animal isolates were assigned to the correct source groups.

They generated DNA fingerprints using whole cell suspensions, which eliminated the need for DNA purification.

Conclusions In almost all cases, a suite of analyses is most suitable for effective identification of inappropriate sanitary sewage discharges. A recent example was reported by Standley *et al.,* (2000), where fecal steroids (including coprostanol), caffeine, consumer product fragrance materials, and petroleum and combustion byproducts were used to identify wastewater treatment plant effluent, agricultural and feedlot runoff, urban runoff, and wildlife sources. They studied numerous individual sources of these wastes from throughout the US. A research grade mass sperctrophotometer was used for the majority of the analyses in order to achieve the needed sensitivities, although much variability was found when using the methods in actual receiving waters affected by wastewater effluent. This sophisticated suite of analyses did yield much useful information, but the analyses are difficult to conduct and costly and may be suitable for special situations, but not for routine survey work.

Another recent series of tests examined several of these potential emerging tracer parameters, in conjunction with the previously identified parameters, during a project characterizing stormwater that had collected in telecommunication manholes, funded by Telcordia (previously named Bellcore), AT&T, and eight regional telephone companies throughout the country (Pitt and Clark, 1999). Numerous conventional constituents, plus major ions, and toxicants were measured, along with candidate tracers to indicate sewage contamination of this water. Boron, caffeine, coprostanol, *E. coli*, enterococci, fluorescence (using specific wavelengths for detergents), and a simpler test for detergents were evaluated, along with the use of fluoride, ammonia, potassium, and obvious odors

and color. About 700 water samples were evaluated for all of these parameters, with the exception of bacteria and boron (about 250 samples), and only infrequent samples were analyzed for fluorescence. Coprostanol was found in about 25 percent of the water samples (and in about 75% of the 350 sediment samples analyzed). Caffeine was only found in very few samples, while elevated *E. coli* and enterococci (using IDEXX tests) were observed in about 10% of the samples. Strong sewage odors in water and sediment samples were also detected in about 10% of the samples. Detergents and fluoride (at >0.3 mg/L) were found in about 40% of the samples which were expected to have been contaminated with industrial activities (lubricants and cleansers) and not sewerage. Overall, about 10% of the samples were expected to have been contaminated with sanitary sewage, about the same rate previously estimated for stormwater systems.

Additional related laboratory tests, funded by the University of New Orleans and the EPA (Barbe' *et al.,* 2000), were conducted using many sewage and laundry detergent samples and found that the boron test was a poor indicator of sewage, possibly due to changes in formulations in modern laundry detergents. Laboratory tests did find that fluorescence was an excellent indicator of sewage, especially when using specialized "detergent whitener" filter sets, but was not very repeatable. Several UV absorbance wavelengths were also examined as sewage indicators and found excellent correlations with 228 nm, a wavelength having very little background absorbance in local spring waters, but with a strong response factor with increasing strengths of sewage.

Table 2.9 summarizes the different measurement parameters discussed above. The originally developed and tested protocol, as reported by Pitt *et al.,* (1993), still should be used as the most efficient routine indicator of sewage contamination of stormwater

drainage systems, with the possible addition of specific *E. coli* and enterococci measurements and UV absorbance at 228 nm. The numerous exotic tests requiring specialized instrumentation and expertise do not appear to warrant their expense and long analytical turn-around times, except in specialized research situations, or when special confirmation is economically justified (such as when examining sewer replacement or major repair options).

Table 2.9 Comparison of Measurement Parameters used for Identifying Inappropriate Discharges into Storm Drainage

Parameter Group	Comments	Recommendation
Fecal coliform bacteria and/or fecal coliform to fecal strep. ratio	Commonly used to indicate presence of sanitary sewage.	Not very useful as many other sources of fecal coliforms are present, and ratio not accurate for old or mixed wastes.
Physical observations (odor, color, turbidity, floatables, deposits, stains, damage to outfalls)	Commonly used to indicate presence of sanitary and industrial wastewater.	Recommended due to easy public understanding and easy to evaluate, but only indicative of gross contamination, with excessive false negatives (and some false positives). Use in conjunction with chemical tracers for greater sensitivity.
Detergents presence (anionic surfactant extractions)	Used to indicate presence of wash waters and sanitary sewage.	Recommended, but care needed during hazardous analyses (only for well-trained personnel). Accurate indicator of contamination during field tests.
Fluoride, ammonia and potassium measurements	Used to identify and distinguish between wash waters and sanitary sewage.	Recommended, especially in conjunction with detergent analyses. Accurate indicator of major contamination sources and their relative contributions.
TV surveys and source investigations	Used to identify specific locations of inappropriate discharges, especially in industrial areas.	Recommended after outfall surveys indicate contamination in drainage system.
Coprostanol and other fecal sterol compounds	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analysis with GC/MSD. Not specific to human wastes or recent contamination. Most useful when analyzing particulate fractions of wastewaters or sediments.

Contd..

Specific detergent compounds (LAS, fabric whiteners, and perfumes)	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Fluorescence	Used to indicate presence of sanitary sewage and wash waters.	Likely useful, but expensive instrumentation. Rapid and easy analysis. Very sensitive.
Boron	Used to indicate presence of sanitary sewage and wash waters.	Not very useful. Easy and inexpensive analysis, but recent laundry formulations in US have minimal boron components.
Pharmaceuticals (colfibric acid, aspirin, ibuprofen, steroids, illegal drugs, etc.)	Used to indicate presence of sanitary sewage.	Possibly useful. Expensive analyses with HPLC. A good and sensitive confirmatory method.
Caffeine	Used to indicate presence of sanitary sewage.	Not very useful. Expensive analyses with GC/MSD. Numerous false negatives, as typical analytical methods not suitably sensitive.
DNA profiling of microorganisms	Used to identify sources of microorganisms	Likely useful, but currently requires extensive background information on likely sources in drainage. Could be very useful if method can be simplified, but with less specific results.
UV absorbance at 228 nm	Used to identify presence of sanitary sewage.	Possibly useful, if UV spectrophotometer available. Simple and direct analyses. Sensitive to varying levels of sanitary sewage, but may not be useful with dilute solutions. Further testing needed to investigate sensitivi Contd..
Stable isotopes of oxygen	Used to identify major sources of water.	May be useful in area having distant domestic water sources and distant groundwater recharge areas. Expensive and time consuming procedure. Can not distinguish between wastewaters if all have common source.
E. coli and enterococci bacteria	More specific indicators of sanitary sewage than coliform tests.	Recommended in conjunction with chemical tests. Relatively inexpensive and easy analyses, especially if using the simple IDEXX methods.

Source : Pitt, 2001b

CHAPTER III

SITE CHARACTERISTICS

3.1 Introduction

This chapter presents detailed descriptions of the physical characteristics of all the sampling sites, both dry as well as wet weather. The major task of this study was to collect samples during wet weather flows from an urban area for evaluation using bacteria analyses. An urban area consists of many different kinds of land uses such as residential, institutional, commercial, industrial open spaces, etc. Each type of land use consists of various kinds of source areas such as roofs, parking lots, landscaped areas, playgrounds, driveways, undeveloped areas, sidewalks, etc. It is a difficult task to take samples from all kinds of source areas during a single storm by one person, so only four major types of source areas were sampled during this research. The source areas sampled were rooftops, parking lots, open spaces and streets, divided into two sets, one affected by birds and animals, and another set with less influence from birds and animals. A major task was selecting the sampling sites. Taking into view the practical limits of time and manpower, eight sampling locations were selected, two from each category of source area. All eight locations were close enough to the departmental graduate student offices so that all sites could likely be sampled during each rain event. Sites were located either on campus of the University of Alabama, or were very near the campus.

Selected rooftops were located in a residential area close to campus. The remaining three source areas were located on campus.

3.2 Wet Weather Sampling Locations

Figure 3.1 shows the locations of all the sampling sites. For each category of source area, two sites were so selected such that one site was more prone to bird or animal use (Marked P in map) and the other was not (Marked NP).

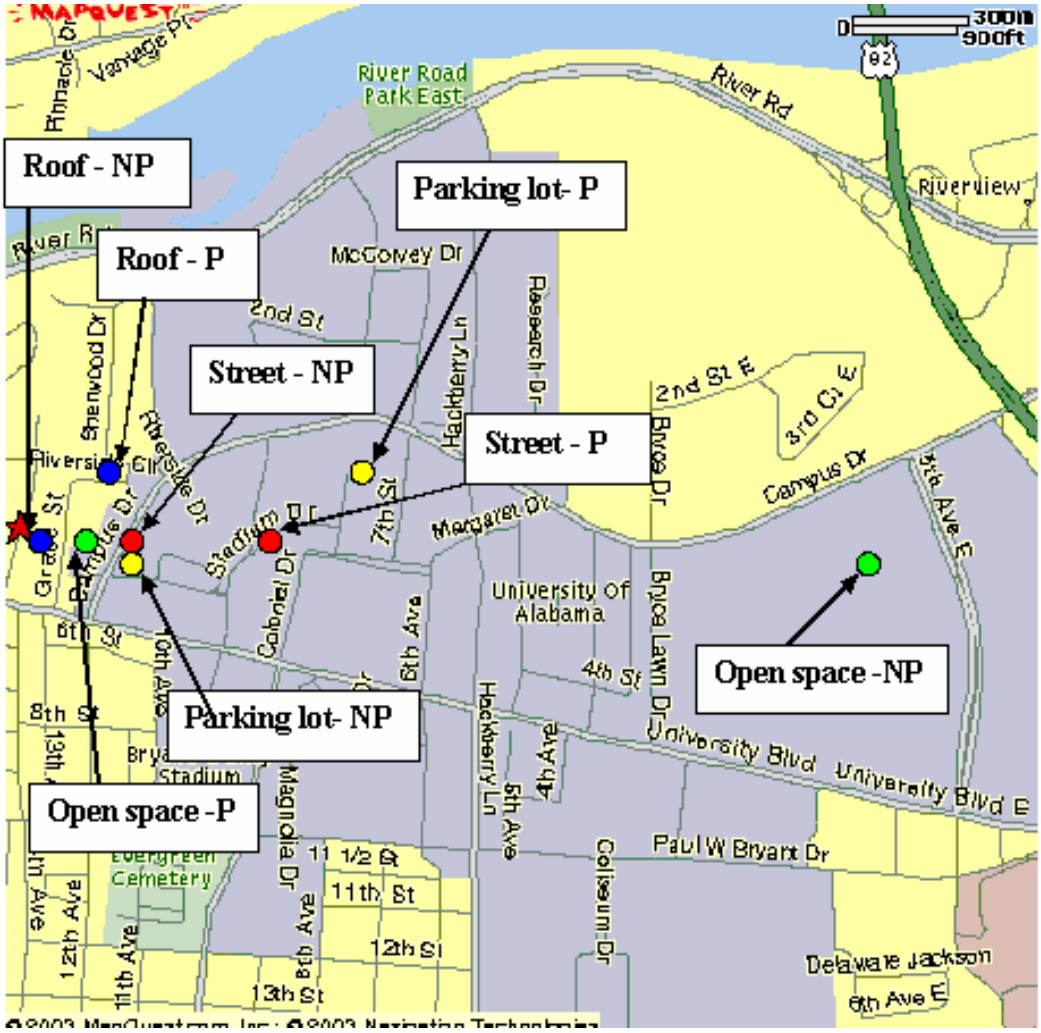

Figure 3.1 Locations of Sampling Sites

3.2.1 Roofs The criteria for selection of roofs for sampling was that one should have a tree canopy cover over it (and high bird and squirrel populations), while the other would not have a tree canopy, and therefore fewer animals. Another important point was that both the roofs should be similar types. Therefore, both roofs were pitched. Both were not directly connected to the drainage system, allowing more convenient sampling. One building (with tree canopy) was located on Riverside Drive. It is in the Tuscaloosa Historical district area (Figure 3.2). Samples were taken from the gutter located on the front corner of the house (as seen in the figure). Bacterial loads due to the feces of birds and animals on the rooftop may vary greatly with location. Taking samples from the downspout helped in getting a well-mixed sample representing sheetflow form a large area of the roof.

The site for the roof not having canopy cover was located on Reed Street (Figure 3.3). This building was higher (3 stories) as compared to the Roof – P (1 story). Since there was no canopy cover over it, it was supposed to be under less influence from birds. Samples were collected from the downspout.

Figure 3.2 Roof with Canopy Cover (Roof – P)

Figure 3.3 Roof without Canopy Cover (Roof – NP)

3.2.2 Streets The main consideration in selection of the sampled streets was that one should be surrounded by trees and other one not. The presence of trees encourages the presence of squirrels and birds. Both streets were paved two-way streets, having curb and gutter drainage systems. There was minimal street dirt and litter accumulation on either street. One street sampling site was near the intersection of Marrs Spring Road and Stadium drive. Squirrels and birds were often seen on the site. This street also has a steep slope (Figure 3.4).

Other street sampling location was close to Campus Drive (Figure 3.5). But the location of the street was so selected that is was high in elevation as compared to adjoining areas and minimal mixture of sheetflow from other source areas took place.

Figure 3.4 Street Surrounded by Trees (Street – P)

Figure 3.5 Street -NP

3.2. 3 Parking Lots Similar criteria were used for selecting the parking lots for sampling. One sampling location was in the Ferguson center parking lot that had trees in the vicinity (Figure 3.6). The second parking lot was located near Campus Drive (Figure 3.7).

Figure 3.6 Parking Lot -P

Figure 3.7 Parking Lot -NP

3.2.4 Open Spaces One of the two open space sampling areas was more prone to animals (Dogs ,cats etc.) as it was in a residential area. This open space area was located on Grace Street and people often brought their pets for morning and evening walks (Figure 3.8). The second open space area was selected in the recreational center grounds of the University of Alabama (Figure 3.9). There were no trees anywhere in the sampling vicinity and no pets are allowed to enter the area.

Figure 3.8 Open Space -P

Figure 3.9 Open Space –NP (Recreation Center Grounds)

3.3 Dry Weather Sampling Locations

A section of Cribbs Mill Creek in Tuscaloosa, Alabama, was selected for dry weather sampling. The section of creek was selected such that the drainage areas contributing to outfalls had either commercial or residential land uses. The section used for the creek investigation begins near the origin of the creek near Veterans Hospital on 15th Street and ends at the intersection of Hargrove Road and 1st Ave. This was approximately a 5 mile stretch.

During the initial creek surveys, the locations of all the outfalls (flowing as well as not flowing) were identified and displayed on the aerial map (this URL displays the map: http://www.eng.ua.edu/~rpitt/Research/ID/ID2.shtml). About 75 outfalls were examined. For most of the outfalls, the drainage areas were in residential areas. Outfalls

60 through 66 were in a commercial area along McFarland Blvd. on the opposite side of University Mall. The drainage areas of outfalls 45 through 52 were also commercial. All of the remaining outfalls are in residential areas. The creek outfalls were examined and sampled five times during this research, as part of an on-going project sponsored by the EPA.

3.4 Library Sampling Locations

In the second phase of dry weather sampling activities, Tuscaloosa source area water samples were obtained, including samples from the Tuscaloosa sewage treatment plant, local springs, irrigation runoff, domestic water taps, car wash, and laundry water. Although, the samples were analyzed for a number of parameters (as a part of the "Inappropriate Discharges" project), this thesis research focused on *E. coli* and enterococci measurements. Twelve samples were collected for each category from different locations and at different times. Section A.1 (Appendix 'A') shows the locations and dates when the different samples were obtained.

Most of the tap water samples were collected from taps in various buildings on campus. Four were also collected from different apartment buildings. Spring water samples were collected from two springs; Mars Spring and Jack Warner Parkway Spring. Carwash samples were collected from various gas stations located in the city. Most were obtained from automatic car wash facilities and some were obtained from self service facilities. Laundry washwater samples were collected by graduate students from the place where they do their laundry. All the sewage samples were taken from the Tuscaloosa wastewater treatment plant. All the industries from where the samples were obtained are

located in Tuscaloosa and were obtained as part of the local pre-treatment monitoring program. Section A.1 (Appendix A) lists the products these industries manufacture and the sources of their water. Irrigation water samples were collected from lawns, or adjacent paved areas, in front of various buildings in the University of Alabama while the adjoining landscaped areas were being watered.

CHAPTER IV

METHODOLOGY

4.1 Experimental Design

In order to achieve the objectives of this thesis, bacterial analyses were conducted for 202 wet weather and 278 dry weather samples. Both *E. coli* and enterococci analyses were conducted. Total coliforms were also evaluated as part of the *E. coli* tests. The following tasks were accomplished during this research:

4.1.1 Effects of Urban Wildlife on Stormwater Bacteria Levels Four source areas were selected for sampling. For each category of source area, two sites were selected, prone and not prone to urban animal use. The prone locations were those where urban wildlife (birds and squirrels for roofs, and dogs for ground-level surfaces) use is common and not prone locations where urban wildlife appears to be generally absent. The number of samples collected in each category during this research is listed in table 4.1.

Table 4.1 Total Number of Sample Pairs Collected From Each Source Area

Site	No. of Paired Samples
Open space- Prone	11
Open space- Not prone	10
Parking lot – Prone	13
Parking lot- Not Prone	10
Roof - Prone	12
Roof - Not Prone	12
Streets- Prone	10
Streets- Not Prone	10

In a few cases, the number of samples from one site analyzed for *E. coli* was different from that of enterococci. A total of 176 samples were analyzed.

4.1.2 Seasonal Variations The climate of Tuscaloosa is subtropical with four distinct seasons including winter (December through February), spring (March and April), summer (May through September) and autumn (October and November). Anticipating that bacterial levels would vary with season, an attempt was made to take samples in every season. Wet weather sampling was conducted from August 2002 to June 2003. No samples were collected during the months of December and March. Most importantly, the objective was to compare cold months (December through February having temperatures below 50 F) with samples collected during the warmer months. However, only two to three samples were taken during the winter months from each site.

4.1.3 Find Variation Within Storm Because of the large variability found for the bacteria analyses in the sheetflow samples, additional tests were conducted to determine the potential causes for this variability. During a single storm on 25 Sep 2002, all the sites were sampled twice, once in the morning and then again in the evening. In addition, six samples from two source areas were collected at an interval of 15 to 30 minutes during a single storm on 17 Oct 2003. A total of 24 samples were analyzed.

4.1.4 Effect of Sample Handling Three factors involving sample handling were also studied which could affect the results. These included holding time, refrigeration, and shaking. For these tests, a single 5 liter sample was taken from one source area from

which 100 mL sub samples were tested after 1, 2, 5, 9, 24, and 48 hrs. The 5 liter sample was split into two components, one was refrigerated, and the other was not. The effect of refrigeration over one to two days was then measured. The effect of shaking was measured by withdrawing an initial 100 mL sample from the unshaken sample bottle, and then shaking the sample bottle and testing another 100 mL sample.

4.1.5 Reference Sample Collection (Library Samples) In order to achieve the second objective of the research, 12 samples were collected from each of several source areas: the influent to a sewage treatment plant, local springs, irrigation runoff, domestic water taps, car wash, industry, and laundry water. Sewage samples were compared with other reference samples and wet weather samples. A total of 142 samples were analyzed.

4.1.6 Outfall Sample Collection (From Local Creek) A five mile stretch of Cribbs Mill Creek in Tuscaloosa, Alabama, was selected for dry weather sampling to test methods to detect inappropriate discharges to the creek. A total of 77 total outfalls were examined over 5 different periods. Although five complete creek walks were done, bacterial analyses were conducted only during the last three creek walks. 20-25 outfalls were found to have dry weather discharges during every creek walk. A total of 136 samples were analyzed during this test phase.

4.2 Sampling Procedures

The main effort of this research involved the collection of samples and their bacterial analysis in the laboratory. This chapter gives information about the sample

collection and analysis procedures. The sample collection procedure was different for dry weather and wet weather sampling, but the analysis procedure was the same for both types of samples. Wet weather sampling started in August 2002 and was completed in June 2003. The objective was to represent all the seasons so that effect of season on bacterial concentrations could be examined. Samples were taken once or twice a month, depending upon rain. December 2002 and March 2003 were the only months when no samples were obtained.

The second part of dry weather sampling involved collection of Tuscaloosa source area samples from domestic water taps, irrigation runoff water, etc. for preparing the Tuscaloosa source area reference sample library. Most of the library samples were collected during the months of May and June 2003.

All samples were analyzed using the same procedure. The IDEXX Quantitray enumeration procedure was used. All samples were analyzed for total coliforms, *E. coli* and enterococci. Although dry weather samples were analyzed for various other constituents, this thesis only presents results for the bacteriological analyses. The quality assurance /quality control (QA/QC) procedures followed are described later.

4.2.1 Wet Weather Sampling Procedure Samples were collected according to procedures given in *Standard Methods for the Examination of Water and Wastewater* (Standard Methods-20th edition, 1998) for microbiological examination. Sterile techniques were used to avoid sample contamination. Sterile gloves were worn during sampling and analysis, and the samples were collected in presterilized 100 mL plastic bottles supplied by IDEXX . The bottles contain sodium thiosulphate ($Na_2S_20_3$) to

prevent problems with chlorine in the samples. $Na_2S_20_3$ is a dechlorinating agent that neutralizes any residual halogen and prevents continuation of bacterial disinfection during sample transit. The use of $Na_2S_20_3$ more accurately results in the true microbial content of the water at the time of sampling (Standard Methods-20th edition, 1998).

All samples were taken manually and no automatic sampling equipment was used. The sample bottle was filled up to the 100 mL mark, leaving ample air space in the bottle to facilitate mixing by shaking, before examination. Sample bottles were filled without rinsing and care was taken so that the inner surface of stopper or cap did not become contaminated. The bottle cap was replaced immediately.

The next step in the sampling procedure was writing the sample I.D on the prelabeled sample bottle. The sample bottle labels listed the date, sample I.D, and time of sampling. The sample bottles have labels on both the cap and the bottle, preventing the caps form being interchanged. Bottles were labeled with name of source area and its proneness to birds and animals. e.g. Roof -NP, Parking Lot -P etc. NP stands for not prone (or less prone) and P stands for prone. Waterproof markers were used because the bottles were exposed to rain. Filled sample bottles were then put in a backpack. The backpack carrying the sample bottles and a permanent marker was always kept ready to be taken to the field at all times to make sure no time was wasted and that sampling could be started soon after the rain starts, enabling more sites to be visited. During the initial five sampling rounds, no sample dilutions were made, so two sample bottles per site (one for *E. coli* and other for enterococci) were taken. From the sixth round on, three 100 mL samples were taken per site to allow for dilution.

Sampling was conducted in a random order for each event to make sure that all the sites were visited an approximately equal number of times. Before leaving for the field, the rain conditions and forecast were checked using Internet weather satellite images and forecasts, and local rain gages, to help ensure that sufficient rain would fall to produce sheetflow. It is almost impossible to obtain satisfactory samples during light rains. The time at which the sample was obtained at a particular site was noted on the sample bottle label right before sampling.

Rooftop samples were obtained by placing the sample bottle directly under the downspout. The bottle was removed soon before it filled to the 100 mL mark. The bottle cap was then used to fill the sample bottle exactly to the 100mL mark.

Sheetflow samples were taken from parking lots and streets. The sampling locations on the street or parking lots were selected so that runoff was not mixed with runoff from other source areas (e.g. samples collected from the curb of the street may not be representative of sheetflow from streets only). Similarly, sampling places inside the parking lots were selected such that there was minimal mixing from other source areas. Samples were taken by holding the sample bottle near its base, keeping it tilted at an angle with mouth facing downstream. Sheetflow samples were placed into the bottle with the cap from the bottle. Care was taken not to scratch the pavement surface with the cap during sampling.

It was difficult to collect sheetflow samples from open spaces. Most open space samples were obtained from ponded water.

Samples collected from different sites were kept in different Zip Lock bags, put in the backpack and transported to the laboratory. Microbiological analysis of the water

samples was started as soon as possible after collection to avoid changes in the microbial population.

4.2.2 Dry Weather Sampling Procedure Cribbs Mill Creek in Tuscaloosa, Alabama, was selected for dry weather sampling. It passes through multiple types of land uses, including residential, commercial, open space areas. Other favorable characteristics were moderate flow, accessibility by road, and it was in a completely urbanized area that has been long developed. A five-mile section of the creek was selected for sampling. The equipment taken to the field included

- One liter HDPE sample bottles
- 100 mL presterlized sample bottles supplied by IDEXX
- Non-mercury thermometer for onsite temperature measurement
- GPS unit to record locations of outfalls
- Reinforced (snake-proof) neoprene waders
- Spray paint for labeling outfalls
- Outfall characterization form
- Street map of area
- First aid kit
- Walkie talkie
- A dipper to sample inaccessible outfalls
- Digital camera
- Duct tape and a permanent marker
- Ice cooler with ice packs to preserve the samples

Before sampling during any day, the field crew contacted the local Tuscaloosa Police Department to let them know where they will be working. The initial sampling excursions produced some citizen calls to the police, and it was decided that pre-notification was efficient and safe.

The field crew consisted of three persons. Upon arriving at the first site, two persons waded the creek in a downstream direction carrying the field equipment in backpacks, while one person with a street map, cooler, and a walkie-talkie drove the vehicle to a convenient downstream location where the creek intersects the street. Collected samples were placed in a portable ice cooler in the vehicle after each stretch was sampled. This collection point was usually about a half mile downstream from the last collection point. About 5 or 6 samples are usually collected from each stretch of creek and iced within a half hour of collection. Heavy-duty waders were always worn while wading which protected from debris (broken glass bottles, bricks etc.) and certain wildlife species, like rattlesnakes, cottonmouth, etc.

The first two creek walks involved a greater effort and time to complete because of the need to locate the outfall locations. After three complete creek walks, no new outfalls were found, and the field time was appreciably shortened. A total of 77 outfalls were eventually found, flowing as well as non-flowing. Outfalls were numbered using black spray paint. The average distance between the outfalls was about 50 feet, and about six flowing outfalls were sampled during in a days creek walk. About 5 to 7 days were needed for every creek walk, or about one mile per day. Out of 77 total outfalls, 20-25 were flowing during every creek walk. When a branch enters the main creek, the sampling crew went to the origin of the branch and walked downstream marking outfalls

along the way. All sorts of outfalls were found, including open ditchs, concrete outfalls, ductal iron pipe outfalls, and PVC outfalls. A number only drained the adjacent paved parking area, while most were conventional outfalls draining 5 to 50 acres each. The following URL includes a large aerial photograph showing all outfalls, along with individual outfall photographs:

http://www.eng.ua.edu/~rpitt/Research/ID/ID2.shtml

During the first two creek walks, a single 1L sample was taken from every flowing outfall and no bacterial analyses were conducted. During the subsequent three creek walks, bacterial analyses were also conducted, so two 100 mL samples were also collected per flowing outfall, in addition to the 1L sample. The 100 mL samples were placed directly into pre-sterilized sample bottles.

The following steps were followed at every outfall:

1) If not already marked, the outfall number was painted on the outfall
2) One 1L sample and two 100 mL grab samples were taken for each flowing outfall.
3) The water temperature measured from the 1L sample bottle.
4) If not already recorded, the latitude and longitude were noted from the GPS.
5) The field characterization forms were filled out for each outfall visit.
6) Photographs of the outfall were taken.

After the third creek walk, some branches of the creek were dropped from further evaluations because of time and a redundancy of the residential land uses in which the branches were located.

The dry weather sampling was conducted at least 24 to 48 hrs after rains, depending upon the rain depths. Samples were collected in the morning and refrigerated, while the 100 mL samples that were collected for bacterial analyses were analyzed immediately after bringing to the lab. All the other constituents were usually analyzed in the afternoon. Other constituents analyzed were ammonia, boron, color, conductivity, detergents, fluorescence, fluoride, hardness, potassium, pH, optical brighteners, and turbidity.

4.2.3 Library (Reference) Sample Collection Procedure All the library samples were collected in 1 litre HDPE bottles and pre-sterlized 100 mL sample bottles. Tap water samples were collected from a service pipe directly connected with the main, not from a cistern or storage tank. The tap water was allowed to flow fully for two to three minutes for clearing the service line and then the sample was taken. It was difficult to collect samples directly from the springs, as the water flow was very slow (dripping). New clean zip lock bags were used to collect samples from Jack Warner Pkwy Spring. Samples from Mars Spring were collected with a dipper sampler

Car wash samples were collected as sheetflow flowing from the washing of the cars. Laundry samples were taken from the washing machine directly when the washing cycle was about to finish and before the rinsing started. Sewage samples were taken from the automatic composite sampler located at the influent of the Tuscaloosa WWTP. Sewage samples collected immediately after rainy days were considered wet season samples.

All the industries that were analyzed (as listed in the previous chapter) send water samples to the Tuscaloosa WWTP weekly.

Irrigation water samples were mostly sheetflow water collected from the sidewalks or road, which flowed during over-watering of lawns. Some samples were collected from small depressions in the lawn itself and not from runoff after flowing across concrete (Figure 4.1).

Figure 4.1 Irrigation Runoff

4.3 Sample Analyses Procedure

All the samples were analyzed for total coliforms, *E. coli*, and enterococci using EPA-approved IDEXX Laboratories methods. EPA suggested water quality criteria based upon *E. coli* and enterococci measurements in 1986. The IDEXX methods used were developed in response to these new EPA microbiological guidelines. All the equipment and supplies needed were obtained from IDEXX, including Colilert or Colilert-18 reagent, Enterolert reagent, presterlized 100 mL sample bottles, Quanti-tray-2000,

Quanti-tray sealer, rubber insert pads, two incubators, two thermometers, comparartor, and a 6 watt, 365nm wavelength UV lamp. Figure 4.2 shows all the equipment used. Two incubators were used, one for the temperature setting for *E. coli* and another for enterococci.

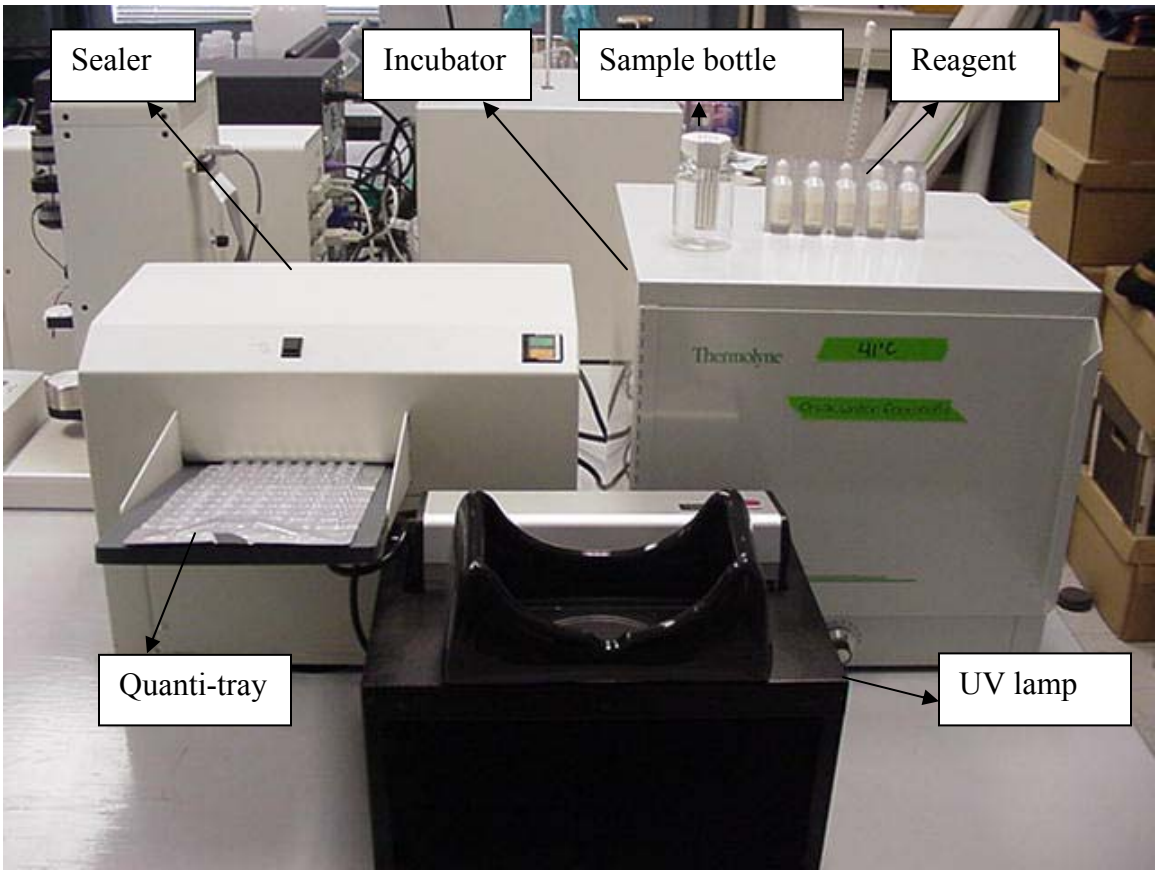

Figure 4.2 Equipment Used

The following steps are followed once the samples reached the laboratory for testing *E. coli* and total coliforms (IDEXX insert notes and Video, 2002):

1) Switch on the sealer. It takes about 10 minutes to warm up. Start using only when both green and amber lights are on.
2) While the sealer is warming up, add one snap pack of either Colilert-18 or Colilert reagent to 100ml sample. Colilert-18 is used for results in 18 hrs, while

Colilert takes 24 hrs. The standard Colilert is preferred when samples are tested for both *E. coli* and enterococci at the same time as both samples can then be removed at the same time, after 24 hrs of incubation, for evaluation (the only time available for enterococci analyses). This saves an extra visit to the laboratory six hours before the enterococci samples are ready.

3) Close the sample bottle tightly and shake well until reagent mixes completely and nothing is left at the bottom. If the sample is sewage, or if obvious clumps of material are present, the sample needs to be blended to break up the material so individual bacteria are correctly counted. A Waring blender on medium speed is used. In all cases, it is important to vigorously manually shake all samples.
4) Label the Quanti-tray with permanent marker on the back. Pour the sample into the tray and place it on the rubber pad and seal by passing through the sealer.
5) Incubate the Quanti-tray in the incubator kept at a temperature of 35± 0.5° C for 18 hrs (if colilert-18 reagent is used) or 24 hrs (if standard colilert reagent is used).
6) Remove Quanti-tray and read the results according to result interpretation table below (Table 4.2). A sample well is considered positive depending upon its appearance.

Table 4.2 Result Interpretation Table.

Appearance of Well	**Result**
Less yellow than comparator[1]	Negative for both total coliforms and *E.*
Yellow equal to or greater than the	Positive for total coliforms
Yellow and fluorescence[2] equal to and	Positive for *E. coli*

Source: IDEXX Insert notes and video, 2002.

Note:

1) Quanti-Tray/ 2000 Comparator # WQT2KC provided by IDEXX.
2) Look for fluorescence with a 6- watt, 365nm, UVlight within 5 inches of the sample, in a dark environment. Light is kept facing away from eyes and towards the sample.
3) Colilert gives definitive results within 24- 28 hours. Wells observed positive for both total coliform and *E. coli* before 24 hrs period and negative wells observed after 28 hrs are also valid

7) The numbers of positive wells are counted and the MPN table is used to determine the most probable numbers.

The following steps are followed for determining enterococci (IDEXX insert notes and video, 2002):

1) Repeat step 1 above.
2) Add Enterolert reagent supplied by IDEXX to 100ml samples.
3) Repeat step 3 and 4 above.
4) Place the Quanti-tray in incubator at 41± 0.5° C for 24 hrs.
5) Remove Quanti-tray and read results at 24 hrs by placing under UV light. See note 2 of Table 4.1.
6) Count the number of fluorescent wells. Positive wells may give different fluorescence intensity.
7) Refer to the MPN table provided with the Quanti-tray 2000 to determine most probable number of enterococci in the sample.

Special procedural notes for enterococci:

1) If due to unavoidable circumstances the samples get incubated for over 28 hrs, then lack of fluorescence is a valid negative. But presence of fluorescence after 28 hours is not a valid positive result.
2) If dilutions are to be made, use sterile water. Since the Enterolert reagent is already buffered, there is no need to add buffered water.
3) There is no comparator available as in case of total coliforms and *E. coli*. Water blank can be used when interpreting results.

4.3.1 Principle The test used for total coliforms and *E. coli* is a commercially available microbiological method included in *Standard Methods for the Examination of Water and Wastewater*, 20th edition (section 9223 B). It is an enzyme substrate test which utilizes hydrolysable substrates which detects total coliforms and *E. coli* enzymes at the same time. Colilert reagent supplied by IDEXX contains two nutrient-indicators, ONPG and MUG, which can be metabolized by the total coliform enzyme *ß*-D-galactosidase and the *E. coli* enzyme *ß*-D-glucuronidase, respectively.
(www.idexx.com/Water/Products/colilert/science.cfm , 2003)

Total coliforms produce enzyme ß-D-galactosidase. Enzyme ß-D-galactosidase, cleaves chromogenic substrate (ONPG), which results in release of the chromogen. When a chromogenic substrate such as ortho-nitrophenyl- ß-D-galactosidase(ONPG) is added, enzyme ß-D-galactosidase hydrolyzes the substrate and produce a color change (Figure 4.3) to yellow in 24 hrs (Standard Methods-20th edition, 1998).

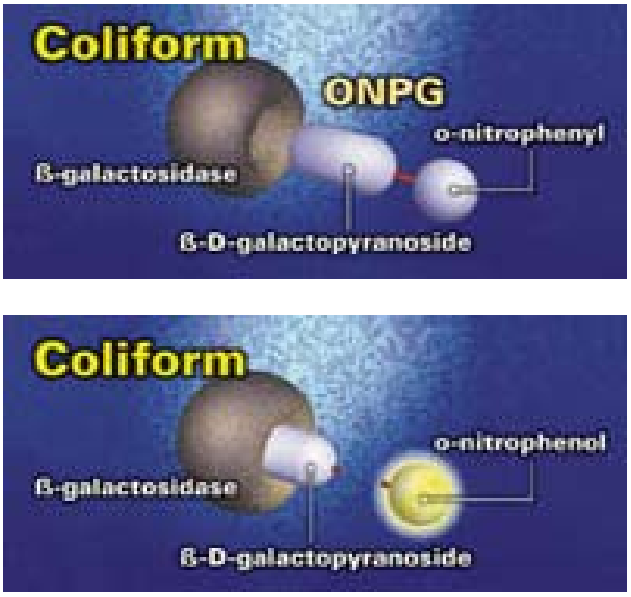

Figure 4.3 ONPG Change from Colorless to Yellow

Source : www.idexx.com/Water/Products/colilert/science.cfm , 2003. With permission.

There is a remote chance that non-coliform bacteria, such as *Aeromonas and Pseudomonas* species may produce a positive response. This is due to the fact that these species may produce a small amount of enzyme ß-D-galactosidase, but are suppressed. These can't produce a positive response within an incubation period of 24 hrs unless more than 10^6 CFU/100 mL are present. Moreover, Colilert's specifically formulated matrix helps selectively suppress the few non-coliforms that do have these enzymes (www.idexx.com/Water/Products/colilert/science.cfm , 2003).

E. coli produces enzyme ß-glucuronidase, which can be detected by adding fluorogenic substrate, such as 4-methyl-umbelliferyl- ß-D- glucuronide (MUG). The enzyme cleaves the fluorogenic substrate resulting in the release of fluorogen which produces fluorescence when viewed under long wavelength (366-nm) ultraviolet (UV) light (Figure 4.4).

Figure 4.4 Fluorescence Produced by MUG Metabolizing

Source : www.idexx.com/Water/Products/colilert/science.cfm , 2003. With permission.

Most non-coliforms do not produce enzymes ß-glucuronidase, so they are unable to grow and interfere. But some strains of *Shigella* spp. may produce a positive response. Since the sanitary quality of water is being tested and *Shigella* spp. are overt human pathogens, this is not considered a detriment.

Enterolert reagent detects enterococci such as *E. faecium, E. faecalis* in fresh and marine water. Enterolert is an official ASTM method (#D6503-99). It can detect up to 1CFU/ 100 mL sample. Similar to the above principle, a substrate present in enterococci fluoresces when metabolized by enterococci (Figure 4.5). This method improves accuracy and avoids the need for hazardous sodium azide suppressants used in traditional media (www.idexx.com/water/products/enterolert/science.cfm, 2003).

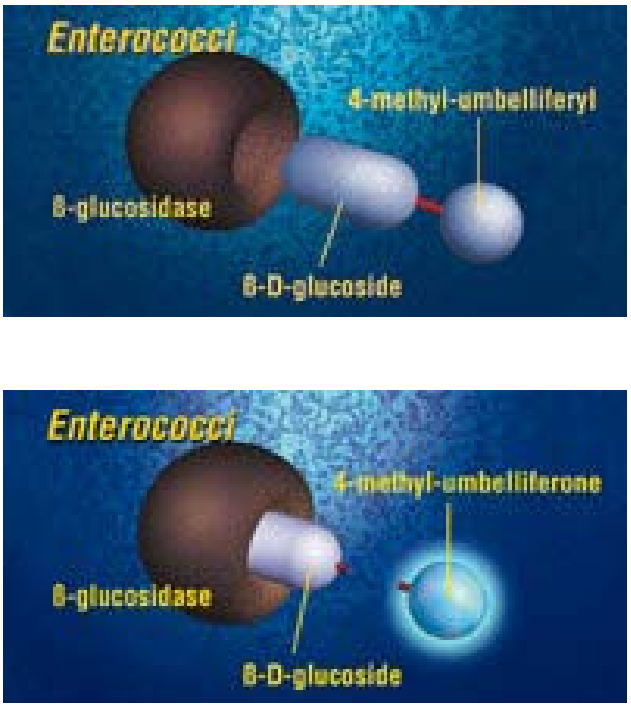

Figure 4.5 Enterococci Detection Principal

Source : www.idexx.com/water/products/enterolert/science.cfm, 2003. With permission

4.4 Quality Assurance / Quality Control

To confirm the quality of results and increase confidence in the data, a quality assurance program was followed. The following aspects were addressed:

1) Personnel: Basic laboratory training was undertaken. The IDEXX training video instructions were followed.
2) Facility: Tests were done in a well-ventilated laboratory having air conditioning that reduced contamination, permitted more stable operation of incubators and decreased moisture problems with media and instruments. The work areas were kept clean and free of unnecessary chemicals. After finishing the tests, the counter and other work surfaces were wiped with an appropriate disinfecting solution

(typically a bleach solution). If any sample or QA/QC solution was spilled, a sorbent material was used to soak up the material and the used sorbent was placed in the proper disposal container (Biohazard bag for on-campus disposal of biohazardous materials).

3) Laboratory equipment and instrumentation: Two separate incubators were used for testing *E. coli* and enterococci. These were maintained at temperatures of 35± 0.5° C and 41± 0.5° C, respectively. A glass thermometer with its bulb and stem submerged in water kept in a beaker inside the incubator was used to verify the incubator temperature. The water levels in the beakers were periodically checked to ensure that the bulb and stem of the thermometers were always submerged. The UV lamp and sealer were periodically cleaned and switched off after use.
4) Supplies: Supplies used for testing were Colilert and Colilert-18 reagent, Enterolert reagent; Quanti-cult bacterial cultures used for quality control, Quanti-trays, and 100mL pre-sterilized sample bottles. The Quanti-cult and analytical reagents were stored in a refrigerator according to the manufacturer requirements. Quanti-trays and sample bottles supplied by IDEXX were sterile (certified by IDEXX) and disposable. This eliminates the use of glassware and any chances of contamination.
5) Analytic methods: The test used for total coliforms and *E. coli*, was the commercially available microbiological method included in *Standard Methods for the Examination of Water and Wastewater*, 20th edition (section 9223 B). Enterolert is an official ASTM method (#D6503-99). These methods are

commonly used by many agencies, including the Alabama Department of Environmental Management (ADEM).

6) Analytical Quality control procedures: Every batch of Colilert and Colilert-18 reagent was checked by testing with known positive and negative control cultures (Quanti-cult®). Quanti-cult® is a set of ready to use bacterial cultures supplied by IDEXX. It consists of three sets each of three different bacterial cultures. Each set consists of 1-50 bacterial cells which were preserved in the colorless cap of a plastic vial. The contents of Quanti-cult® were kept stored in a refrigerator until time of use. Following are the contents:
    - 3 *E. coli* capped vials labeled "EC" in foil packs and 2 reusable labels
    - 3 Klebsiella pneumoniae –capped vials labeled "KP" in foil packs and 2 reusable labels. This is a total coliform bacterium.
    - 3 Pseudomonas aeruginosa – capped vials labeled "PA" in foil packs and 2 reusable labels. This is a non-coliform bacterium.
    - 12 rehydration fluid vials
    - 1 autoclavable foam vial holder

The following steps were followed every time a new batch of reagents was tested:

1) Three rehydration fluid vials were pre-warmed for 5 minutes in the incubator.
2) The blue cap of all the three rehydration fluid vials were replaced with colorless caps of organisms containing vials, one cap from each of three different organisms vials.
3) The vials were then inserted back into a foam rack and incubated for 10 minutes at 35-37° C. Vials were kept inverted.

4) Vials were then removed and the caps were gently taped to mix. If undissolved particles were still present on the inside surface of cap; the vials were again reincubated for 10 more minutes.
5) Fill three 100 mL presterlized sample bottles with sterile water. Water was taken from DI water plant (18 meg-ohm sterile water) of environmental lab. Reusable labels were stuck on the sample bottles, one for each kind of organism.
6) Entire contents of each vial were added to the respective 100 mL prewarmed sterile water.
7) One snap pack of Colilert or Colilert-18 reagent was added to each of the sample bottles. The reagent was mixed and put in the Quanti-tray. Same procedure was followed as described above for testing *E. coli*.
8) The following results were expected from the control cultures (Table 4.3):

Table 4.3 Results Expected from Quality Control Cultures

Organism Type	**Expected Color**	**Expected Fluorescence**
Pseudomonas aeruginosa (non-coliform)	Colorless	No fluorescence
Klebsiella pneumoniae (Total coliform)	Yellow	No fluorescence
E. coli	Yellow	Fluorescence

Source : IDEXX insert notes and Video, 2002

Quality control tests were run three times on different batches (Table 4.4 through 4.6).

The following are the results obtained during our QA tests:

Date of test: 30th July 2002

Reagent tested: colilert-18

Table 4.4 First Batch Quality Control Test Results

Organism Present in Quanti-tray	**Positive Cells Observed**		**Total Coliforms (MPN/100 mL)**	***E. coli* (MPN/100 mL)**
	Color	Fluorescence		
Pseudomonas aeruginosa (non-coliform)	Small +ve 0 Large +ve 0	Small +ve 0 Large +ve 0	<1	<1
Klebsiella pneumoniae (Total coliform)	Small +ve 0 Large +ve 12	Small +ve 0 Large +ve 0	13.5	<1
E. coli	Small +ve 2 Large +ve 10	Small +ve 2 Large +ve 10	13.2	13.2

Date of test: 12 November 2002

Reagent tested: colilert

Table 4.5 Second Batch Quality Control Test Results

Organism Present in Quanti-tray	**Positive Cells Detected**		**Total coliforms (MPN/ 100mL)**	***E. coli* (MPN/ 100 mL)**
	Color	Fluorescence		
Pseudomonas aeruginosa (non-coliform)	Small +ve 0 Large +ve 0	Small +ve 0 Large +ve 0	<1	<1
Klebsiella pneumoniae (Total coliform)	Small +ve 0 Large +ve 2	Small +ve 0 Large +ve 0	2	<1
E. coli	Small +ve 2 Large +ve 15	Small +ve 2 Large +ve 15	19.9	19.9

Date of test: 22 April 2003

Reagent tested: colilert

Table 4.6 Third Batch Quality Control Test Results

Organism Present in Quanti-tray	**Positive Cells Detected**		**Total Coliforms (MPN/100 mL)**	***E. coli* (MPN/100 mL)**
Pseudomonas aeruginosa (non-coliform)	Color Small +ve 0 Large +ve 0	Fluorescence Small +ve 0 Large +ve 0	<1	<1
Klebsiella pneumoniae (Total coliform)	Small +ve 0 Large +ve 6	Small +ve 0 Large +ve 0	6.3	<1
E. coli	Small +ve 0 Large +ve 13	Small +ve 0 Large +ve 13	14.8	14.8

The results obtained were as expected and required. To insure the quality of water used for dilutions, periodical bacterial analysis of water blanks was also done along with the QC samples (Table 4.7). The following observations were made:

Table 4.7 Dilution Water Quality Test Results

Date Blank Tested	**Positive Cells Detected**		**Total Coliforms (MPN/ 100 mL)**	***E. coli* (MPN/ 100 mL)**
30th July 2002	Color Small +ve 0 Large +ve 0	Fluorescence Small +ve 0 Large +ve 0	<1	<1
12 November 2002	Small +ve 0 Large +ve 0	Small +ve 0 Large +ve 0	<1	<1
22 April 2003	Small +ve 0 Large +ve 0	Small +ve 0 Large +ve 0	<1	<1

There are no QA/QC bacterial cultures available for enterococci.

CHAPTER V

RESULTS AND DISCUSSION

This chapter presents the results of the wet weather and dry weather sampling and bacteria analyses. Summary tables only are included in this chapter, with detailed results attached in Appendix A. Statistical analyses were conducted using MINITAB, EXCEL and Pro-Stat software. Results are presented both in tabular and in graphical forms for better understanding. Finally, brief discussions and explanations of the results are included.

5.1 Results

5.1.1 Wet Weather Sampling Table 5.1 summarizes the *E. coli* and enterococci levels (MPN/100 mL) obtained from wet weather source area sampling conducted from August 2002 to June 2003 as part of this research. The remaining set of wet weather sampling data is shown in Section A.2 of Appendix A. In addition to the *E. coli* and enterococci levels, various other factors are described, including the time of day at which the sample was taken; the total rainfall occurring before the sampling (inches); maximum rainfall rate (5 min peak intensity, inches/hour) that occurred before the sample was taken; and total coliform levels detected in the samples.

Table 5.1 Wet Weather Source Area Sampling Results

Sample I.D	Date Sample Taken	*E. coli*	Enterococci
		MPN/100 mL***	MPN/100 mL
OPEN SPACE -Prone*	21-Sep-02	1732.9	>2419.2
	25-Sep-02	15.5	>2419.2
	25-Sep-02	41.3	>2419.2
	10-Oct-02	Not Sampled	Not Sampled
	27-Oct-02	Not Sampled	Not Sampled
	5-Nov-02	2419.2	19863
	29-Jan-03	35.4	216
	6-Feb-03	1	395
	6-Feb-03	1	Not Sampled
	24-Apr-03	82	322
	14-May-03	52	2489
	12-Jun-03	>2419.2	>24192
	27-Jun-03	3.1	4106
OPEN SPACE – Not Prone**	21-Sep-02	Not Sampled	Not Sampled
	25-Sep-02	2419.2	>2419.2
	25-Sep-02	866.4	>2419.2
	10-Oct-02	Not Sampled	Not Sampled
	27-Oct-02	Not Sampled	Not Sampled
	15-Oct-02	217.8	>2419.2
	5-Nov-02	44.8	8664
	29-Jan-03	17.7	195
	6-Feb-03	2	505
	24-Apr-03	8.6	2755
	14-May-03	307.6	9804
	12-Jun-03	63.1	>24192
	27-Jun-03	6.2	>24192
PARKING LOT- Not Prone	25-Sep-02	83.9	>2419.2
	25-Sep-02	69.7	2419.2
	10-Oct-02	14.2	>2419.2
	27-Oct-02	1553.1	48.2
	5-Nov-02	15.8	238
	29-Jan-03	4.1	238
	6-Feb-03	<1	31
	24-Apr-03	72.3	9804
	14-May-03	25.6	1130
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	5.2	613
	21-Sep-02	1046.2	529.8
	25-Sep-02	137.6	>2419.2

Contd..

PARKING LOT- Prone	25-Sep-02	66.3	344.8
	10-Oct-02	980.4	>2419.2
	27-Oct-02	866.4	>2419.2
	5-Nov-02	17.3	158
	29-Jan-03	52	199
	29-Jan-03	54.6	160
	29-Jan-03	37.3	145
	6-Feb-03	6.3	150
	24-Apr-03	8.3	127
	14-May-03	290.9	805
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	29.5	416
ROOF- Prone	29-Aug-02	145.5	Not Sampled
	21-Sep-02	461.1	>2419.2
	25-Sep-02	18.7	>2419.2
	25-Sep-02	1413.6	980.4
	10-Oct-02	410.6	67.9
	27-Oct-02	>2419.2	1
	5-Nov-02	>2419.2	9.3
	29-Jan-03	2	16.4
	6-Feb-03	<1	31
	24-Apr-03	517.2	>24192
	14-May-03	Not Sampled	Not Sampled
	12-Jun-03	727	24192
	27-Jun-03	2419.2	15531
ROOF- Not Prone	29-Aug-02	<1	Not Sampled
	21-Sep-02	30.5	8
	25-Sep-02	2	2
	25-Sep-02	5.2	21.1
	10-Oct-02	344.8	69.1
	27-Oct-02	161.6	43.5
	5-Nov-02	29.2	1
	29-Jan-03	<1	<1
	6-Feb-03	>2419.2	3
	24-Apr-03	6.3	<1
	14-May-03	2	7
	12-Jun-03	5.2	9.5
	27-Jun-03	Not Sampled	78
STREET- Prone	21-Sep-02	1553.1	>2419.2
	25-Sep-02	920.8	>2419.2
	25-Sep-02	1119.9	>2419.2
	10-Oct-02	>2419.2	>2419.2
	27-Oct-02	>2419.2	>2419.2
	5-Nov-02	>2419.2	>24192
	29-Jan-03	Not Sampled	Not Sampled

Contd..

STREET- Prone	6-Feb-03	12.1	332
	24-Apr-03	95.9	8164
	14-May-03	>2419.2	3130
	12-Jun-03	NT	NT
	27-Jun-03	2419.2	15531
STREET- Not Prone	25-Sep-02	>2419.2	>2419.2
	25-Sep-02	980.4	>2419.2
	10-Oct-02	1046.2	>2419.2
	27-Oct-02	>2419.2	>2419.2
	5-Nov-02	1299.7	1785
	29-Jan-03	131.3	563
	6-Feb-03	52.8	749
	24-Apr-03	77.6	1401
	14-May-03	114.5	435
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	32.3	683

*Prone: locations where urban wildlife (birds and squirrels for roofs, and dogs for ground-level surfaces) frequent.
**Not prone: locations where urban wildlife appear to be generally absent.
*** MPN/100 mL : most probable number of organisms per 100 mL of sample

The upper detection limit (UDL) of this method was 2419.2 MPN/100 mL and the lower detection limit (LDL) was 1 MPN/100 mL for all three indicator organisms. After completion of the first five rounds of sampling, it was observed that most enterococci levels exceeded the UDL. Therefore, three 100mL samples per site were collected in the subsequent rounds (two for enterococci and one for *E. coli*). One 100 mL sample was diluted 10 times to increase the range of the UDL to 24,192 MPN/100 mL. Enterococci levels were found in both diluted as well as not diluted samples. Enterococci levels found in the diluted samples were found to better represent the bacterial levels. Therefore, to maintain uniformity, the dilution results were used whenever they were available. For most of the statistical analyses, the values greater than UDL and less than LDL were replaced with the UDL and LDL values, respectively, generally resulting in conservative results. As can be seen from the table, wide ranges of bacterial levels were detected from each of the source areas. *E. coli* levels varied from <1 to >2419.2 for most of the source

areas. Since no dilutions were done for *E. coli* samples, the range was limited by the LDL and UDL values. However, the enterococci levels had a wider range due to the dilution (<1 to > 24192). The enterococci values were much higher than the *E. coli* values.

5.1.2 Dry Weather Sampling Results Another part of this research included bacterial analyses of dry weather samples taken from outfalls flowing into Cribbs Mill Creek in Tuscaloosa, AL. Although the samples were analyzed for a number of parameters (as part of the EPA-funded Inappropriate Discharge Detection and Elimination "IDDE" project) this thesis focused on bacterial analyses, i.e. *E. coli* and enterococci. Section A.3 (Appendix A) shows results of bacterial analyses of dry weather samples. An outfall is considered a problem outfall if the bacteria levels exceeded the observed limits shown in the flow chart (Figure 5.22) used for identifying the most significant component of flow from an outfall. If the bacteria levels exceeded the values listed, it is highly likely that sanitary sewage contamination is present (the source area bacteria loads are not high enough to cause such high values). Section 5.2.3 describes how the values in the flow chart were determined.

In the last research phase, "library" samples (reference samples) collected from various source areas were analyzed for various tracer materials, including *E. coli* and enterococci. This included samples from influent to sewage treatment plants, local springs, irrigation runoff, domestic water taps, car wash, and laundry water. Tables 5.2 and 5-3 show the results of the bacterial analyses of the library samples.

Table 5.2 *E. coli* Levels in Reference Samples (MPN/100 mL)

Sample No.	Tap Water	Spring Water	Irrigation	Laundry	Carwash	Industrial	Sewage (Dry Weather)**	Sewage (Wet Weather)
NO.1	NA	4.1	27.8	NA	1553.1	66.3	>2419.2	
NO.2	NA	1	8.3	NA	1413.6	>2419.2	NA	
NO.3	NA	NA	>2419.2	<1	4.1	0	>2419.2	
NO.4	NA	NA	>2419.2	<1	14.6	3	816.4	
NO.5	NA	NA	31.8	<1	>2419.2	NA	NA	
NO.6	<1	<1	>2419.2	>2419.2	1413.6	NA	12033000	
NO.7	<1	290.9	>2419.2	20.1	15.8	NA		2851000
NO.8	<1	172.3	>2419.2	<1	11.9	NA		3654000
NO.9	<1	<1	>2419.2	19.7	235.9	<1		2187000
NO.10	<1	9.7	1299.7	<1	15.5	>2419.2		1785000
NO.11	<1	1	>4838.4	<1	1553.1	<1		3255000
NO.12	<1	<1	>4838.4	<1	<1	<1		2282000
Geometric mean*	1	5.01	771.9	3.96	94.06	19.73	15484.78	2590319
Median *	1	1	2419.2	1	125.85	2	2419.2	2566500
COV*	0	1.96	0.76	3.09	1.21	1.81	1.99	0.26

* Values calculated by replacing <1 by 1 and >2419.2 by 2419.2

** The initial dry weather sewage samples were not well shaken before analyses and are therefore considered artificially low. The wet weather sewage samples were therefore used during this research to represent local sanitary sewage.

Table 5.3 Enterococci Levels in Reference Samples (MPN/100 mL)

Sample No.	Tap Water	ing Wa	Irrigation	Laundry	Carwash	Industrial	Sewage (Dry)**	Sewage (Wet)
NO.1	NA	4.1	>2419.2	NA	>2419.2	0	>2419.2	
NO.2	NA	36.4	2	NA	6.2	>2419.2	NA	
NO.3	NA	NA	>2419.2	<1	5.2	0	>2419.2	
NO.4	NA	NA	>2419.2	<1	3.1	>2419.2	43.6	
NO.5	NA	NA	>2419.2	<1	1	NA	NA	
NO.6	<1	<1	287.7	<1	>2419.2	NA	613000	
NO.7	<1	412	>2419.2	<1	<1	NA		833000
NO.8	<1	140.8	>2419.2	<1	11.1	NA		598000
NO.9	<1	3.1	>2419.2	<1	<1	<1		292000
NO.10	<1	65.7	>2419.2	<1	<1	866.4		328000
NO.11	<1	<1	>4838.4	<1	2419.2	22.2		369000
NO.12	<1	<1	>4838.4	<1	<1	<1		609000
Geometric mean*	1	10.65	1258.62	1	12.57	69.48	3536.49	469578
Median*	1	4.1	2419.2	1	4.15	11.6	2419.2	483500
COV*	0	1.82	0.57	0	1.79	1.52	1.97	0.41

* Values calculated by replacing <1 by 1 and >2419.2 by 2419.2

** The initial dry weather sewage samples were not well shaken before analyses and are therefore considered artificially low. The wet weather sewage samples were therefore used during this research to represent local sanitary sewage.

5.2 Statistical Analysis and Discussion

5.2.1 Statistical Analyses of Wet Weather Data Statistical analyses of data were conducted using the following software packages: MINITAB, ProStat, and MS-Excel. Although total coliforms were also detected (as part of the *E. coli* analyses), only *E. coli* and enterococci data were analyzed. Most of the total coliform observations were greater than the upper detection limit, and additional dilution analyses were not warranted for this secondary parameter. Observations from each of the source areas prone to urban animals (including the presence of trees) were compared to observations from similar source areas not prone to urban animal use.

Due to the presence of large numbers of non-detected values, three types of paired and unpaired statistical tests were used to determine if significant differences occurred between the sites. MINITAB was used to plot box plots. For both, *E. coli* and enterococci, two separate box plots were prepared, one for warm months and the other for the whole year. Figure 5.1 through 5.4 shows these box plots contrasting the observations from the sites. The box plots show the normal range box, extreme value symbols (stars) and the median symbols (circle). In order to prepare undistorted plots, values less than the lower detection limit (<1) were replaced by 0.5, and values greater than the upper detection limit values (>2419.2) were removed. The number of observations greater than the UDL removed for each site is noted at the bottom of box plot. Only two sampling rounds were conducted during the winter.

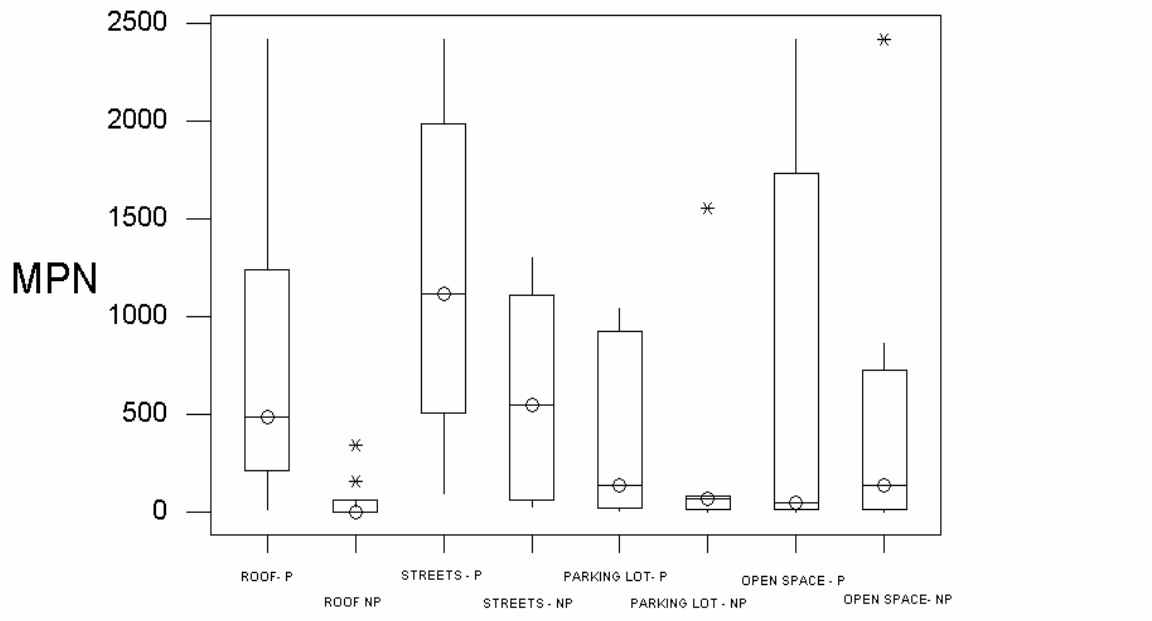

Figure 5.1 Group Box Plot for *E. coli* for all Warm Months*

*No. of values >2419.2 removed: Roof- P: 2; Street-P: 4; Street- NP: 2

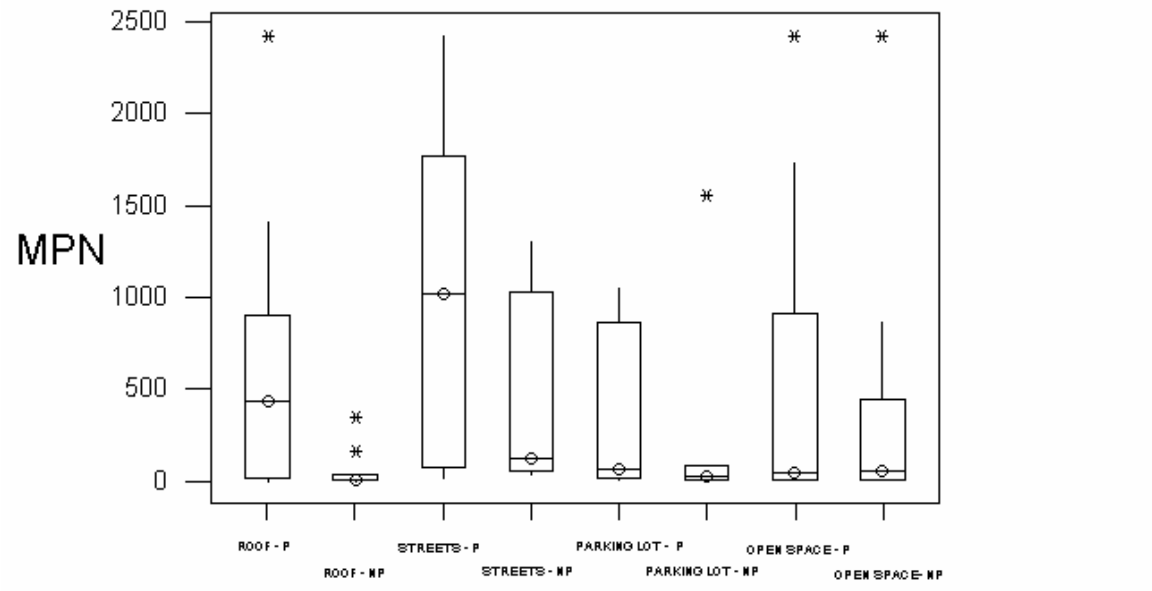

Figure 5.2 Group Box Plot for *E. coli* for Whole Year*

* No. of values >2419.2 removed: Roof- P- 2, Roof- NP- 1, Street-P-4 , Street- NP- 2, Open space- P- 1

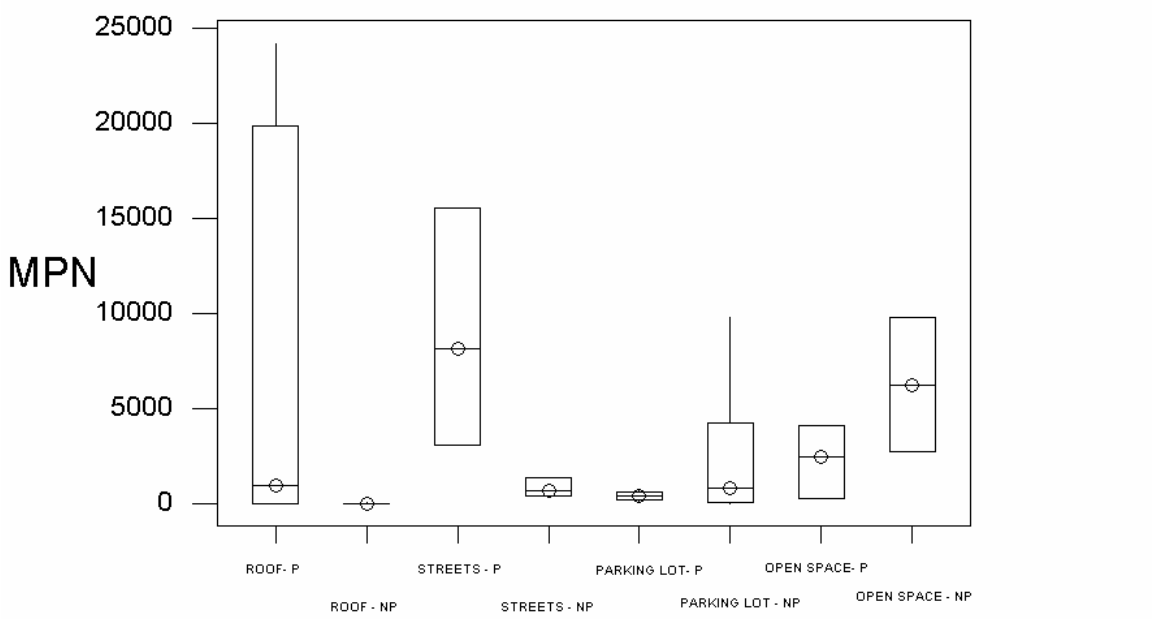

Figure 5.3 Group Box Plot for Enterococci for all Warm Months *

* No. of values >2419.2 removed: Roof- P- 3, Street-P-6 , Street- NP- 4, Parking lot -P-3, Parking lot -NP- 2, Open space- P- 4 and Open space- NP-5

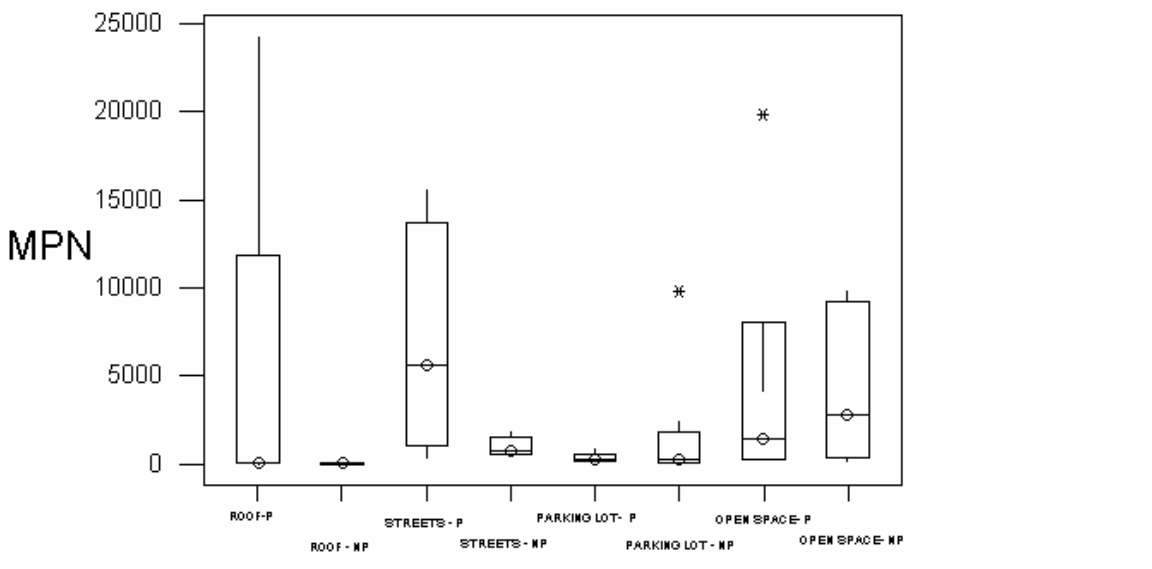

Figure 5.4 Group Box Plot for Enterococci for Whole Year*

* No. of values >2419.2 removed: Roof- P- 3, Street-P-6 , Street- NP- 4, Parking lot -P-3, Parking lot -NP- 2, Open space- P- 4 and Open space- NP-5

As is common for most wet-weather bacteria observations, large overlaps exist between different sampled categories. The overlapping values observed for the sites prone and not prone to urban wildlife made it difficult to visually interpret if these sites had significantly different bacteria levels. A number of factors may be responsible for the overlapping values, such as mixing of runoff from other source areas, presence of building materials, effects of tree shading, moisture content, etc. Considerable care was taken to prevent mixing of runoff from other source areas, but it was still possible that some contamination could have occurred due to splashing of rain water by speeding cars traveling along the opposite side of the road, and mixing of runoff from small landscape areas in a parking lot.

The plots were supplemented with statistical tests to measure the significance of the likely differences between paired data sets. The two-tail Wilcoxon Rank sum test (same as Mann Whitney U test) was performed using MINITAB. The two-tail Wilcoxon Rank test was performed because it was not clear if the animal prone sites had significantly higher, or lower, levels than the not prone sites. This test performs a hypothesis test of the equality of the two population medians and calculates the corresponding point estimate and confidence interval. The probability of these two medians being the same (within the confidence interval) is then calculated. Probability (p) values less than, or equal to, 0.05 are usually used to signify a significant difference. This would correspond to a 1 in 20 chance that the medians were really the same when they were assumed to be different. This test is a nonparametric alternative to the two-sample T-test. Nonparametric tests are preferred when the values are not normally distributed. Different nonparametric tests have different restrictions, so it is important

that the most suitable tests are used that correspond to data characteristics. An assumption for the Mann-Whitney test is that the data are independent random samples from two populations that have the same shape (MINITAB help menu). To make sure that the populations have the same shape, over-laying probability plots were made for prone and not prone data (As shown in section A.4 of Appendix A). In all the cases, the straight lines were very close to each other and the bandwidths were quite similar. Therefore, the distributions can be reasonably assumed to be the same shape. Table 5.4 shows the output obtained using MINITAB for comparison between prones (exposed to animal activity) and Not prones (not likely exposed to animal activity). A summary of all Wilcoxon Rank Sum or Mann-Whitney tests performed is shown in Table 5.5 and 5.6.

Table 5.4 Comparisons between Data Collected at Sites Prone to Urban Animal Use and at Sites Not Prone to Urban Animal Use for *E. coli**

Mann-Whitney Test and CI: ROOFPRONE, ROOF NOT PRONE

```
ROOFPRONE       N =   8     Median =       489.2
ROOF NOTPRONE   N =  10     Median =         5.8
Point estimate for ETA1-ETA2 is       457.5
95.4 Percent CI for ETA1-ETA2 is (140.2,1383.2)
W = 110.0
Test of ETA1 = ETA2  vs  ETA1 not = ETA2 is significant at 0.0029
The test is significant at 0.0027 (adjusted for ties)
```

Mann-Whitney Test and CI: STREETS PRONE, STREETS NOT PRONE

```
STREETS    N =   5     Median =      1119.9
STREETS    N =   6     Median =       547.5
Point estimate for ETA1-ETA2 is       689.5
96.4 Percent CI for ETA1-ETA2 is (-379.0,1520.9)
W = 37.0
Test of ETA1 = ETA2  vs  ETA1 not = ETA2 is significant at 0.2353
The test is significant at 0.2300 (adjusted for ties)

Cannot reject at alpha = 0.05
```

Mann-Whitney Test and CI: PARKING LOT-PRONE, PARKING LOT -NOT PRONE

```
PARKING    N =   9     Median =       137.6
PARKING    N =   9     Median =        69.7
Point estimate for ETA1-ETA2 is        53.7
95.8 Percent CI for ETA1-ETA2 is (-53.7,852.3)
W = 97.0
Test of ETA1 = ETA2  vs  ETA1 not = ETA2 is significant at 0.3314
The test is significant at 0.3304 (adjusted for ties)

Cannot reject at alpha = 0.05
```

Mann-Whitney Test and CI: OPEN SPACE-PRONE, OPEN SPACE NOT PRONE

```
OPEN SPA   N =   7     Median =        52.0
OPEN SPA   N =   8     Median =       140.5
Point estimate for ETA1-ETA2 is        -4.5
95.7 Percent CI for ETA1-ETA2 is (-686.4,1552.7)
W = 53.5
Test of ETA1 = ETA2  vs  ETA1 not = ETA2 is significant at 0.8170
The test is significant at 0.8163 (adjusted for ties)

Cannot reject at alpha = 0.05
```

* Excluding winter months and greater than UDL values; less than LDL replaced by 0.5

Table 5.5 Wilcoxon Rank Sum Test Results Summary (*E. coli*)

Source Areas Compared	**Ratio of Medians (Prone/Not Prone)**	**p- value (Two tail)***	**Significant Difference Observed? (At the 0.05 level)**
Roof prones v/s Roof not prones	84	0.002	Yes
Streets Prone v/s Streets not prones	2	0.23	No
Parking lot prone v/s Parking lot not prone	1.9	0.33	No
Open space prone v/s Open space not prone	0.37	0.81	No

* Values adjusted for ties.

Table 5.6 Wilcoxon Rank Sum Test Results Summary (Enterococci)

Source Areas Compared	**Ratio of Medians (Prone/Not Prone)**	**p- value (Two Tail)***	**Significant Difference Observed? (at the 0.05 level)**
Roof prones v/s Roof not prones	59.5	0.11	No
Streets Prone v/s Streets not prones	7.83	0.04	Yes
Parking lot prone v/s Parking lot not prone	0.62	0.58	No
Open space prone v/s Open space not prone	0.38	0.59	No

* Values adjusted for ties

Since greater than UDL values were removed for the Mann Whitney test, the number of observations compared were reduced to three in some cases. To supplement the above Mann Whitney test results, Kruskal Wallis tests were also conducted. For these tests, values greater than UDL and less than LDL values were replaced by UDL and LDL values. The Kruskal-Wallis test performs a hypothesis test of the equality of the population medians for a one-way design (two or more populations). This test is a generalization of the procedure used by the Mann-Whitney test and offers a nonparametric alternative to the one-way analysis of variance (ANOVA). The Kruskal-Wallis test looks for differences among the population medians. The Kruskal-Wallis hypotheses are:

H0: the population medians are all equal versus H1: the medians are not all equal

An assumption for this test is that the samples from the different populations are independent random samples from continuous distributions, with the distributions having the same shape (same as Mann Whitney). The Kruskal-Wallis test is more powerful (the confidence interval is narrower, on average) than Mood's median test for analyzing data from many distributions, including data from the normal distribution, but is less robust against outliers (MINITAB help menu). Table 5.7 shows the results of the Kruskal Wallis tests.

Table 5.7 Kruskal Wallis Test Results Summary

Source Areas Compared		**p- Value***	**Difference Observed? (At The 0.05 Level)**
Roof prones v/s Roof not prones	*E. coli*	0.030	Yes
	Enterococci	0.010	Yes
Streets Prone v/s Streets not prones	*E. coli*	0.164	No
	Enterococci	0.017	Yes
Parking lot prone v/s Parking lot not prone	*E. coli*	0.259	No
	Enterococci	0.683	No
Open space prone v/s Open space not	*E. coli*	0.778	No
	Enterococci	0.514	No

* Values adjusted for ties.

In order to see if the data patterns were reasonably similar, additional tests using the paired sign method were conducted. The sign test does not require the distributions to be of same shape, or for the variance to be the same. Moreover, the values greater than and less than the quantification range can also be included. Paired tests were conducted because, except for the presence of trees, all other physical parameters that may affect the results, such as temperature, rainfall, type of land use, location etc. were very similar in both cases during each sampled event. First, the differences between the prone observations and not prone observation were found. The sign test of the median = 0 v/s >0 was performed on the difference using MINITAB. Table 5.8 shows the results of the paired sign tests.

Table 5.8 Paired Sign Test Results

Source Areas Compared	Indicator Organism	p- Value	Difference Observed? (At The 0.05 Level)
Roof prones v/s Roof not prones	*E. coli*	0.005	Yes
	Enterococci	0.03	Yes
Streets Prone v/s Streets not prones	*E. coli*	0.14	No
	Enterococci	0.18	No
Parking lot prone v/s Parking lot not prone	*E. coli*	0.11	No
	Enterococci	0.91	No
Open space prone v/s Open space not prone	*E. coli*	0.74	No
	Enterococci	0.89	No

Tree coverage (i.e canopies over the roofs) encouraged higher bird and squirrel populations. Samples taken from the roofs with tree canopies were therefore expected to show significantly higher values of *E. coli.* and enterococci, compared to roofs without tree canopies. This assumption was confirmed during these analyses and statistical tests. However, no significant differences in bacterial levels were observed between the open space and parking lot sites that were prone and not prone to urban wildlife. The streets sites that were prone to urban animal use showed significantly higher enterococci levels as compared to streets that were not prone to urban animals, but the *E. coli* levels were not significantly different. These results indicated that urban birds may be a significant source of bacterial contamination in stormwater. However, the tests were not all consistent, as the open space and parking areas never showing significant differences between areas that may have more urban wildlife than other areas. These areas are likely exposed to many more interferences than the roofs and streets.

The levels of indicator bacteria present in the source area stormwater exceeded the EPA 1986 water quality criteria (single sample max value) in 31% (*E. coli*) and 74%

(enterococci) of the samples, and the geometric mean criteria was exceeded in 100% of the source area areas. Since none of these sites could be contaminated by sewage, urban birds and animals were found to be significant, but variable, contributors to elevated levels of stormwater bacteria.

5.2.2 Variability in Bacterial Levels Because of the large variability found for the bacteria analyses in the sheetflow samples, additional tests were conducted to determine the potential causes for this variability. This discussion is divided into three parts, variability between different storms, variability within storms, and factors effecting variability.

Variability between Storms Figures 5.5 and 5.6 show the variation of bacterial levels in wet weather flows by month since the beginning of the project. These time series plots were made to identify potential effects of season on both types of sampling locations (prones and not prones) simultaneously. Contrary to what was expected, the bacterial levels did not follow a smooth increase or decrease with changing temperatures during the different months.

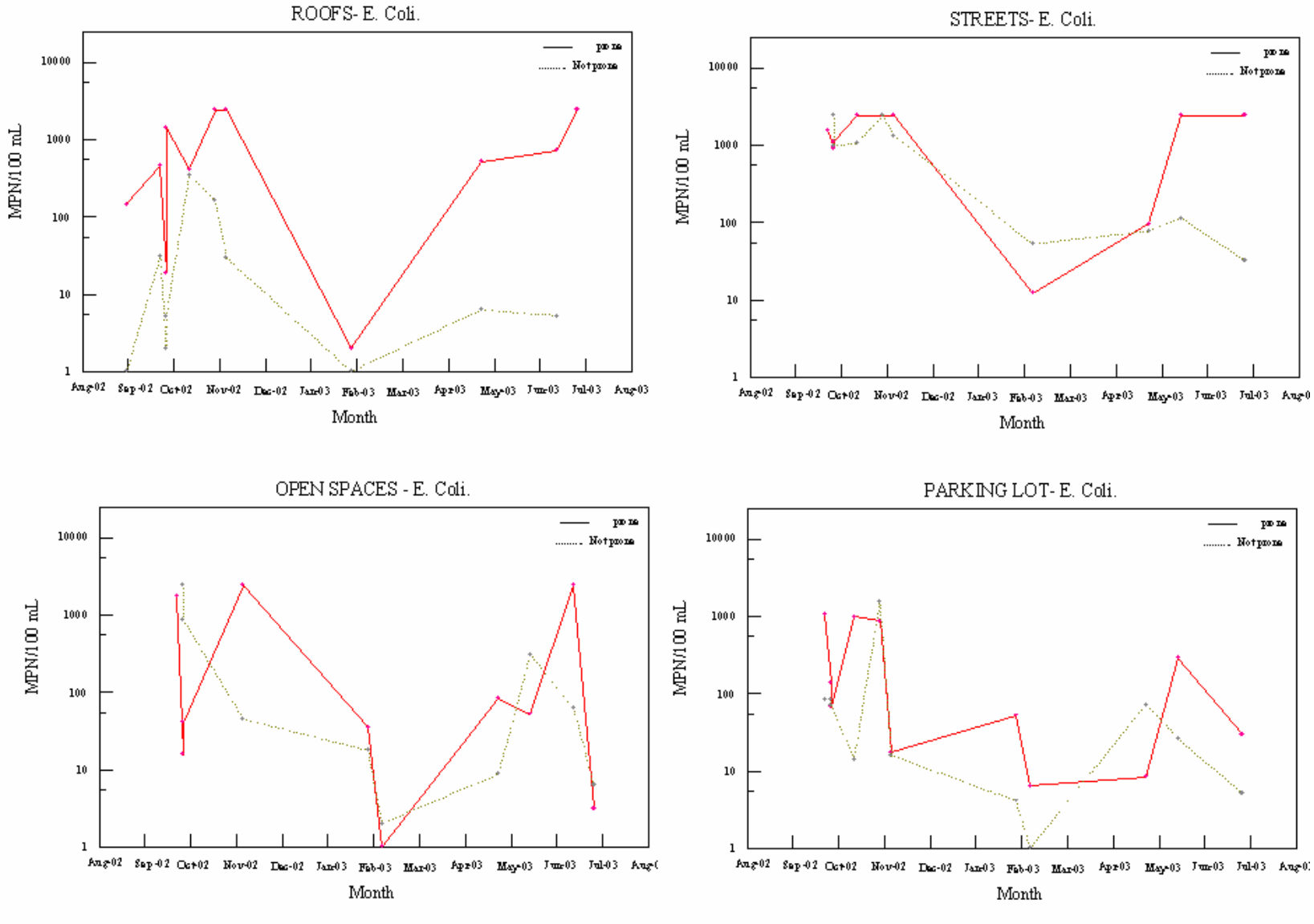

Figure 5.5 Variations of *E. coli* Levels from Various Source Areas by Months

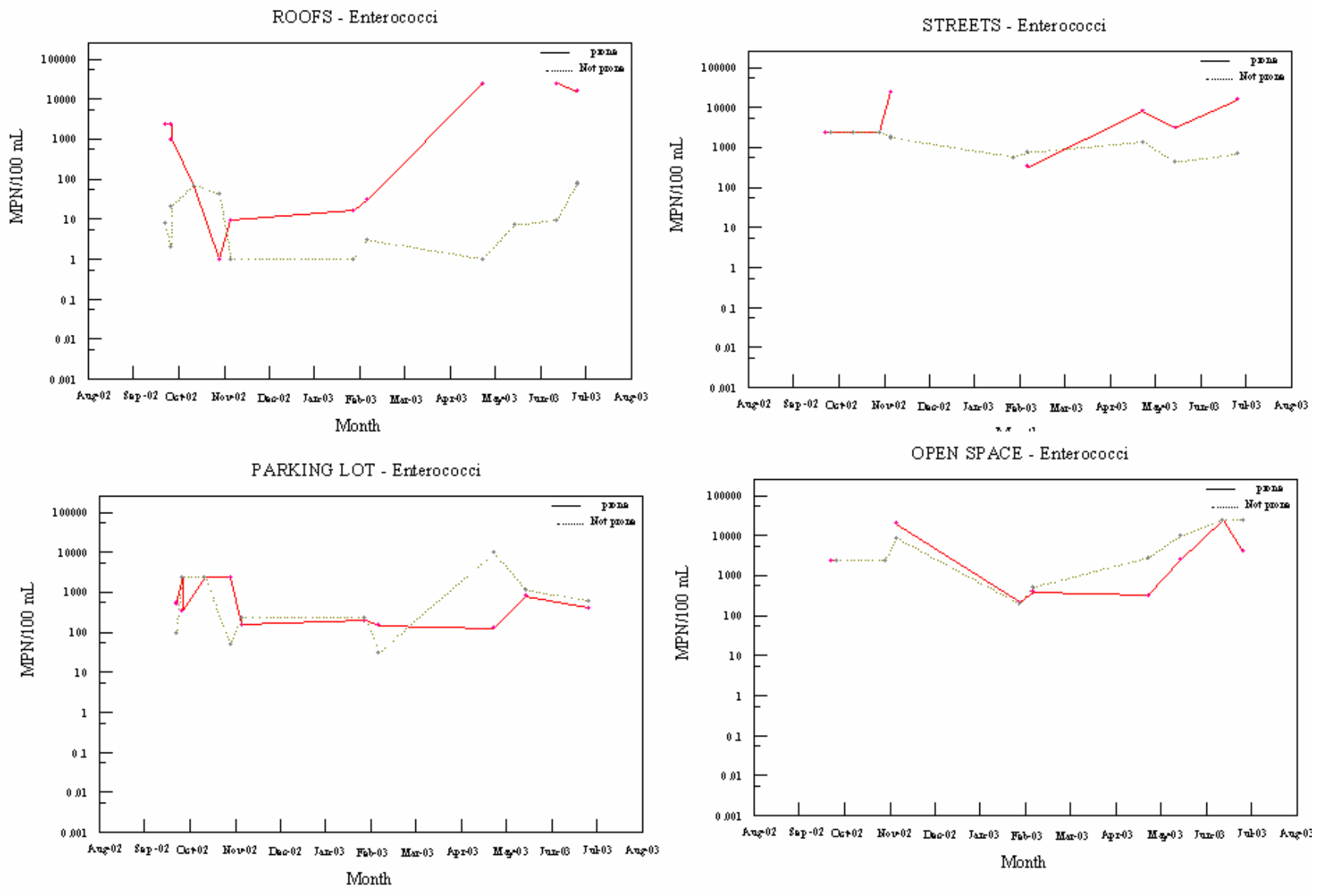

Figure 5.6 Variations of Enterococci Levels from Various Source Areas by Month

Variability within Storms During a single storm on 25 Sep 2002, all the sites were sampled twice, once in the morning and then again in the evening (Figures 5.7 and 5.8). From these figures, it is clear that bacterial levels in urban runoff from various source areas vary within storms, but there is no consistent pattern: some areas may have an increase in bacteria levels, while other areas may experience a decrease. Paired sign tests for morning v/s evening sampling gave probability (p) value of 1 for both *E. coli.* and enterococci i.e. no significant differences were observed at the 0.05 level (not enough data is available to indicate they are the same). Since no dilutions were made for enterococci samples for this storm, most of the values remained above the upper detection limit.

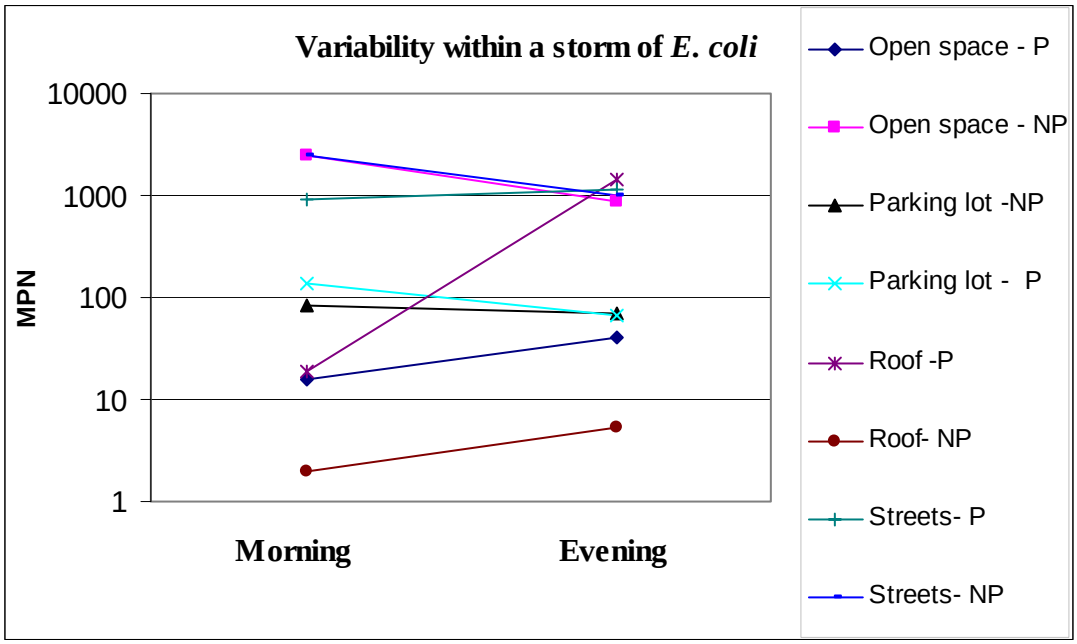

Figure 5.7 Variability within a Storm for *E. coli*

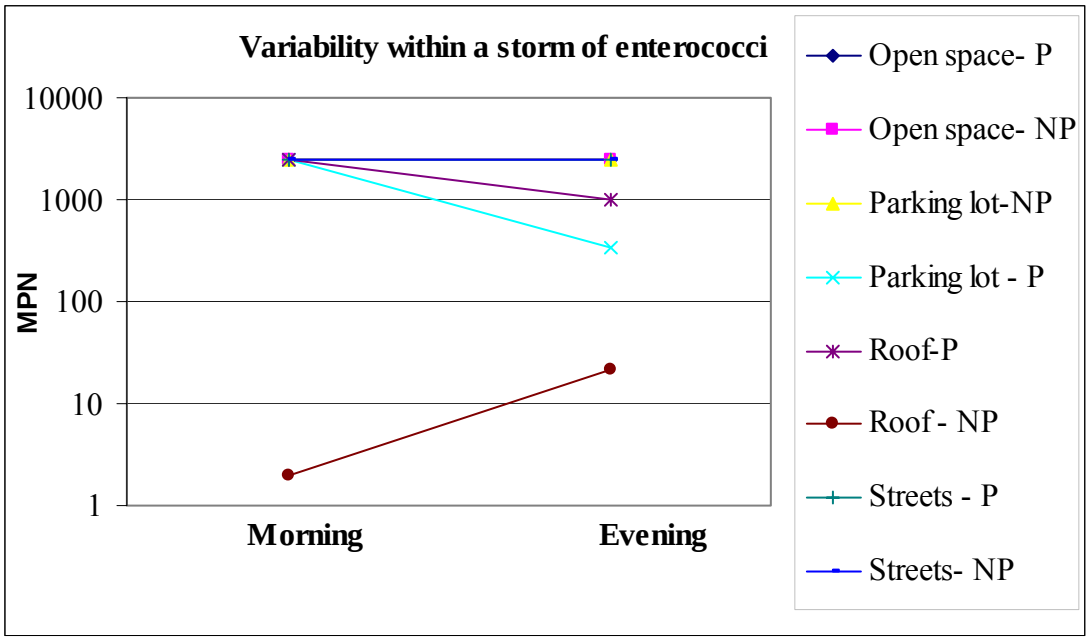

Figure 5.8 Variability within a Storm for Enterococci

Factors Effecting Variation in Bacterial Levels in Wet Weather Flow In order to explain large variations in bacterial levels within a storm, and between storms, various factors were examined.

1) Climate The climate of Tuscaloosa, Alabama, is subtropical with four distinct seasons, and is humid with no dry season. It is an area of high humidity. December through February are winter months. Frosts and freezes are possible during these months. Cold periods, which are short lived, are associated with cold fronts, which may be accompanied by large amounts of rain. The average monthly temperature during these months is below 50^0 F. March and April are considered to be spring months. During this period, daily high temperatures are usually less than 80 degrees F., and freezes are rare. Spring-like temperatures are common from late February through most of April. Summer-like conditions usually begin in late April, or early May, and last until the end of September or early October. May through September are considered summer months. Summer temperatures above 90 degree F. are normal, and summer high temperatures almost never drop below 80 degrees F. October and November are considered to be the autumn

months. The temperatures during these months are similar to spring, but there is less rainfall. (www.math.ua.edu/weather.htm#data, 2002).

As seen in Figures 5.5 and 5.6, there is no smooth increase or decrease in bacterial levels with changing seasons. However, the geometric mean values for samples collected during the cold months (December through February, with temperature below 50^0 F) were compared with samples collected during the other months. Table 5.9 shows this comparison of warm and cold weather geometric mean bacteria values. Cold weather values were found to be much lower than the warm weather, except in the case of Roof- NP where one unusually high value was found. Thus, seasonally low temperatures may cause decreases in bacterial levels. Due to only two observations for winter months, statistical test could not be performed.

Table 5.9 Comparison of Geometric Means

Site	***E. coli* (MPN/100 mL)**		**Enterococci (MPN/100 mL)**	
	Warm Above 50^0 F	**Cold** Below 50^0 F	**Warm** Above 50^0 F	**Cold** Below 50^0 F
Roof - Prone	>574	1	>684	22.5
Roof - Not prone	10.5	>34.7	8.7	1.2
Streets- Prone	>1330	12.1	>4530	332
Streets- Not prone	>470	83.2	>1500	650
Parking lot - Prone	129	28.5	>640	160
Parking lot- Not prone	45.8	1.4	>1010	85.8
Open space- Prone	>130	3.2	>3500	292
Open space- Not prone	110	5.9	>6100	310

2) Amount of Rain Occurred before Sampling Six samples from two different source areas were collected at an interval of 15 to 30 minutes. The total rain that occurred (in inches) before the sample was taken was noted from the weather station installed above the CEE departmental building. Table 5.10 shows the collected data. As can be seen from Figures 5.9 and 5.10, bacterial levels may increase or decrease with increasing amounts of rain with time, but stayed within a generally narrow band.

Table 5.10 Effect of Total Rain and Rain Intensity on Bacterial Levels

Time of Sampling	Total Rain Occurred (inches)	5 Minute Rain Rate (in/hr)	Street - NP		Parking Lot - NP	
			E. coli MPN/ 100 mL	Enterococci MPN/100 mL	*E. coli* MPN/100 mL	Enterococci MPN/100 mL
9 A.M	0.29	0.29	1553.1	130	16	3654
9.15 A.M	0.35	0.46	547.5	107	18.7	3255
9.30 A.M	0.4	0.06	1046.2	738	10.9	3255
9.45 A.M	0.44	0.17	517.2	364	17.3	4352
10 A.M	0.47	0.09	920.8	712	7.4	1014
10.30 A.M	0.48	0.04	980.4	1106	16	1376

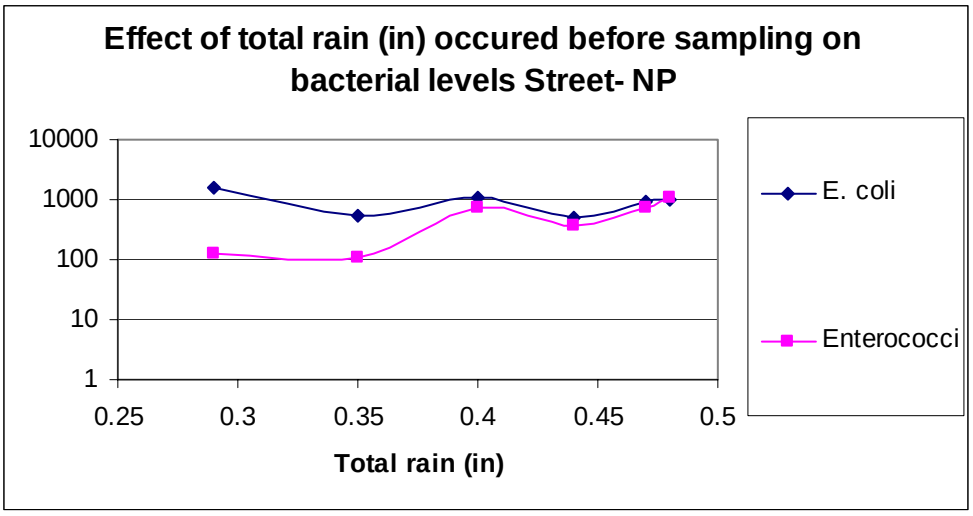

Figure 5.9 Effect of Total Rain on Bacterial Levels (Street- NP)

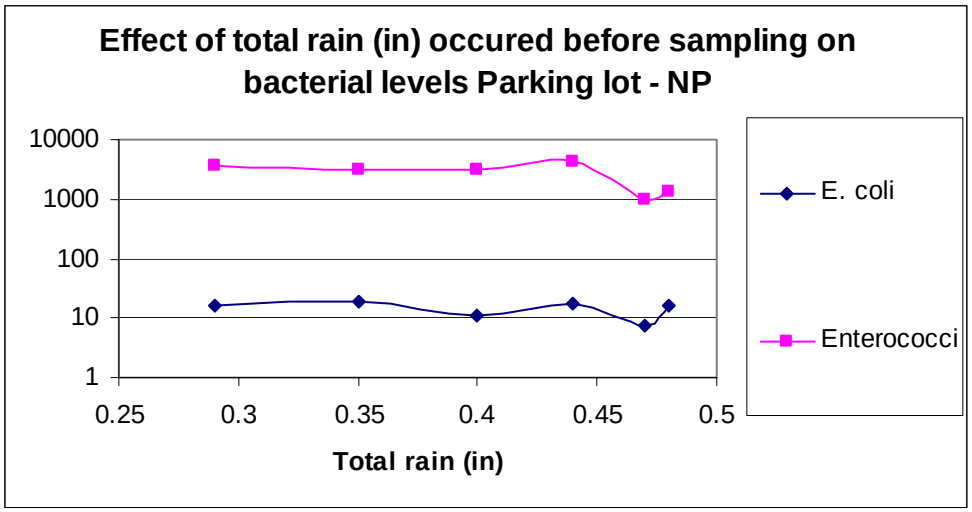

Figure 5.10 Effect of Total Rain on Bacterial Levels (Parking Lot- NP)

Regression analyses and associated ANOVA tests were conducted to determine the significance of the slope term in the relationship between total rain depth and bacterial levels. Table 5.11 shows the p-value for slope term, and the lower and upper 95 % values. Since the p-value for the slope term is greater than 0.05 and the confidence interval includes zero values for all cases, no significant relationship likely exists between total rain and bacterial levels. The enterococci, streets, not prone condition had a p value close to 0.05 (0.052) and may therefore be considered marginally significant, with possible increasing bacteria levels associated with larger rains.

Table 5.11 Regression Analysis Results to Find Effect of Total Rain (Slope Coefficients, MPN/100 mL/in)

Source Area	Indicator Organisms	p- Value for Slope Coefficient	Lower 95 %	Upper 95 %
Streets-NP	*E. coli*	0.37	-8667.9	4100.0
	Enterococci	0.052	-69.5	8657.8
Parking Lot -NP	*E. coli*	0.43	-97.6	51
	Enterococci	0.20	-30626.9	9122

3) Rain Rate (in/hr) Table 5.10 also shows the 5 minute peak rain intensity found for each of these sampling intervals and these are plotted on Figures 5.11 and 5.12.

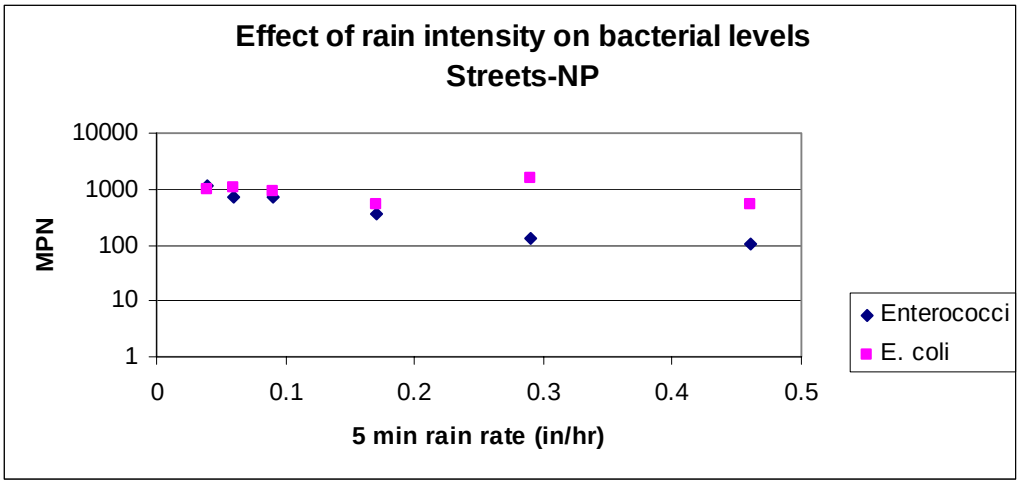

Figure 5.11 Effect of Rain Rate on Bacterial Levels (Streets-NP)

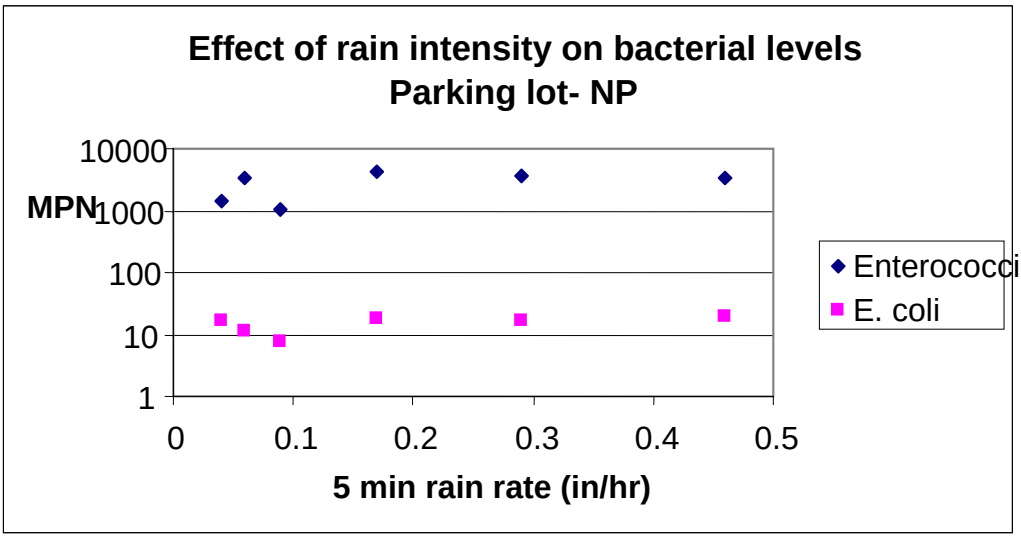

Figure 5.12 Effect of Rain Rate on Bacterial Levels (Parking Lot- NP)

Regression analyses and associated ANOVA tests were conducted to determine the significance of the slope term in the relationship between rain intensity and bacterial levels (Table 5.12). Except for enterococci levels from street- NP, the p-value for the slope term is greater than 0.05 and the confidence interval includes zero, so the slope term relating the rain rate and the bacterial levels were not significant, except for this one

case. The p-value for the intercept was 0.002 for street- NP (enterococci) and the confidence interval did not include zero. The enterococci levels decreased with rain rate for this site and condition.

Table 5.12 Regression Analysis Results to Find Effect of Rain Rate (Slope Coefficients, MPN/100 mL/In/Hr)

Source Area	Indicator Organisms	P- Value For Slope Coefficients	Lower 95 %	Upper 95 %
Streets-NP	*E. coli*	0.73	-3598.4	2775.6
	Enterococci	0.02	-3713	-551.6
Parking lot - NP	*E. coli*	0.18	-12.4	45.3
	Enterococci	0.34	-6089.1	13796.2

4) Effect of sample handling Three factors involving sample handling were also studied which could affect the results. These included holding time, refrigeration, and the effects of shaking. For these tests, a single 5 liter sample was obtained from one source area. Subsamples, each as 100 mL duplicates, were tested after 1, 2, 5, 9, 24, and 48 hrs (Table 5.13). After the 9 hr samples were taken, the 5 liter sample was split into two components, one was kept refrigerated while the other was not.

Table 5.13 Effect of Holding Time

Holding Time* Hrs	*E. coli* MPN/100 mL	Enterococci MPN/100 mL
1	1413.6	360.9
1	1413.6	91
2	1119.9	248.9
2	>2419.2	435.2
5	1203.3	461.1
5	1732.9	248.1
9	1299.7	213
9	1046.2	269
24	920.8	419
48	1046.2	128

* Not refrigerated and not shaken

Figure 5.13 shows variation of bacterial levels with sample holding time Since the p-value, found by regression analyses and associated ANOVA tests, for the slope term is greater than 0.05 (Table 5.14) and the confidence interval includes zero values for both cases, no significant relationship likely exists between holding time and bacterial levels for samples that are not refrigerated and not shaken.

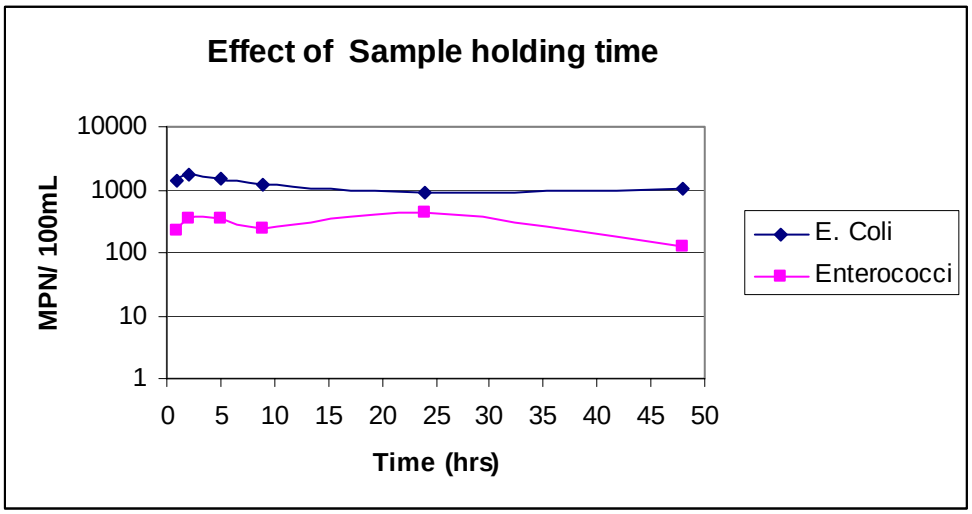

Figure 5.13 Variations with Sample Holding Time

Table 5.14 Regression Analysis Results to Find Effect of Holding Time (Slope Coefficients, MPN/100 mL/hr)

Indicator Organisms	P- Value For Slope Coefficient	Lower 95 %	Upper 95 %
E. coli	0.10	-28.7	4.3
Enterococci	0.37	-9.7	4.6

The effect of refrigeration over one to two days was then measured (Table 5.15). All these samples were shaken before analyses.

Table 5.15 Effect of Refrigeration

Holding Time Hrs	Refrigeration	*E. coli* MPN/100 mL	Enterococci MPN/100 mL
24	Refrigerated	1046.2	689
24	Not Refrigerated	920.8	419

48	Refrigerated	1299.7	240
48	Not Refrigerated	1046.2	128

The effect of shaking was measured by first taking a 100 mL sample from the unshaken larger sample container, and later shaking the larger sample bottle and testing another 100 mL sample (Table 5.16). None of these samples were refrigerated to produce a worst-case for the holding time period.

Table 5.16 Effect of Shaking

Holding Time Hrs	**Shaking**	***E. coli* MPN/100 mL**	**Enterococci MPN/100 mL**
24	Shaken	920.8	419
24	Not shaken	920.8	298.7
48	Shaken	1046.2	128
48	Not shaken	488.4	30

A 2^3 factorial evaluation was conducted to identify the main effects and effects of interactions between these factors. Table 5.17 show the factorial design. The calculated main effects and interaction effects are shown in table 5.18

Table 5.17 Factorial design

Experiment no.	**Time (T) - 24 hr + 48hr**	**Refrigeration (R) _ Not + Yes**	**Shaking (S) _ No + Yes**	***E. coli* MPN/100 mL**	**Enterococci MPN/100 mL**
1	-	-	-	920.8	298.7
2	+	-	-	488.4	30
3	-	+	-	1553.1	413
4	+	+	-	1119.9	173
5	-	-	+	920.8	419
6	+	-	+	1046.2	128
7	-	+	+	1046.2	689
8	+	+	+	1299.7	240
Average				1049.4	298.8

Table 5.18 Main Effects and Interaction Effects

Indicator	Main Effects			Interaction Effects			
	Time (T)	Refrigeration (R)	Shaking (S)	TS	TR	RS	TRS
E. coli	-121.6	410.6	57.6	311.1	31.8	-221.2	32.2
Enterococci	-312.1	159.8	140.3	-57.8	-32.3	31.1	-46.6

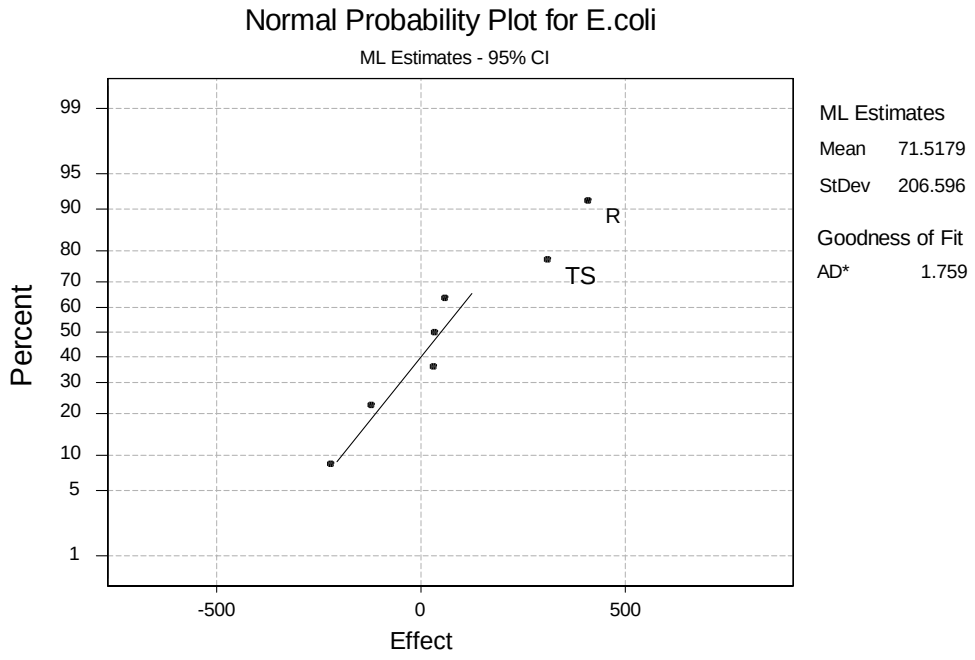

Figure 5.14 Normal Probability Plots for Effects (*E. coli)*

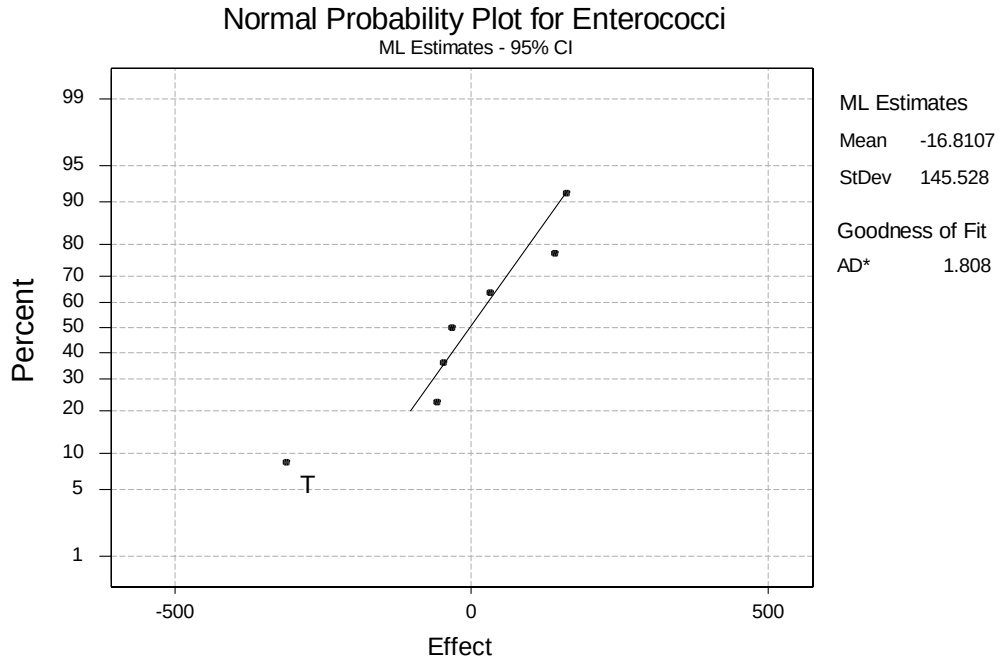

Figure 5.15 Normal Probability Plot for Effects (Enterococci)

Interpretations are needed for R and TS for *E. coli* and T only for enterococci, as can be seen from probability plots of effects (Figure 5.14 and 5.15). Based on these effects, the calculated values were found using the equations:

$$Value = Avg. \pm (effects/2)(\ factor)$$

$$E.Coli = 1049 \pm (411/2)(R) \pm (311/2)(TS)$$

$$Enterococci = 298.8 \pm (-312.1/2)(T)$$

Table 5.19 and 5.20 shows the calculated and observed values for various conditions.

Table 5.19 Calculated and observed values (*E. coli*)

Condition		**Calculated Values**	**Observed Values**
TS	R		
+	+	1410	1553, 1300
+	_	1098	921, 1046

_	+	1000	1120, 1046
_	_	688	488, 921

Table 5.20 Calculated and Observed Values (Enterococci)

Condition (T)	**Calculated Values**	**Observed Values**
+ (48 Hrs)	142.75	30, 173, 128, 240
- (24 Hrs)	454.85	298.7, 413, 419, 689

Residuals were calculated and normal probability plots were prepared for the residuals (Figure 5.16 and 5.17). From these plots and analyses, it is clear that refrigeration (R) and time- shaking interaction (TS) affect the *E. coli* levels. Only the effect of refrigeration over a period of two days was studied, not for shorter time periods. Refrigeration of samples reduced the die-off rates of *E. coli,* and refrigerated samples showed correspondingly higher levels of *E. coli* compared to samples that were not refrigerated, all as expected. During this research, precautions were taken to minimize the effect of these adverse factors. Samples were always transported from the field to the laboratory in an ice cooler and analyzed as soon as possible to reduce the holding time. All samples were vigorously shaken before analyses.

In the case of enterococci, only the holding time had a significant affect for the test conditions examined. The longer the holding time, the lower the enterococci levels, as expected. Refrigeration and shaking had a reduced effect on the measured levels for the test conditions. As previously noted, all samples were analyzed within a few hours of sample collection.

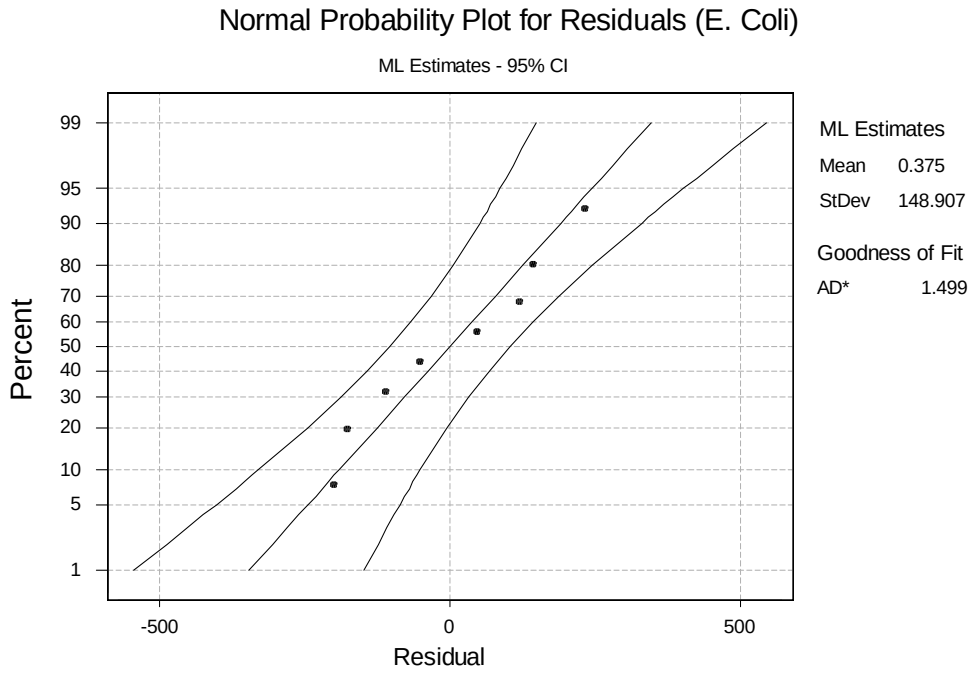

Figure 5.16 Normal Probability Plot for Residuals (*E. coli*)

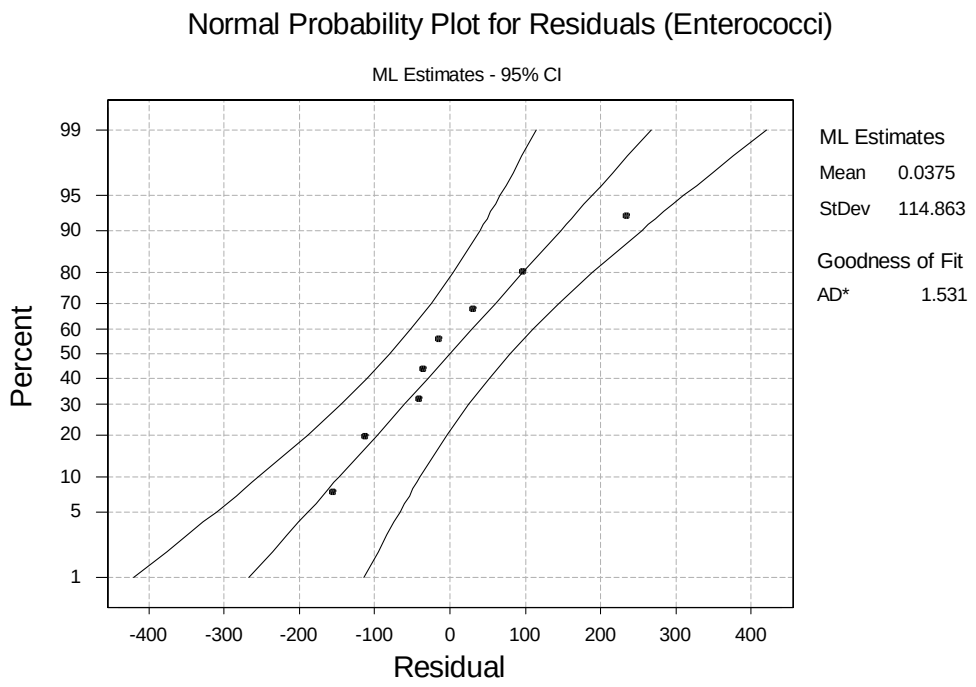

Figure 5.17 Normal Probability Plot for Residuals (Enterococci)

5.2.3 Comparison of Sewage Data With Wet Weather and Dry Weather Data The secondary objective of this study was to find how *E. coli* and enterococci could be effectively used to identify sources of inappropriate discharges in storm drainage systems. For this purpose, sewage samples were compared with wet weather and dry weather source area samples (from the project reference sample library). The most important comparison was between sewage samples collected during wet weather and wet weather urban runoff source area samples. Mann Whitney tests were conducted using MINITAB and probability (p-values) calculated to identify significant differences in the data sets.

Bacteria levels were originally measured in sewage samples collected from the Tuscaloosa wastewater treatment plant by dilution to 0.01% sewage. Calculations were then conducted to determine bacteria levels in 0.05, 1, 1.5, 2, and 5 and so on up to 100

% sewage mixtures. Runoff data from each source area were compared with the calculated values for every dilution ratio. The probability of the sewage and source area sample bacteria levels being significantly different was determined using the Mann Whitney test. Figures 5.18 and 5.19 are plots showing the resultant p-value and percentage sewage dilution. When the values of the probabilities were ≤ 0.05, the diluted sewage sample bacteria levels were determined to be significantly higher as compared to bacterial levels in the urban runoff source area samples (with a 1 in 20 error level). *E. coli* levels in diluted sewage start showing significantly higher values ($p \leq 0.05$) as compared to urban runoff (compared to streets prone which had the highest *E. coli* values) at 0.13% sewage in clear water (Figure 5.18). The mean value of *E. coli* corresponding to 0.13 % sewage in clear water is 3470 MPN/100 mL. Thus, if the *E. coli* levels found from a storm drain outfall exceed 3470 MPN/100 mL during wet weather, the only likely source (with a 1 in 20 error level) is sewage contamination (other possible contaminating sources have significantly lower bacteria levels).

Similarly, enterococci levels in sewage start showing significantly higher values as compared to urban runoff source area samples (from Open spaces-NP which had the highest values) at 3.7% and higher sewage in clear water (Figure 5.19). The mean value of enterococci corresponding to 3.7% sewage in clear water is 18,530 MPN/100 mL. Thus, if the enterococci levels found at a storm drain outfall exceed 18,530 MPN/100 mL during wet weather, the high bacteria levels are most likely from sewage contamination. Lower bacteria levels at the outfalls are likely from urban animals, or sewage diluted further than these levels.

Similar plots and analyses were made between reference library samples (collected during dry weather) and percentage sewage in clear water (Figures 5.20 and 5.21). Dry weather outfall samples having *E. coli* and enterococci levels equal to or higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL respectively, are most likely contaminated by sanitary sewage. Based on these observations and analyses, the earlier simple flow chart developed by Lalor (1994) to identify the most significant component of flow from an outfall has been modified, as shown in Figure 5.22.

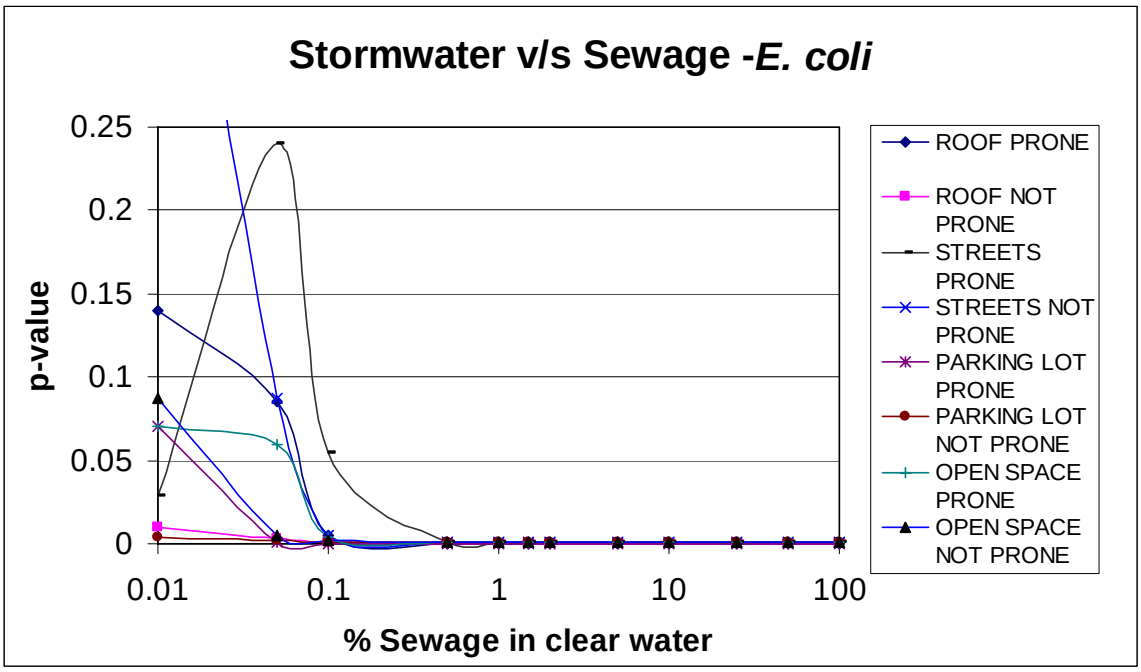

Figure 5.18 Comparison of Sewage with Wet Weather Data (*E. coli*)

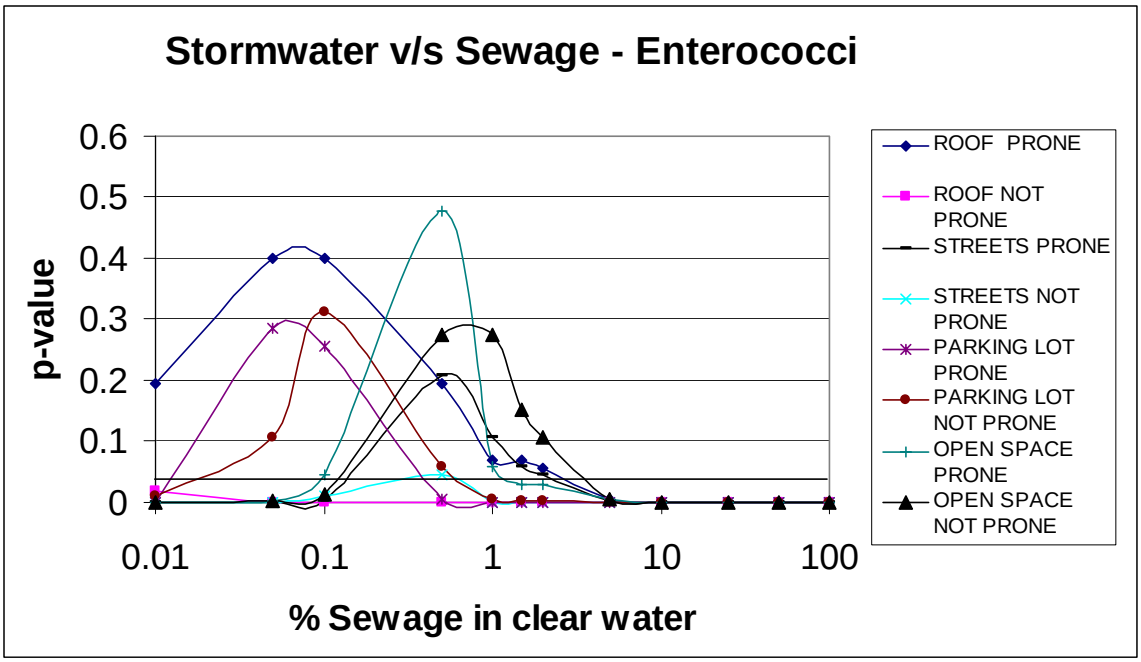

Figure 5.19 Comparison of Sewage with Wet Weather Data (Enterococci)

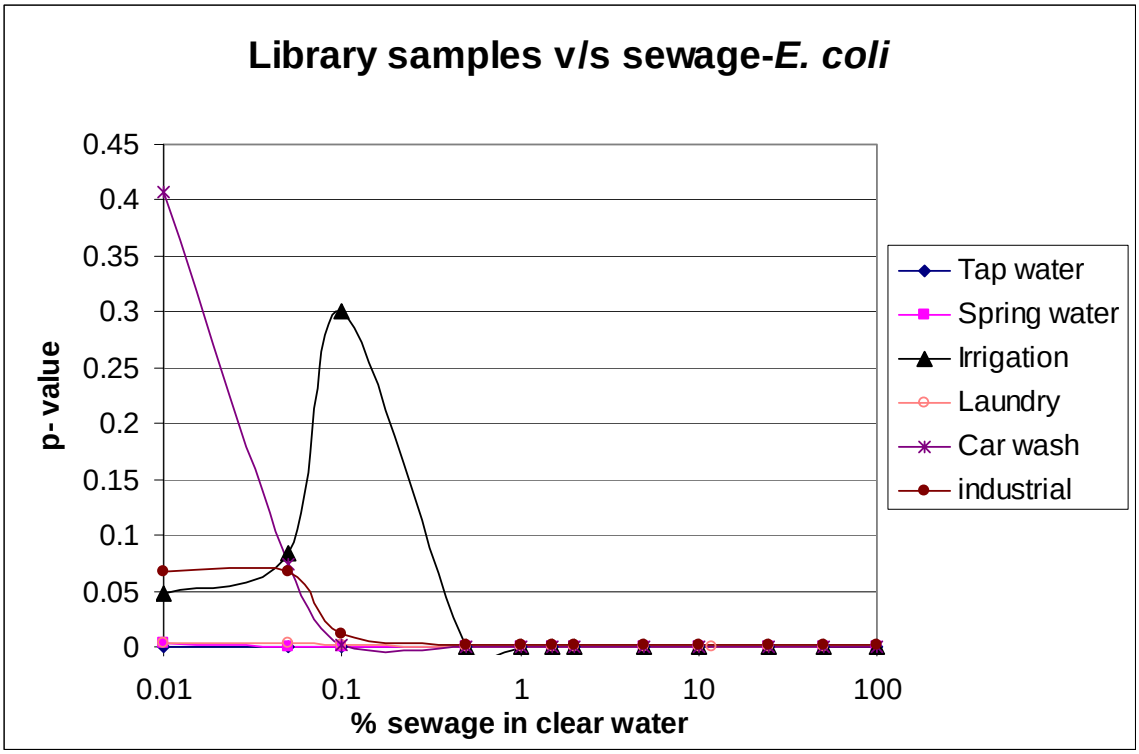

Figure 5.20 Comparison of Sewage with Dry Weather Data (*E. coli*)

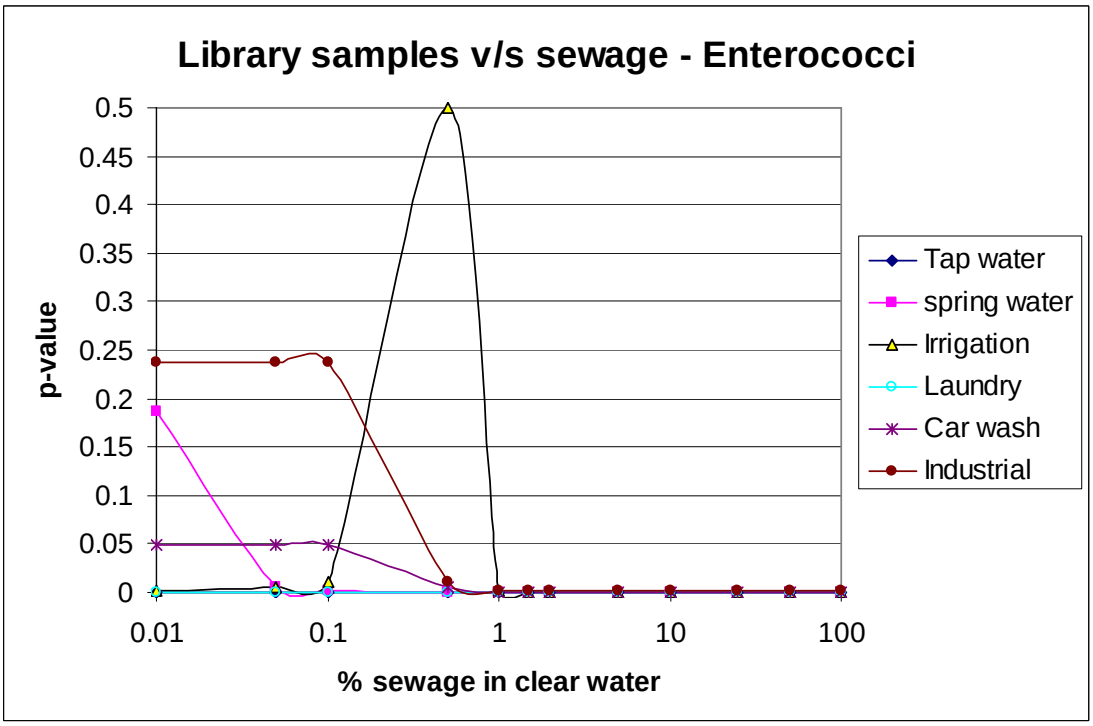

Figure 5.21 Comparison of Sewage with Dry Weather Data (Enterococci)

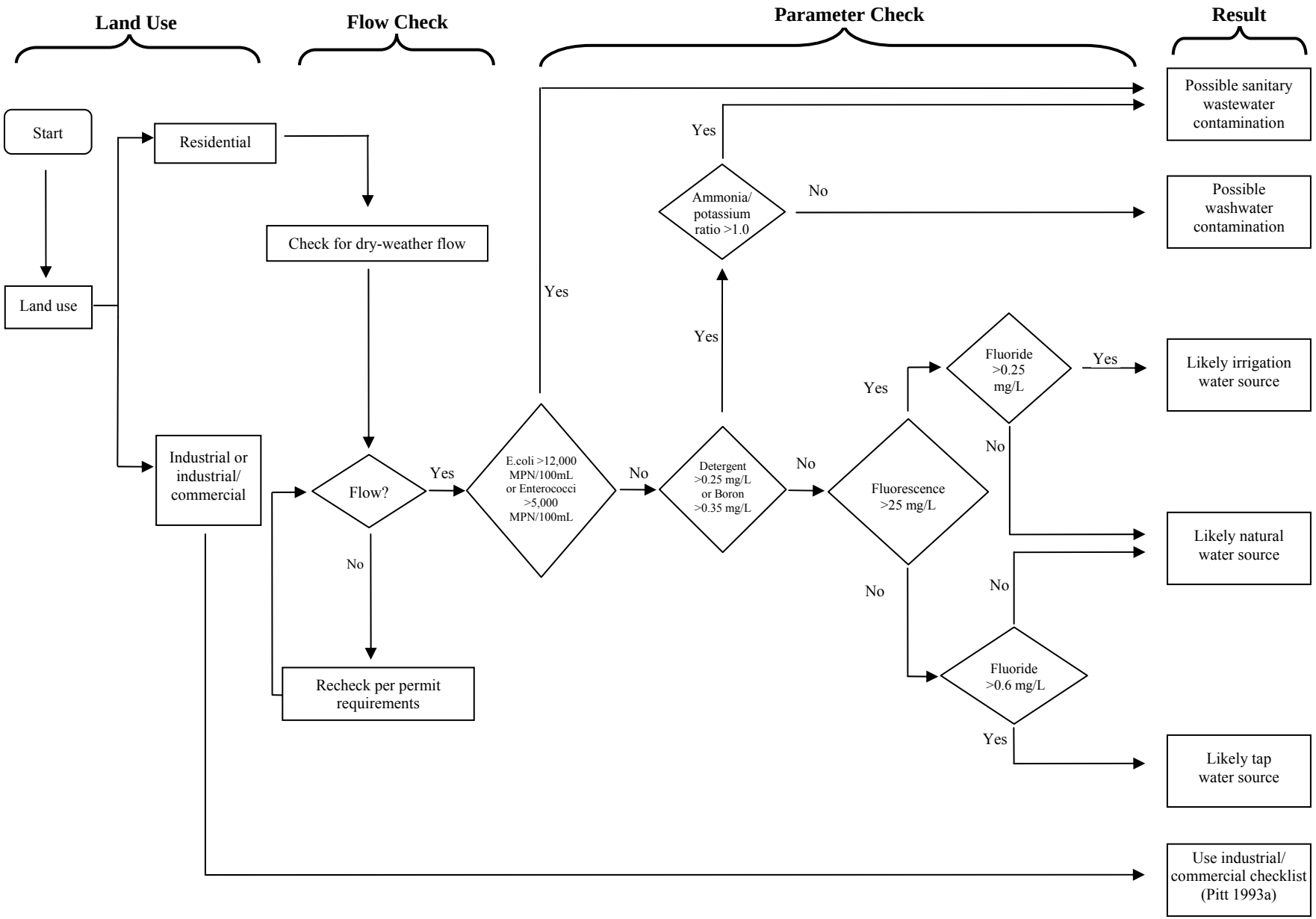

Figure 5.22 Modified Flow Chart to Identify Most Significant Flow Component

CHAPTER VI

CONCLUSIONS AND FUTURE RECOMMENDED STUDIES

6.1 Conclusions

The main objective of this research was to identify possible sources of *E. coli* and enterococci bacteria in dry and wet weather flows. All of the eight sites sampled periodically during wet weather for bacteria analyses were sheetflow samples from various source areas. None could possibly be contaminated with sanitary sewage. Even then, *E. coli* and enterococci levels higher than 2,400 and 24,000 MPN/100 mL, respectively, have been observed in wet weather samples, although the maximum value varied from site to site. The levels of indicator bacteria present in the urban runoff source area samples exceeded the EPA 1986 water quality criteria (single sample max value) in 31% (*E. coli*) and 74% (enterococci) of the samples and the geometric mean criteria was exceeded in 100% of the source area samples. The presence of high levels of bacteria in wet weather samples (both sheetflows and at outfalls) show that apart from sewage, there exist other potential sources that contribute to elevated levels. Since both the indicator organisms studied (*E. coli* and enterococci) are not of soil origin and are found in

intestines of warm-blooded animals, urban birds and other animals can be considered significant sources of bacteria in stormwater.

Comparisons of samples collected from areas prone to urban animal use and those that are not, show that large overlaps exist between the bacterial concentrations found from both types of areas. Bacterial levels from roofs prone to urban animal use (squirrels and birds) were significantly higher than from roofs not exposed to such use. The other source areas did not show any significant difference between areas prone and not prone to urban animal use, except for some street areas. This could be the result of persistence of bacteria in soil, or due to mixing of runoff from other areas affecting the source areas tested.

The secondary objective of this study was to find how *E. coli* and enterococci could be effectively used for identifying sources of inappropriate discharges in storm drainage systems. Until now, no set levels were available to determine if the discharges from an outfall were likely contaminated by sewage on the basis of indicator organism levels. Many believe that the presence of any indicator bacteria indicates the likely presence of sanitary sewage, especially if they exceed the regulatory standard levels. It was found during this research that the dry-weather outfall samples showing *E. coli* and enterococci levels higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL respectively, are likely contaminated by sanitary sewage. Levels lower than this are most likely caused by other sources, such as irrigation runoff, carwash water, laundry water, etc.

Some of the other findings of the research are:

- Bacteria levels in urban areas are not source limited, i.e. measured bacteria levels did not decrease with increasing amounts of rain, or even with increasing rain intensities. The levels may increase, or decrease, somewhat with time, but stayed generally level (Figure 5.5 and 5.6).
- Bacterial levels are higher in summer months than during colder winter months.
- The ratio of *E. coli* /enterococci varied greatly for all conditions.
- Wet weather samples have mostly higher enterococci levels than *E. coli* while dry weather source area samples (such as springs and irrigation runoff) have higher *E. coli* levels that enterococci levels.
- Both the indicators followed the same general trend for every site; i.e. both *E. coli* and enterococci levels increase or decrease simultaneously, although by different amounts.
- Sewage samples need vigorous agitation before analyses to break up the lumps of fecal matter in which bacteria are present.
- Samples must be kept refrigerated and analyzed shortly after sample collection. Samples a day old and unrefrigerated can be expected to have decreased bacteria levels compared to chilled and fresh samples.

6.2 Recommendations for Future Research

Additional research is needed to refine the results of this study. Due to limitations of time and manpower, the number of samples that could be taken was limited, especially during the winter months (December through February). Since there are some variations for different times within storms, it is recommended that sampling efforts collect as many samples as possible during each storm and try to sample more events. The number of sample pairs required to evaluate the differences between prone and not prone sites is given in Table 6.1 and 6.2. The numbers of sample pairs required per season can be calculated using the following equation (Burton and Pitt, 2002).

Number of sample pairs required, $n = 2 [(Z_{1-\alpha} + Z_{1-ß})/(\mu_1 - \mu_2)]^2 \sigma^2$

Values used in equation were

α = False positive rate (0.05)

ß = False negative rate (0.2)

$Z_{1-\alpha}$ = Z score corresponding to (1- α) (1.645)

$Z_{1-ß}$ = Z score corresponding to (1- ß) (0.847)

Table 6.1 Number of Sample Pairs Required for *E. coli*

Site	**Means*** **(μ_1, μ_2)**	**COV**	**μ_1-μ_2**	**n**
Roof- P	611.5	1.25	558.1	8
Roof- NP	53.4	2.01		
Streets – P	1020.1	0.89	553.3	22
Streets – NP	466.8	1.15		
Parking lot - P	318.2	1.33	143	119
Parking lot - NP	175.2	2.61		
Open Spaces- P	486.9	1.88	91.5	1041
Open Spaces- NP	395.3	1.92		

* Values calculated by replacing <1 by 0.5 and and removing >2419.2

Table 6.2 Number of Sample Pairs Required for Enterococci

Site	Means* (μ_1, μ_2)	COV	μ_1-μ_2	n
Roof- P	5103.6	1.8	5083.3	11
Roof- NP	20.2	1.3		
Streets – P	6789.2	0.9	5853.2	5
Streets – NP	936	0.5		
Parking lot - P	341.2	0.6	- 1282.7	22
Parking lot - NP	1623.9	1.9		
Open Spaces- P	4565.1	1.6	180.5	14191
Open Spaces-	4384.6	1.0		

* Values calculated by replacing <1 by 0.5 and and removing >2419.2

As the difference in means of two data sets goes decreases, more sample pairs are required to detect significant differences between them. In case of open spaces it is almost impossible to take so many samples. However, such small differences are seldom important; they would seldom result in different management decisions. During this research, seven to nine sample pairs were collected during the warm months. The number of sample pair requirement is only close in case of roofs and streets (for enterococci). As discussed in Chapter 5, significant differences were observed in both of these cases. Differences of about 50% in bacteria concentrations could be evaluated if about 25 sample pairs were available, a likely reasonable maximum value.

Finally, in order to get the optimum use of the IDEXX methods, it is recommended that all sheetflow, stormwater, and dry-weather samples be diluted by 1/10 to minimize the number of samples exceeding the upper detection limit.

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APPENDIX A

A.1 Library Sampling Locations

Type of Sample	Sample ID	Date	Sampling Location
Tap water	Tap-No.1	5/17/2002	B.B.Commer Hall
	Tap-No.2	5/17/2002	Rose Towers
	Tap-No.3	5/17/2002	H.C.Commer Hall
	Tap-No.4	5/17/2002	REC Centre
	Tap-No.5	5/17/2002	Coleman Coloseum
	Tap-No.6	5/29/2003	MIB (UA)
	Tap-No.7	5/30/2003	Alex Appt.
	Tap-No.8	6/3/2003	Georgas Library(UA)
	Tap-No.9	6/8/2003	Rodgers Library
	Tap-No.10	6/8/2003	Alexander Property Appt.
	Tap-No.11	6/8/2003	Pslidea Court Appt.
	Tap-No.12	6/8/2003	University Plaza Appt.
Spring water	Spring-No.1	9/30/2002	Marrs Spring
	Spring-No.2	10/11/2002	Jack Warner Pkwy
	Spring-No.3	11/3/2002	Marrs Spring
	Spring-No.4	11/3/2002	Jack Warner Pkwy
	Spring-No.5	3/11/2003	Marrs Spring
	Spring-No.6	5/16/2003	Jack Warner Pkwy
	Spring-No.7	5/17/2003	Jack Warner Pkwy
	Spring-No.8	5/18/2003	Marrs Spring
	Spring-No.9	5/30/2003	Marrs Spring
	Spring-No.10	6/3/2003	Marrs Spring
	Spring-No.11	6/3/2003	Jack Warner Pkwy
	Spring-No.12	6/5/2003	Jack Warner Pkwy
Carwash	Carwash-No.1	10/31/2002	Gee's Car Wash-Self Service
	Carwash-No.2	10/31/2002	Texaco Gas Station - Automatic Carwash
	Carwash-No.3	5/16/2003	Chevey Gas Station - Automatic Carwash
	Carwash-No.4	5/17/2003	Self Service Carwash-University Blvd
	Carwash-No.5	5/17/2003	Self Service Carwash-University Blvd
	Carwash-No.6	5/17/2003	Chevey Gas Station – Automatic Carwash
	Carwash-No.7	5/29/2003	Chevey Gas Station-Mcfarland - Automatic Carwash
	Carwash-No.8	6/3/2003	Parade Gas Station (Mcfarland) - Automatic Carwash
	Carwash-No.9	6/3/2003	Stop and Go Self Service Carwash-Skyland
	Carwash-No.10	6/3/2003	Parade Gas Station-Skyland - Automatic Carwash

Contd..

	Carwash-No.11	6/3/2003	Shell Gas Station (Skyland Blvd) - Automatic Carwash
	Carwash-No.12	6/8/2003	Parade Gas Station (Skyland Blvd) - Automatic Carwash
Laundry(Household)	Laundry-No.1	11/3/2002	Renee's House (unknown)
	Laundry-No.2	12/14/2002	Renee's House (unknown)
	Laundry-No.3	5/11/2003	Renee's House (unknown)
	Laundry-No.4	5/11/2003	Renee's House (unknown)
	Laundry-No.5	5/11/2003	Renee's House (unknown)
	Laundry-No.6	5/30/2003	Yukio's apartment (Purex)
	Laundry-No.7	5/31/2003	Yukio's apartment (Purex)
	Laundry-No.8	5/30/2003	Suman (Tide)
	Laundry-No.9	6/3/2003	Yukio's apartment (Purex)
	Laundry-No.10	6/3/2003	Soumya (Tide)
	Laundry-No.11	6/3/2003	Veera (Gain)
	Laundry-No.12	6/8/2003	Sanju (Tide)
Sewage	Sewage-No.1	12/18/2002	Tuscaloosa WWTP (Wet Season)
	Sewage-No.2	1/8/2003	Tuscaloosa WWTP (Wet Season)
	Sewage-No.3	1/15/2003	Tuscaloosa WWTP (Wet Season)
	Sewage-No.4	3/11/2003	Tuscaloosa WWTP (Wet Season)
	Sewage-No.5	5/18/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.6	5/29/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.7	5/30/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.8	6/2/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.9	6/3/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.10	6/4/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.11	6/5/2003	Tuscaloosa WWTP (Dry Season)
	Sewage-No.12	6/6/2003	Tuscaloosa WWTP (Dry Season)
Industrial	Industrial-No.1	12/18/2002	DELPHI (Automotive manufacture),Unknown
	Industrial-No.2	12/18/2002	PECO FOODS (Poultry Supplier), City Water
	Industrial-No.3	12/18/2002	CINTAS (Cooperate uniform manufacture), City Water
	Industrial-No.4	12/18/2002	TAMKO (Roofing Products), Unknown
	Industrial-No.5	1/8/2003	DELPHI (Automotive manufacture),Unknown
	Industrial-No.6	1/8/2003	PECO FOODS (Poultry Supplier), City Water
	Industrial-No.7	1/8/2003	CINTAS (Cooperate uniform manufacture), City Water
	Industrial-No.8	1/8/2003	TAMKO (Roofing Products), Unknown

	Industrial-No.9	1/15/2003	DELPHI (Automotive manufacture),Unknown
	Industrial-No.10	1/15/2003	PECO FOODS (Poultry Supplier), City Water
	Industrial-No.11	1/15/2003	CINTAS (Cooperate uniform manufacture), City Water
	Industrial-No.12	1/15/2003	TAMKO (Roofing Produc
Irrigation Water	Irrigation-No.1	5/16/2003	Furgason Parking (UA) - Run through concrete
	Irrigation-No.2	5/18/2003	B.B. Commer (UA) - Run through concrete
	Irrigation-No.3	5/16/2003	Art Building (UA) - Taken at a little puddle, NO concrete
	Irrigation-No.4	5/19/2003	MIB (UA) - Run through concrete
	Irrigation-No.5	5/30/2003	MIB (UA) - Run through concrete
	Irrigation-No.6	5/30/2003	Art Building (UA) - Taken at a little puddle, NO concrete
	Irrigation-No.7	5/30/2003	Quad(UA) - Taken at a little puddle, NO concrete
	Irrigation-No.8	6/5/2003	MIB (UA) - Run through concrete
	Irrigation-No.9	6/5/2003	MIB (UA) - Taken at a little puddle, NO concrete
	Irrigation-No.10	6/5/2003	Bevil (UA) - Taken at a little puddle, NO concrete
	Irrigation-No.11	6/9/2003	MIB (UA) - Run through concrete
	Irrigation-No.12	6/9/2003	MIB (UA) - Taken at a little puddle, NO concrete

Contd...

A.2 Wet Weather Sampling Results and Data

Table 5.1 in Chapter 5 shows only the *E. Coli* and enterococci levels observed in wet weather samples. The following table also shows the rain characteristics and total coliform data for the wet weather samples.

SAMPLE I.D	DATE SAMPLE TAKEN	TIME SAMPLE TAKEN	TOTAL RAIN BEFORE SAMPLING (INCHES)**	MAX. RAIN RATE BEFORE SAMPLE TAKEN (INCHES/HR)***	TOTAL COLIFORMS MPN/ 100 mL
OPEN SPACE -Prone	21-Sep-02	10.50PM	1.12	2.55	>2419.2
	25-Sep-02	10.30AM	0.61	0.69	>2419.2
	25-Sep-02	4.40PM	1.12	0.74	>2419.2
	10-Oct-02	NT*	NT	NT	NT
	27-Oct-02	NT	NT	NT	NT
	5-Nov-02	11.25A.M	0.78	2.13	>2419.2
	29-Jan-03	8.20PM	0.99	1.36	>2419.2
	6-Feb-03	4.00PM	0.19	0.39	>2419.2
	6-Feb-03	4.00PM	0.19	0.39	>2419.2
	24-Apr-03	2.35PM	NA****	NA	>2419.2
	12-Jun-03	3.15PM	NA	NA	>2419.2
	27-Jun-03	12.35PM	NA	NA	>2419.2
	21-Sep-02	NT	NT	NT	NT
	25-Sep-02	10:20AM	0.59	0.69	>2419.2

	25-Sep-02	6.00PM	1.17	0.74	>2419.2
	10-Oct-02	NT	NT	NT	NT
	27-Oct-02	NT	NT	NT	NT
OPEN SPACE	15-Oct-02	7.30 PM	1.14	0.76	2419.2
– Not Prone	5-Nov-02	12.40P.M	1.02	2.13	>2419.2
	29-Jan-03	8.40 PM	1	1.36	>2419.2
	6-Feb-03	5.40PM	0.33	0.39	>2419.2
	24-Apr-03	3.30PM	NA	NA	>2419.2
	14-May-03		NA	NA	>2419.2
	12-Jun-03	3.30PM	NA	NA	>2419.2
	27-Jun-03	12.45PM	NA	NA	>2419.2
	21-Sep-02	3:47PM	0.64	2.55	>2419.2
	25-Sep-02	9.22AM	0.51	0.69	>2419.2
	25-Sep-02	4.30PM	1.12	0.74	>2419.2
	10-Oct-02	6.45PM	0.1	0.05	>2419.2
	27-Oct-02	7.45 PM	0.09	0.3	>2419.2
PARKING LOT	5-Nov-02	12.00A.M	0.95	2.13	>2419.2
– Not Prone	29-Jan-03	9.25PM	1.02	1.36	Contd...
	6-Feb-03	3.50PM	0.16	0.27	1299.7
	24-Apr-03	2.20PM	NA	NA	>2419.2
	14-May-03		NA	NA	>2419.2
	12-Jun-03	NT	NA	NA	NT
	27-Jun-03	12.10PM	NA	NA	>2419.2
	21-Sep-02	4.02PM	0.65	2.55	>2419.2
	25-Sep-02	9.45AM	0.53	0.69	>2419.2
	25-Sep-02	4.00PM	1.11	0.74	>2419.2
	10-Oct-02	6.30 PM	0.09	0.05	>2419.2
	27-Oct-02	7.25 PM	0.09	0.3	>2419.2
PARKING LOT	5-Nov-02	12.15PM	0.98	2.13	>2419.2
-Prone	29-Jan-03	11.45 AM	0.25	1.36	290.9
	29-Jan-03	12	0.27	1.36	>2419.2
	29-Jan-03	12.15 PM	0.28	1.36	>2419.2
	6-Feb-03	3.25PM	0.8	0.14	191.8
	24-Apr-03	2PM	NA	NA	>2419.2
	14-May-03		NA	NA	>2419.2
	12-Jun-03	NT	NA	NA	NT
	27-Jun-03	12PM	NA	NA	>2419.2
	29-Aug-02	4:55 PM	0.14	1.48	>2419.2
	21-Sep-02	3:35 PM	0.63	2.55	1986.3
	25-Sep-02	9.00AM	0.5	0.69	>2419.2
	25-Sep-02	5.40PM	1.16	0.74	>2419.2
	10-Oct-02	7:20 PM	0.13	0.13	>2419.2

ROOF- Prone	27-Oct-02	9.00 PM	0.11	0.3	>2419.2
	5-Nov-02	11.35A.M	0.87	2.13	>2419.2
	29-Jan-03	9.20PM	1.02	1.36	146.4
	6-Feb-03	4.15PM	0.22	0.39	25.6
	24-Apr-03	2.45PM	NA	NA	>2419.2
	14-May-03	NT	NA	NA	NT
	12-Jun-03	3PM	NA	NA	>2419.2
	27-Jun-03	12.20PM	NA	NA	>2419.2
ROOF- Not Prone	29-Aug-02	5:00 PM	0.17	1.48	>2419.2
	21-Sep-02	3:37PM	0.63	2.55	980.4
	25-Sep-02	8.45 AM	0.48	0.69	>2419.2
	25-Sep-02	4.50PM	1.12	0.74	>2419.2
	10-Oct-02	7.00 PM	0.12	0.13	>2419.2
	27-Oct-02	8.00 PM	0.09	0.3	>2419.2
	5-Nov-02	11.20 AM	0.76	2.13	1299.7
	29-Jan-03	8.15PM	0.99	1.36	920.8
	6-Feb-03	4.07PM	0.2	0.39	>2419.2
	24-Apr-03	2.40PM	NA	NA	>2419.2
	14-May-03		NA	NA	>2419.2
	12-Jun-03	3.10PM	NA	NA	>2419.2
	27-Jun-03	12.30PM	NA	NA	>2419.2
	21-Sep-02	3:57PM	0.64	2.55	>2419.2
	25-Sep-02	9.40AM	0.52	0.69	>2419.2
	25-Sep-02	4.20PM	1.12	0.74	Contd...
	10-Oct-02	6.40 PM	0.09	0.05	>2419.2
STREET- Prone	27-Oct-02	7.40 PM	0.09	0.3	>2419.2
	5-Nov-02	11.50A.M	0.91	2.13	>2419.2
	29-Jan-03	NT	NT	NT	NT
	6-Feb-03	3.45PM	0.14	0.14	>2419.2
	24-Apr-03	2.10PM	NA	NA	>2419.2
	14-May-03		NA	NA	>2419.2
	12-Jun-03	NT	NA	NA	NT
	27-Jun-03	12.05PM	NA	NA	>2419.2
STREET - Not Prone	21-Sep-02	NT	NT	NT	NT
	25-Sep-02	9.17AM	0.51	0.69	>2419.2
	25-Sep-02	4.35PM	1.12	0.74	>2419.2
	10-Oct-02	6.50 PM	0.11	0.11	>2419.2
	27-Oct-02	7.50PM	0.09	0.3	>2419.2
	5-Nov-02	11.45A.M	0.91	2.13	>2419.2
	29-Jan-03	9.35PM	1.02	1.36	>2419.2
	6-Feb-03	3.55PM	0.17	0.39	>2419.2
	24-Apr-03	2.30PM	NA	NA	>2419.2

	14-May-03		NA	NA	>2419.2
	12-Jun-03	NT	NA	NA	NT
	27-Jun-03	12.15PM	NA	NA	>2419.2

* Not tested - Unable to sample
** Gap of 8 hrs between rains considered as new event
*** 5 Min peak intensity
**** Data not available

A.3 Results of Bacterial Analyses of Dry Weather Samples

OUTFALL #	**SAMPLE #**	**Date**	**Total Coliform MPN/ 100 mL**	***E. Coli* MPN/ 100 mL**	**Enterrococci MPN/ 100 mL**	**PROBLEM INDICATED BY INDICATOR ORGANISMS***
1	S001041702	4/17/2002				
	S01021803	2/18/2003	866.4	206.3	1	NO
	S01033103	3/31/2003	>2419.2	>2419.2	8	**YES**

3	S003041702	4/17/2002				
	S003053102	5/31/2002				
	S003100902	10/9/2002	>2419.2	>2419.2	6	**YES**
	S03021803	2/18/2003	275.5	143.9	6.2	NO
	S03033103	3/31/2003	1413.6	325.5	48.3	NO
3a	S03a053102	5/31/2002				
	S03a100302	10/3/2002	>2419.2	130.1	3	NO
	S03a021803	2/18/2003	>2419.2	166.4	28.5	NO
	S03a033103	3/31/2003	>2419.2	29.2	36.3	NO
3b	S003b053102	5/31/2002				
	S003b100302	10/3/2002	>2419.2	5.2	<1	NO
	S3b021803	2/18/2003	139.6	18.5	<1	NO
	S03b033103	3/31/2003	980.4	111.9	98.8	NO
3c	S003c053102	5/31/2002				
	S003c100302	10/3/2002	<1	<1	<1	NO
	S3c021803	2/18/2003	<1	<1	<1	NO
	S03c033103	3/31/2003	<1	<1	<1	NO
	S003d060402	6/4/2002				
	S003d100902	10/9/2002	>2419.2	272.3	142.3	NO
	S3d021903	2/19/2003	344.8	160.7	39.1	NO
3e	S003e060402	6/4/2002				
	S3e021903	2/19/2003	148.3	3.1	<1	NO
	S03e040103	4/1/2003	57.3	1	<1	NO
4	S004042202	4/22/2002				
	S004060402	6/4/2002				
	S004100902	10/9/2002	>2419.2	12.1	17.9	NO
	S04021903	2/19/2003	290.9	3	1	NO
	S04040103	4/1/2003	387.3	1	2	NO
5	S005042202	4/22/2002				
	S005060402	6/4/2002				
	S05021903	2/19/2003	344.8	12.1	10.8	NO
	S05040103	4/1/2003	1553.1	3.1	4.1	NO
9	S009042202	4/22/2002				
10a	S010a060602	6/6/2002				
12	S012042502	4/25/2002				
	S012d060602	6/6/2002				
23	S023042502	4/25/2002				
	S023061002	6/10/2002				
24	S024042502	4/25/2002				
	S024061002	6/10/2002				
26a	S026a061102	6/11/2002				
	S26a021903	2/19/2003	1299.7	387.3	23.5	NO
27	S027042602	4/26/2002				

Contd...

	S027061102	6/11/2002				
	S027101402	10/14/2002	>2419.2	1203.3	100.8	NO
	S27022403	2/24/2003	435.2	63.1	5.2	NO
	S27041703	4/17/2003	>2419.2	410.6	3	NO
27a	S027a101402	10/14/2002	>2419.2	172.3	11.6	NO
	S27a041703	4/17/2003	>2419.2	547.5	21.8	NO
28	S28022403	2/24/2003	547.5	224.7	7.3	NO
29a	S029a101402	10/14/2002	>2419.2	2419.2	116	NO
	S29a030403	3/4/2003	140.1	12.2	<1	NO
31	S031042602	4/26/2002				
	S031061702	6/17/2002				
	S031101402	10/14/2002	>2419.2	125	10.7	NO
	S31030403	3/4/2003	488.4	68.3	<1	NO
	S31a041703	4/17/2003	770.1	33.6	9.2	NO
31a	S31a041703	4/17/2003	>2419.2	60.5	33.2	NO
33	S033042602	4/26/2002				
	S033d061702	6/17/2002	1			
	S33030403	3/4/2003	140.1	3	2	NO
	S33041703	4/17/2003	>2419.2	5.2	21.3	NO
36	S036042602	4/26/2002	1			
	S036061902	6/19/2002				
	S036101702	10/17/2002	>2419.2	61.3	3	NO
	S36030503	3/5/2003	>2419.2	1	3.1	NO
	S36a041703	4/17/2003	>2419.2	22.8	25.3	NO
37a	S037a061902	6/19/2002				
	S037a101702	10/17/2002	>2419.2	24.3	2	NO
	S37a030503	3/5/2003	76.6	1	12	NO
	S37a041703	4/17/2003	290.9	1	4.1	NO
38	S038042902	4/29/2002				
	S038062402	6/24/2002				
	S038101702	10/17/2002	>2419.2	866.4	>2419.2	**YES**
39	S039042902	4/29/2002				
	S039062402	6/24/2002				
	S039101702	10/17/02	>2419.2	178.9	112.4	NO
	S39030503	3/5/2003	178.9	<1	1	NO
	S39041703	4/17/2003	>2419.2	38.6	12	NO
45	S045050802	5/8/2002				

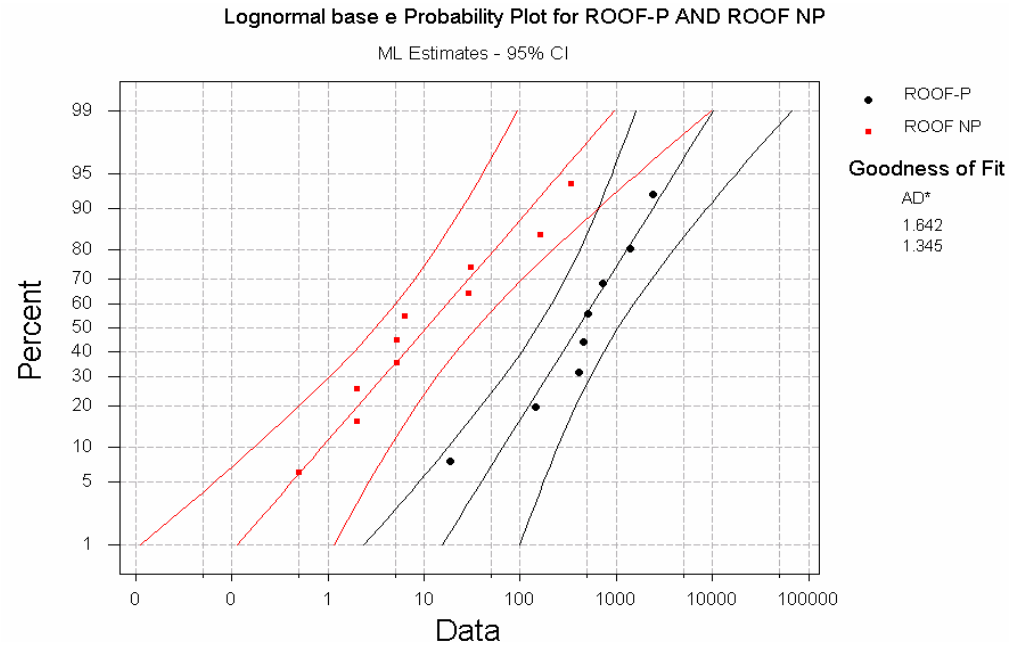
Contd...

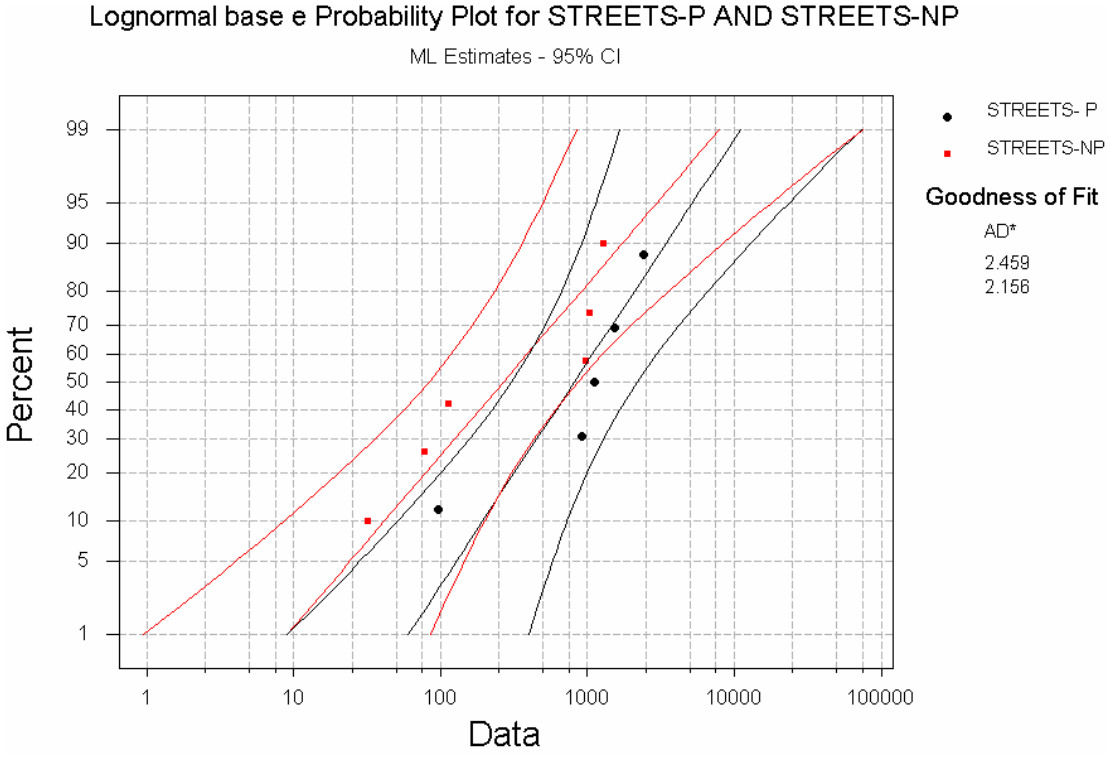
	S045d062402	6/24/2002				
	S045101802	10/18/02	>2419.2	37.4	3.1	NO
	S45030503	3/5/2003	>2419.2	74.4	14.6	NO
			980.4	8.6	<1	NO
49	S049050802	5/8/2002	1			
	S049062402	6/24/2002				
	S49041803	4/18/2003	325.5	<1	<1	NO
53	S053050802	5/8/2002				
	S053062402	6/24/2002				
	S053101802	10/18/2002	>2419.2	16.6	18.7	NO
	S53030503	3/5/2003	488.4	<1	<1	NO
	S55041803	4/18/2003	1413.6	1	<1	NO
55	S055050802	5/8/2002				
	S055d062402	6/24/2002				
	S055101802	10/18/2002	>2419.2	2419.9	727	NO
	S55030503	3/5/2003	>2419.3	1	12.2	NO
	S55041803	4/18/2003	>2419.2	307.6	10.5	NO
61	S061101802	10/18/2002	>2419.2	124.6	228.2	NO
65	S065101802	10/18/2002	>2419.2	307.6	172.3	NO
66	S066051002	5/10/2002				
73	S073051002	5/10/2002				
	S073070203	7/2/2002				
Creek sample I	CS01101402	10/14/2002	>2419.2	410.6	4.1	NO
Creek Sample II	CS02101802	10/18/2002	>2419.2	686.7	517.2	NO
Pond Entrance I	PS01100902	10/9/2002	>2419.2	579.4	113.3	NO
	PS01021903	2/19/2003	2	<1	<1	NO
			>2419.2	261.3	2	NO
Pond Entrance II	PS02101402	10/14/2002	29.2	2	3	NO
			2419.2	240	3.1	NO
Pond Outlet	PS03101402	10/14/2002	1986.3	47.5	<1	NO
	PS03021903	2/19/2003	>2419.2	275.5	4.1	NO
			2419.2	21.8	<1	NO

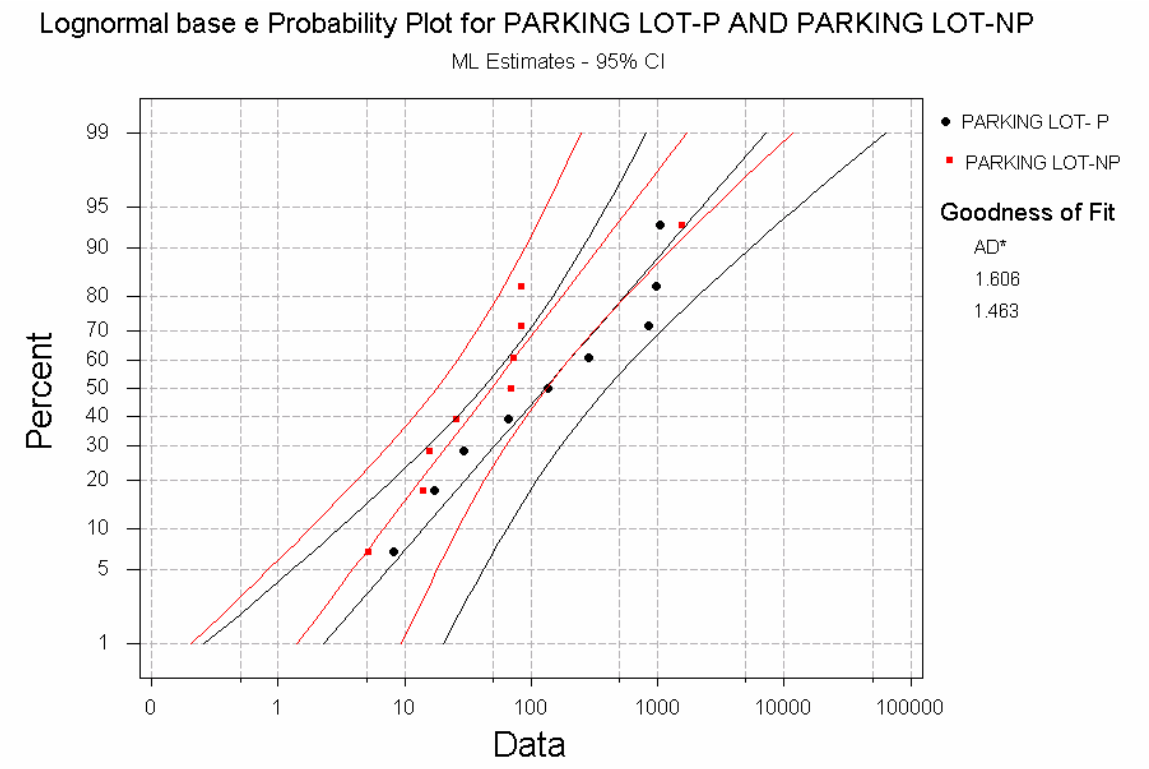
***** An outfall is considered a problem outfall if the bacteria levels exceeded the observed limits shown in the flow chart (Figure 5.22)

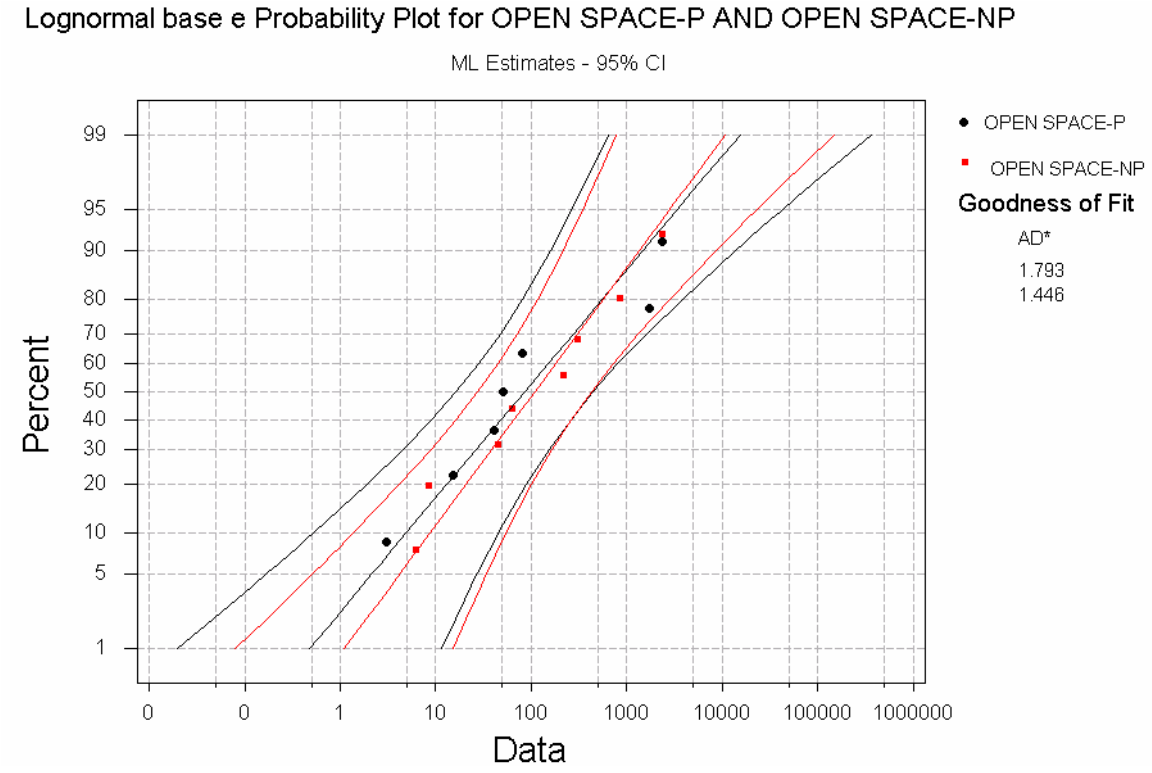
A.4 Normal Probability Plots

To make sure that the populations have the same shape, over-laying probability plots were made for prone and not prone data. Following four probability plots are for *E. Coli* data.

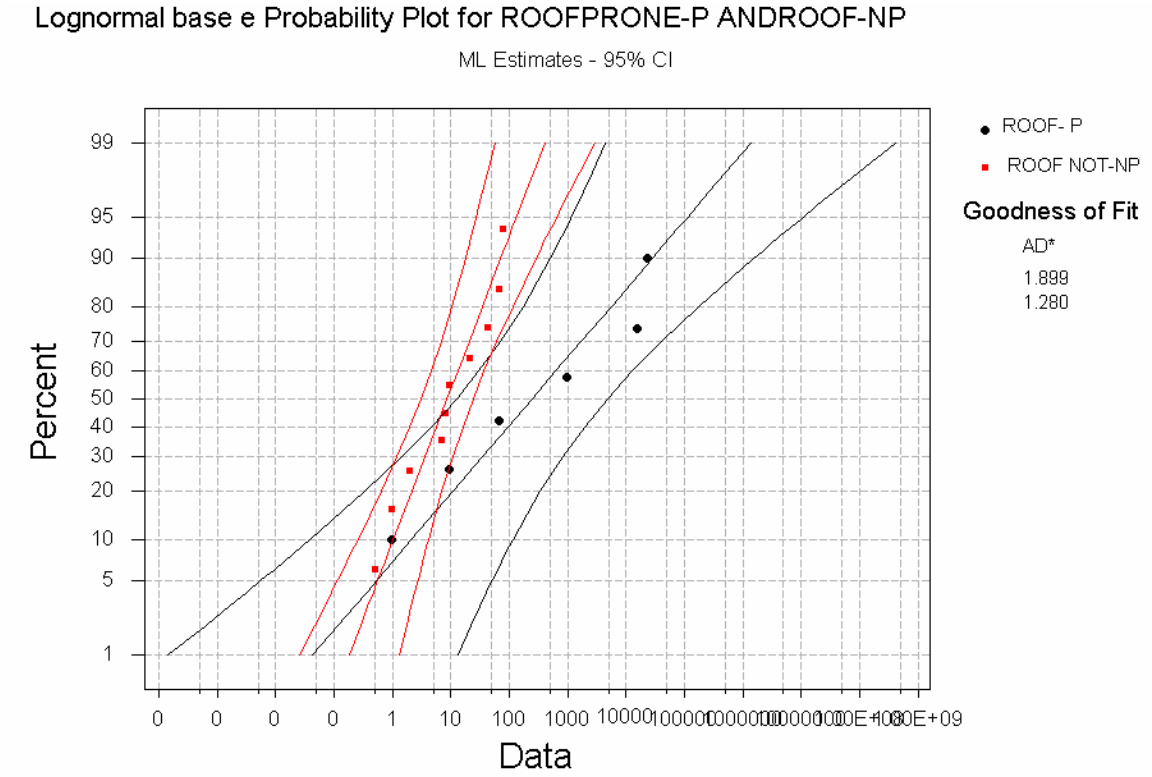


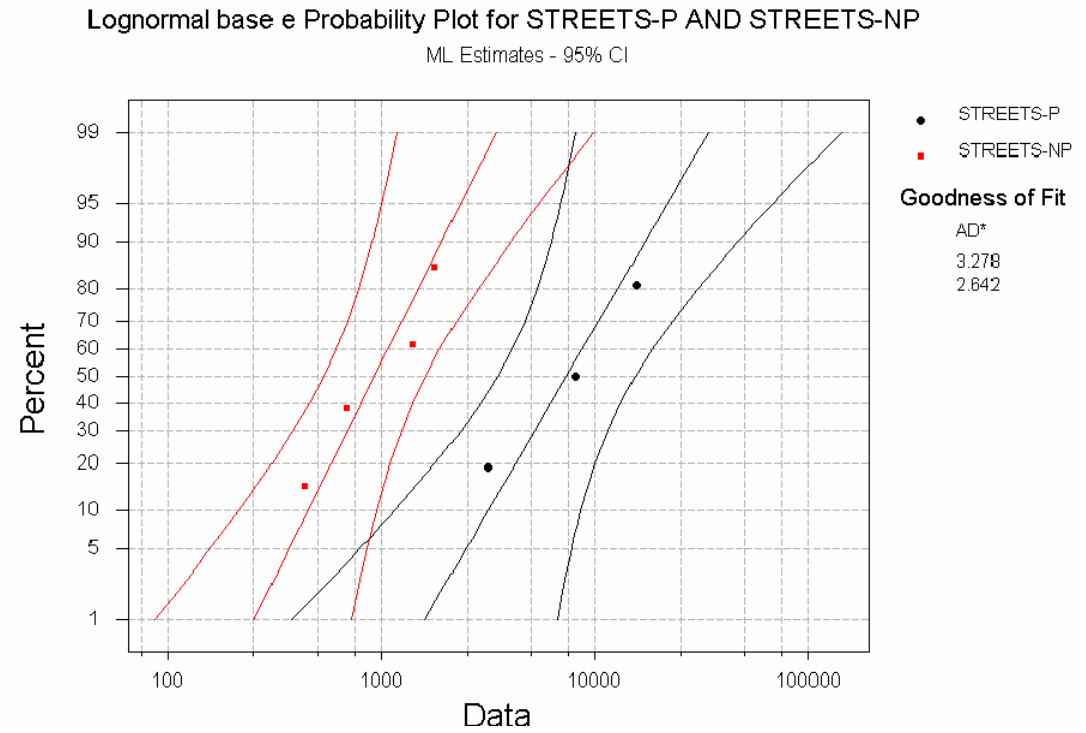

Lognormal base e Probability Plot for STREETS-P AND STREETS-NP
ML Estimates - 95% CI
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
1
10
100
1000
10000
100000
Data
STREETS- P
STREETS-NP
Goodness of Fit
AD*
2.459
2.156

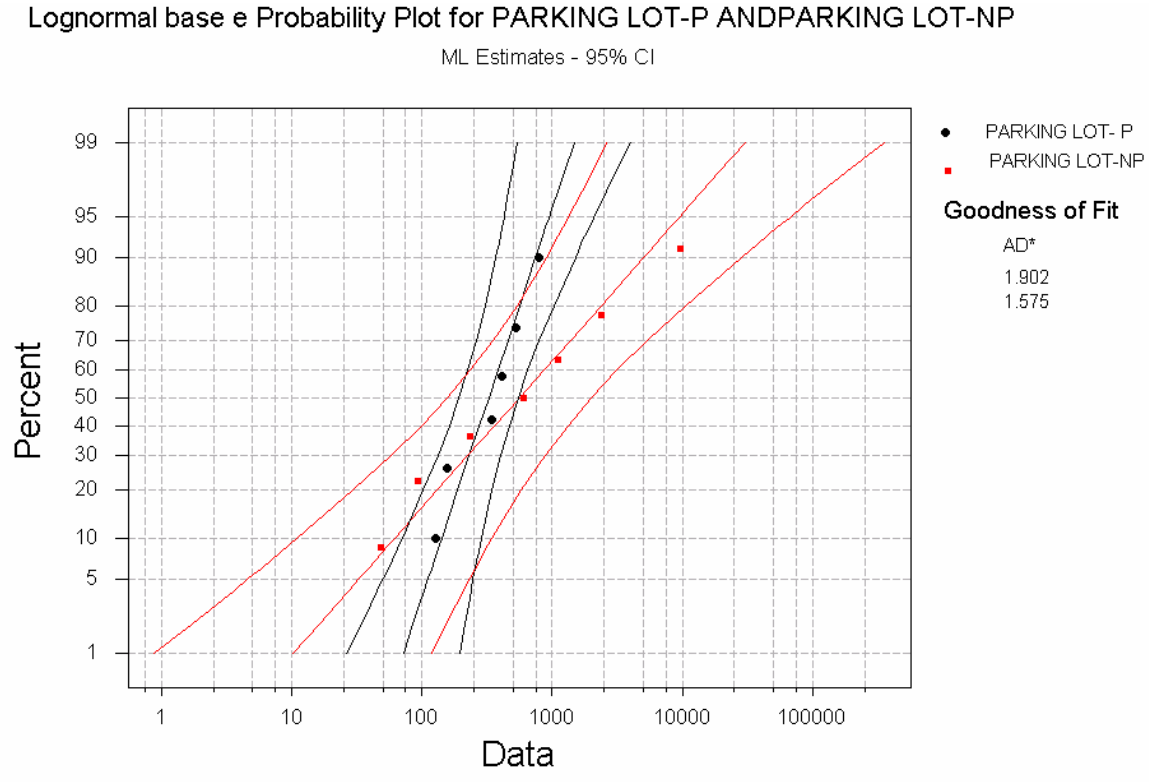

Lognormal base e Probability Plot for PARKING LOT-P AND PARKING LOT-NP
ML Estimates - 95% CI
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
0
1
10
100
1000
10000
100000
Data
PARKING LOT- P
PARKING LOT-NP
Goodness of Fit
AD*
1.606
1.463

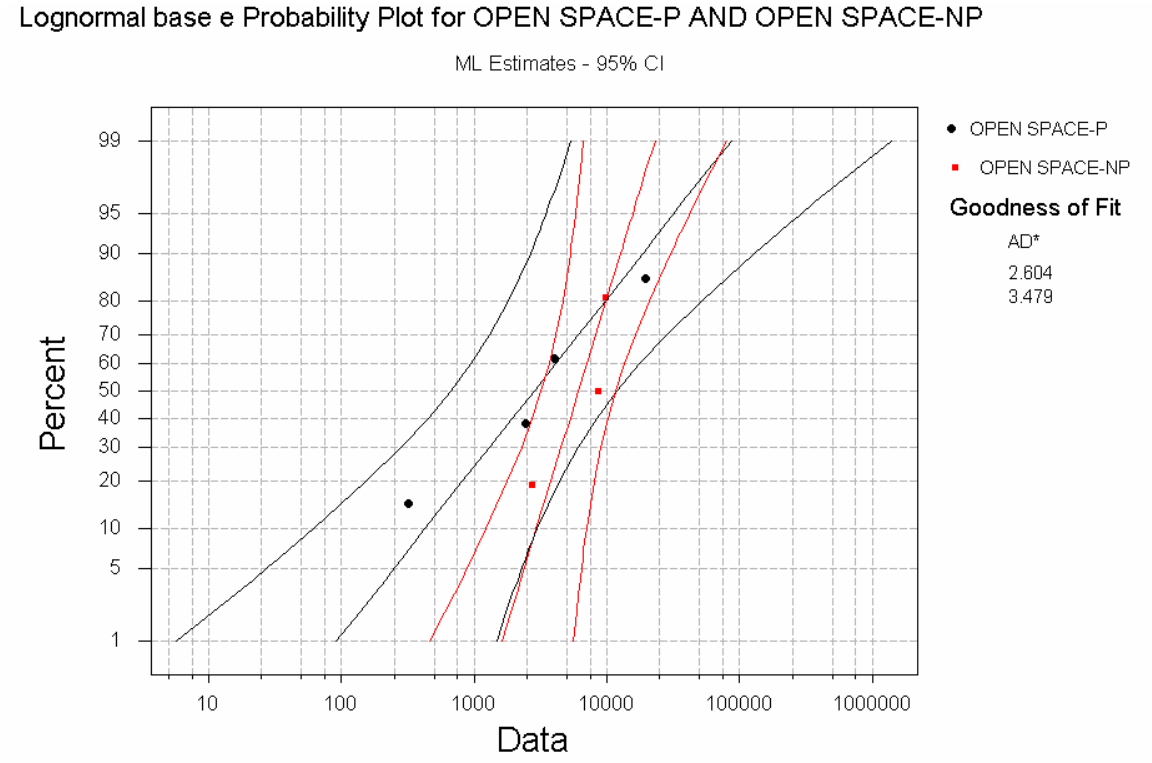


Enterococci - Following four probability plots are for enterococci data.


Lognormal base e Probability Plot for ROOFPRONE-P ANDROOF-NP
ML Estimates - 95% CI
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
0
0
0
0
1
10
100
1000
Data
ROOF- P
ROOF NOT-NP
Goodness of Fit
AD*
1.899
1.280


Lognormal base e Probability Plot for STREETS-P AND STREETS-NP
ML Estimates - 95% CI
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
100
1000
10000
100000
Data
STREETS-P
STREETS-NP
Goodness of Fit
AD*
3.278
2.642


Lognormal base e Probability Plot for PARKING LOT-P ANDPARKING LOT-NP
ML Estimates - 95% CI
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
1
10
100
1000
10000
100000
Data
PARKING LOT- P
PARKING LOT-NP
Goodness of Fit
AD*
1.902
1.575



A.5 Permission letter

You have my permission to use the pictures for the sole purposes disclosed below.

Sincerely

Elisabeth L. Perry

Manager, Business Communications

IDEXX Laboratories, Inc.

One IDEXX Drive

Westbrook, Maine USA

PH: 207-856-8348

FX: 207-856-0319

Mobile: 207-329-1488

-----Original Message-----

<sherg001@bama.ua.edu> -----

Date: Wed, 15 Oct 2003 13:59:16 -0500

From: "Sumandeep S. Shergill" <sherg001@bama.ua.edu>

Reply-To: "Sumandeep S. Shergill" sherg001@bama.ua.edu

Subject: Permission to publish pictures and notes.

To: betsy-perry@idexx.com

Hi Betsy,

I need to use some procedural notes (which are enclosed in packs of reagents sold) and copy 6 pictures from your web site for putting it in my thesis only .Following are there details.

1) 4 pictures showing how Colilert uses the patented Defined Substrate Technology® (DST®) to simultaneously detect total coliforms and E. coli. The url is

http://www.idexx.com/Water/Products/colilert/science.cfm

2) 2 pictures showing how Enterolert uses Defined Substrate

Technology® (DST®) nutrient-indicator to detect enterococci. The url is http://www.idexx.com/water/products/enterolert/science.cfm

I shall be thankful if you allow me to do so. Please mention your designation in your reply .

Sumandeep Shergill