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MICHAEL J. LANG

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**A STUDY FOCUSED ON THE RISK OF ILLNESS FROM *E. COLI* IN
RECREATIONAL-USE WATER, USING THE RED CEDAR WATERSHED AS A
MODEL**

By

Michael J. Lang

A DISSERTATION

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ABSTRACT

A STUDY ON THE RISK OF ILLNESS FROM *E. COLI* IN RECREATIONAL-USE WATER, USING THE RED CEDAR WATERSHED AS A MODEL

By

Michael J. Lang

With news reports of deaths from the ingestion of *E. coli* over the past few years, there is heightened public awareness of the risk of illness from *E. coli* from swimming and other recreational uses of surface water. Locally, the *State News* has run stories on the high *E. coli* levels that have been occasionally reported from the weekly sampling of the Red Cedar on the MSU campus. In addition, high *E. coli* levels have lead to unsafe conditions in the Clinton River and Lake St. Clair and cost the State of Michigan \$2.5 million in testing to determine the sources of the contamination. Nationwide, a majority of bodies of water that are used for recreational purposes, are considered to have *E. coli* counts above acceptable levels. In developing nations, 90% of untreated sewage from urban areas is dumped into streams and oceans causing unsafe conditions in both recreational-use water and drinking water due to high *E. coli* levels. The sources of *E. coli* are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow. Runoff from heavy rains may impact *E. coli* levels in nearby surface water.

In this study, data collected through a collaboration of State agencies, county health departments and MSU resources were analyzed and linked to land use and physical characteristic of the watershed, such as rainfall and nutrient concentrations, using statistical methods. In addition, a small number of samples from the Red Cedar

were analyzed for both total *E. coli* and pathogenic *E. coli* levels using the traditional methods, as well as, a new innovative method.

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LIST OF ABBREVIATIONS

AFO	Animal Feeding Operations
AGNPS	Agricultural Non-Point Source
AU	Animal Units
BEACH	Beach Environmental Assessment Closure and Health Act
BMP	best management practices
CAFO	Concentrated Animal Feeding Operation
CFU	Colony Forming Unit
CSO	Combined Sewer Overflow
CWA	Clean Water Act
DAEC	Diffusely Adherent <i>E. coli</i>
DMF	N, N Dimethylformamide
<i>E. coli</i>	<i>Escherichia coli</i>
EAEC	Enteraggregative <i>E. coli</i>
EHEC	Enterohemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EPA	Environmental Protection Agency
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
g	grams
GIS	Global Information Systems
HUS	Hemolytic Uremic Syndrome
ICHD	Ingham County Health Department
IMS	Immunomagnetic separation
MDCH	Michigan Department of Community Health
MDEQ	Michigan Department of Environmental Quality
ml	milliliters
MSU	Michigan State University
NC	Nitrocellulose
NLCD	National Land Cover Data
NPDES	National Pollution Discharge Elimination System
NRDC	National Resources Defense Council
NWS	National Weather Service
PBS	phosphate buffer saline
PCR	Polymerase Chain Reaction
PFGE	Pulsed-field gel electrophoresis
POTW	Publicly Owned Treatment Works
SSO	Sanitary Sewer Overflows
TMDL	Total Maximum Daily Load
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USPHS	United States Public Health Services
USGS	United States Geological Services
WHO	World Health Organization
WWTP	Waste Water Treatment Plant

Chapter 1

Introduction and Overview

Introduction

With news reports of deaths from the ingestion of *E. coli* over the past few years, there is heightened public awareness of the risk of illness from *E. coli* from swimming and other recreational uses of surface water. Locally, the *State News* has run stories on the high *E. coli* levels that have been occasionally reported from the weekly sampling of the Red Cedar on the MSU campus (Johnson 2003; Cynecki 2003). In addition, high *E. coli* levels have lead to unsafe conditions in the Clinton River and Lake St. Clair and cost the State of Michigan \$2.5 million in testing to determine the sources of the contamination (Morris 2002). Nationwide, a majority of bodies of water that are used for recreational purposes, are considered to have *E. coli* counts above acceptable levels (Dorfman 2002). In developing nations, 90% of untreated sewage from urban areas is dumped into streams and oceans causing unsafe conditions in both recreational-use water and drinking water due to high *E. coli* levels (Crossette 1996). The sources of *E. coli* are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow (USEPA 1986 and 2000). Runoff from heavy rains may impact *E. coli* levels in nearby surface water.

A causal connection between fecal coliform and gastrointestinal sickness was first identified in 1953. (Stevenson 1953). In the late 1970s, in a landmark prospective cohort study, reported a linear relationship between the incidence of gastroenteritis among swimmers and marine bacterial counts (Cabelli 1982). There has a large body of

literature involving the epidemiology of illness from the use of recreational water. An excellent review of this body of literature has been published which included 37 studies. This review concluded that a causal dose-related relationship between gastrointestinal symptoms and recreational water quality measured by bacterial indicator counts exists (Pruss 1998).

E. coli O157 was first reported in the United States in 1982, when it was associated with a multi-state outbreak of hemorrhagic colitis that was traced to hamburger from a restaurant chain (Riley 1983; Tarr 1995). Exposure through ingestion of *E. coli* O157:H7 can cause severe bloody diarrhea and abdominal cramps although in some cases the infection causes non-bloody diarrhea. Usually little or no fever is present, and the illness resolves in 5 to 10 days. The clinical diagnosis of this illness is haemorrhagic colitis. In some people, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail (Moake 1994; CDC 2001).

Table 1-1. *E. coli* O157:H7 major outbreaks in the news.

Timeframe	Location	Company	Vector for Infection
1982	Washington State	Jack in the Box	Hamburger
1982	Michigan	Small cider mills	Cider
June 1997	Nationwide	Hudson Meats	Hamburger
May 2000	Walkerton, Ont.	Municipal water	Drinking water
August 2000	Washington, NY	State Fair	Drinking water
July 2002	Nationwide	ConAgra	Hamburger

The original intent of the research was to perform a risk assessment for illness from *E. coli O157:H7* to humans when using the Red Cedar River for recreational purposes. Since there are no dose response data for illness caused by *E. coli O157:H7* in recreational use water or even from ingestion via contaminated food, epidemiological studies on illness from *E. coli O157:H7* in recreational use water were to be collected and meta-analysis used to determine the dose response for *E. coli O157:H7* in recreational use water. Unfortunately, there is not enough data from the few epidemiological studies that have been reported (see Chapter 2). Since the data that was collected in this study was *E. coli* concentration levels, a relationship between *E. coli* concentration and *E. coli O157:H7* concentration was necessary. In an extensive literature search, no such relationship has been reported or estimated. Since the original goals of the research were not possible, the research questions were changed so that some of the identified holes in the reported literature could possibly be filled by this study. Results from this study may provide mechanisms for improved identification of potential risk factors for illness from *E. coli* in recreational-use water and may provide a solution for the real-time communication of such risks.

Research Questions

1. Are there physical measurements that are taken in a watershed that maybe indicators of an increased risk to humans using recreational water from exposure form *E. coli*?
2. Does of the type of land use surrounding the sampling point impact change in the reported physical measurement that are taken in the Red Cedar River watershed?

3. Do the levels of *E. coli* O157:H7 in recreational water in the Red Cedar River follow the levels of the indicator organism that is monitored and regulated?
4. What is the risk to humans using in recreational water from pathogenic *E. coli* O157:H7 in the Red Cedar River?

Overview

In this study, data collected through a collaboration of State agencies, county health departments and MSU resources were analyzed and linked to land use and physical characteristic of the watershed, such as rainfall and nutrient concentrations, using statistical methods. In addition, a small number of samples from the Red Cedar were analyzed for both total *E. coli* and pathogenic *E. coli* levels using the traditional methods, as well as, a new innovative method. This dissertation is set up so that chapters 2 through 5 are stand-alone manuscript written for submission to appropriate journals.

Chapter 2 entitled “A Review of the Impact of *E. coli* and *E. coli* O157:H7 on Human Health in a Watershed: Risk Factors, Risk Assessment Tools, Epidemiological Evidence, and Public Policy” is an extensive literature review of *E. coli* contamination in recreational use water and the effect on human health. Many of the references in this review are also used in later chapters. This chapter is written with the intent to submit it as a manuscript to Epidemiological Reviews.

Chapter 3 entitled “The Effect of Rainfall, River Flow and other Meteorological Aspects on *E. coli* Levels in the Red Cedar River” has the following objectives. An objective of this study is to determine if there is a seasonal effect on the concentration levels of *E. coli* in the Red Cedar River watershed. Another objective is to determine if

there is relationship between the flow rate of the river and concentration levels of *E.coli*. In addition, an objective of this study is to determine the statistical significance of concentration levels of *E.coli* as related to the watershed physical characteristics of rainfall, air temperature, water temperature, intensity of the sun, humidity, soil moisture, ground temperature, wind speed, duck population, as well as river flow. Finally, an objective of this study is to use the statistical data to model risk factors so that water safety can be determined by the changes in physical characteristics which may be determinable in real time. It is hypothesized, based on the results reported in the literature, that the model risk factors are directly related to rainfall and associated factors. This chapter is written with the intent to submit it as a manuscript to Journal of Environmental Quality.

Chapter 4 entitled “The Effect of Rainfall, Nutrient Levels, and Land-use on *E. coli* Levels in the Red Cedar River” has the following objectives. The objective of this study is determine if the type of land-use around a sampling point is associated with elevated *E. coli* concentration levels. Another objective of this study is to determine if the type of land-use around a sampling point is associated with elevated levels of nutrients. In addition, an objective of this study is determine if there is any association between the concentration levels of nutrients and the concentration levels of *E. coli*. It is hypothesized that agricultural land-use has the highest discharges of *E. coli* and nutrients and therefore has a greater negative impact to the Red Cedar River than other types of land-use. This chapter is written with the intent to submit it as a manuscript to Journal of American Water Resources.

Chapter 5 entitled “A Linkage between Technology and Policy in Evaluating the Risk of Microbial Safety in Recreational-use Water” has the following objectives. The main focus of this project is to improve the safety of recreational-use water through better communication of the actual risk. An objective is to determine if rain or river flow has an influence on the concentration of *E. coli* and *E. coli* O157:H7. Another objective is to evaluate if total *E. coli* measured maybe used as an indicator of pathogenic *E. coli* O157:H7 contamination. In addition, an objective is to evaluate if a biosensor maybe employed to measure quickly the presence of *E. coli* and *E. coli* O157:H7. It is hypothesized that a linear relationship will exist between *E. coli* concentrations and *E. coli* O157:H7 concentrations. This chapter is written with the intent to submit it as a manuscript to Environmental Health Perspectives

Table 1.2. Collaborators on Research.

- Ingham County Health Department
- Livingston County Health Department
- Michigan Department of Environmental Quality
- Michigan Department of Community Health
- MSU Physician’s office
- MSU Biosensor Lab
- MSU WATER Human Health Subcommittee
- MSU Department of Crop and Soil Sciences
- East Lansing WWTP

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USEPA Human Health Risk Assessment Guidelines (1986), and supplement (August 2000)

Chapter 2

A Review of the Impact of *E. coli* and *E. coli* O157:H7 on Human Health in a Watershed: Risk Factors, Risk Assessment Tools, Epidemiological Evidence, and Public Policy

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Introduction

Escherichia coli

Risk Factors for Illness in Recreation-use Water

- Physical Characteristics of a Watershed
- Agricultural Animals and Activities
- Other Animals Reservoirs

Causal Connection of Illness and Agriculture

- Human Sewage
- Causal Connection of Illness and Sewage

Tools for *E. coli* Risk Assessment

- Modeling of the Watershed
- Detection Methods for *E. coli*
- Risk Assessment Models

Epidemiological Evidence

- Indicator Bacteria
- E. coli* O157:H7

Public Policy

Conclusions

References

Introduction

Beach closings and illness after exposure to marine water may be increasing in frequency (Harvell 1999; Harvard Med School 1998)). In the United States from 1988 to 1994, there were over 12,000 coastal beach closings and advisories (an increase of 400% over that period), with over 75% of the closings due to microbial contamination (Barton 1995). A recent report compiled by the Natural Resources Defense Council (NRDC), which surveyed more than 200 waterfront communities, found that during 1999 there were at least 6,160 beach days of closings and advisories at beaches (Dorfman 2000). According to NRDC's twelfth annual beach report, at least 13,410 closings and advisories were issued across the country in 2001, a 19 percent jump over the previous year (Dorfman 2002). Although not all cases can be traced to anthropogenic discharges, the belief that marine pollution has no significant impact on health (Carson 1951; Moore 1959) must now be challenged. Infectious diseases and toxin-related illness may be caused by enteric pathogens or chemicals that enter the marine environment from terrestrial ecosystems (e.g., through fecal contamination from a number of point and diffuse sources) (Weiskel 1996). Alternatively, indigenous organisms and their associated biotoxins that may have increased in number or virulence as a result of ecological imbalance (Epstein 1993; Smayda 1993) can cause negative health effects. Anthropogenic inputs to the coastal environment may be contributing to both terrestrial and marine stress (Epstein 1993).

There is a large body of literature involving the illness caused by *E. coli* strains in water. In the early 1980s, *E. coli* O157:H7 was recognized as a pathogen (Riley 1982).

The pathogenicity to humans from this pathogen has been reported to be as low as 10 cells (Phillips CA 1999). There are an estimated 73,000 cases of *E. coli O157* infections per year in the United States, of which approximately 62,000 are food-borne and 11,000 are waterborne (Mead PS et al.1999). These estimations consider waterborne cases from ingestion of water as food not from recreational use of a waterbody. Examples of waterborne cases include Walkerton, Ontario, where the town's water supply was contaminated by *E. coli O157:H7* (not included in the estimations because it happened in Canada in the year 2000) (Nikiforuk 2000; Brooke 2000; Wickens 2000) and Washington County fair in the state of New York (Bopp 2003). Although there are an estimated 73,000 cases of *E. coli O157* infections per year in the United States since 1982 (CDC 2000), reports of clinical cases from *E. coli O157:H7* are less than ten worldwide.

Escherichia coli

Escherichia coli (*E. coli*) is the type species of the genus *Escherichia*, which contains mostly motile rod-like gram-negative bacilli within the family *Enterobacteriaceae* and the tribe *Escherichia*. *E.coli* bacteria live in the digestive systems of humans and other warm-blooded animals. *E. coli* can be found in the fecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls (Hancock 2001; Niemis 1991).

The organism typically colonizes the infant gastrointestinal tract within hours of life, and, thereafter, *E. coli* and the host derive mutual benefit (Drasser 1974). *E. coli* usually remains harmlessly confined to the intestinal lumen; however, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal

"nonpathogenic" strains of *E. coli* can cause infection. Most strains of the *E. coli* bacteria are not dangerous, but they can indicate the presence of other disease-causing bacteria.

There are a variety of sources that contribute bacteria and other pathogens to the surface water. These sources include illicit waste connections to storm sewers or roadside ditches, septic systems, combined and sanitary sewer overflows, storm (rain) runoff, wild or domestic animal waste, and agriculture runoff.

E. coli O157 was first reported in the United States in 1982, when it was associated with a multi-state outbreak of hemorrhagic colitis that was traced to hamburger from a restaurant chain (Riley 1982; Tarr 1985). The current teachings suggest that there are six categories of pathogenic *E. coli* as described in Table 2-1.

Table 2-1. Categories of Pathogenic *E. coli* (adapted from Nataro and Kater 1998)

Acronym	Name	Associated Clinical Syndromes	Examples of Serotypes
ETEC	Enterotoxigenic <i>E. coli</i>	Traveler's diarrhea	
EPEC	Enteropathogenic <i>E. coli</i>	Watery diarrhea of infants	
EHEC	Enterohemorrhagic <i>E. coli</i>	Hemolytic-uremic syndrome (HUS)	O157:H7
EAEC	Enteraggregative <i>E. coli</i>	Persistent diarrhea	O127:H2
EIEC	Enteroinvasive <i>E. coli</i>		
DAEC	Diffusely Adherent <i>E. coli</i>		

Of these six, two categories, EIEC and DAEC, are considered "emerging pathogens." There is very little documentation on these categories and the identification of these strains has been limited to infants in less developed countries. In addition, these emerging strains have not been identified in the developed countries. For the other categories, three of these, ETEC, EPEC, and EAEC, have doses that cause illness in

humans that are far above the standards of total *E. coli* that is acceptable for “safe” human recreational use of surface water. If the waterbody has levels of *E. coli* below the regulated standard then there is no risk from these three categories of pathogenic *E. coli*. The final category of pathogenic is EHEC which includes the famous serotype *O157:H7*. The infectious dose for this strain has been estimated in the literature to be anywhere from 1000 organisms to just 10 organisms (Philips 1999). These levels are far below the total *E. coli* standard that is acceptable for “safe” human recreational use of surface water. No literature references are available for what the percentage of EHEC in a total *E. coli* measurement from surface water may be, therefore, a huge gap in the literature exists and research is necessary to help answer these questions.

Among the most important virulence characteristics of *E. coli O157* is its ability to produce one or more Shiga toxins also called verocytotoxins and formerly known as Shiga-like toxins. The first of these, Shiga toxin 1 (Stx1) is indistinguishable from Shiga toxin produced by *Shigella dysenteriae* type 1 (Nataro and Kaper 1998). The second, Shiga toxin 2 (Stx2) is a more divergent molecule with only 56% amino acid homology with Stx1. Most *E. coli O157* strains produce Stx2 with the percentage that also produce Stx1 ranges from less than 25% in series from Europe (Thomas 1996) to greater than 80% in a series from North America (Cimolai 1994; Slusker 1997) and Japan (NIH 1996).

Shiga toxin infection often causes severe bloody diarrhea and abdominal cramps although in some cases the infection causes non-bloody diarrhea. Usually little or no fever is present, and the illness resolves in 5 to 10 days. The clinical diagnosis of this illness is haemorrhagic colitis. Ground beef and other bovine products have often been

implicated as sources, along with other food products (Ackers 1998, Besset 1993; Hilborn 1999; Michino 1999; Tamblyn 1999; Watanabe 1996) and person-to-person transmission (Pavia 1990; Bender 1997). Occasional outbreaks have also been associated with public drinking water (Swerdlow 1992) and swimming in contaminated water (Friedman 1999).

In some people, particularly children under 5 years of age and the elderly, the infection can also cause a complication called hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail (Riley 1983; Wells 1983; Moake 1994). About 2%-7% of infections lead to this complication. In the United States, HUS is the principal cause of acute kidney failure in children, and most cases of HUS are caused by *E. coli* O157:H7. HUS is a life-threatening condition usually treated in an intensive care unit. Blood transfusions and kidney dialysis are often required. With intensive care, the death rate for HUS is 3%-5% (CDC 2001). About one-third of persons with HUS have abnormal kidney function many years later, and a few require long-term dialysis. Another 8% of persons with HUS have other lifelong complications, such as high blood pressure, seizures, blindness, paralysis, and the effects of having part of their bowel removed (CDC 2001).

Human infection with *E. coli* O157 has been reported from over 30 countries on six continents. Annual incidence rates of 8 per 100,000 population or greater have been reported in regions of Scotland (Rielly 1997; Coia 1998; Licence 2001), Canada (Waters 1994), and the U.S. (Griffin 1991). High rates may be present in regions of South America, especially in Argentina where HUS has an incidence 5-10 times higher than North America (Lopez 1989; Rivas 1998). Infection rates appear to be rather low in Australia (Fegan 2002).

Risk Factors for Illness in Recreation-use Water

Ever since fecal contamination of water was determined a human health risk, there has always been a great deal of concern regarding the level of coliform bacteria counts in water. Many bodies of water throughout the world are considered to have counts above acceptable levels. The sources of these coliforms are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow (EPA 2000).

Physical Characteristics of a Watershed

Physical characteristics of the watershed are potential risk factors to the levels of *E. coli* and its various strains. The physical factors that will be reviewed in this section include seasonal variability, rainfall, river flow, nutrient, and the rate of survival of bacteria in water.

Over a twelve-year period (1984-1995) a study examined admissions for HUS in Scotland and determined that seasonality was present with the highest amount of admissions in July and August (Douglas 1997). It should be noted that this study was not limited to recreational use of water and showed the seasonal pattern only for patients under 15 years old. In contrast, a study in Florida showed that concentration of fecal indicator organisms during the late fall and early winter months which corresponds to the wet-weather months in Florida (Lipp 2001). In addition the study determined that the levels of fecal indicators were significantly associated with rainfall, stream flow, and temperature. Moreover, a study of two streams in Arkansas over a three year period concluded that concentrations of indicator bacteria increased with increasing flow rates

and seasonal effects were observed on the indicators with the highest levels occurring during the summer months (Edwards 1997). Furthermore in a study of a dairy herd reported that *E. coli* O157:H7 was found in 4.3% of the herd and peaked during the May to July timeframe but was not found in the herd from November to May (Mechie 1997). However a study in northwest England reported that bacteria indicators showed no seasonal variations over a two-year period (Obiri-Danso 1999).

Other studies support the association of the levels of fecal indicators in a water body and rainfall events. In a study that examined 99 samples from three tributaries that contributed to different drinking water reservoirs showed that *E. coli* levels along with other bacteriological parameters increased considerably during extreme rainfall events (Kistemann 2002). In a related study on Delaware River, increased concentrations of *Giardia*, *Cryptosporidium* and other microorganisms were associated with rainfall (Atherholt 1998). In a prospective study on waterborne disease outbreaks in the U.S. for 1948 to 1994, analyzed 548 reported outbreaks as documented by the USEPA database. This results from this study showed that 51% of waterborne disease outbreaks were preceded by precipitation events above the 90th percentile and 67% by events above the 80th percentile (Curriero 2001). This study concluded that there is a statistical significance association between rainfall and waterborne disease outbreaks. Other studies also conclude that *E. coli* levels increase with rainfall (Briski 2000; Ferguson 1996; Atherholt 1998; Pettibone 1996; Niemi 1991; Mallin 2001). A recent study suggests that an increase in rainfall or snowmelt increases the impact of diseases caused by microbiologic agents (Rose 2001).

Increased river flow has been related to increases in the levels of *E. coli* in the river. Early studies in Idaho and Oregon found that fecal coli concentrations were higher during periods of high flow than during period of lower flow (Stephenson 1978; Tiedemann 1988). These finding were confirmed in a study of two rivers in Arkansas which concluded that concentrations of indicator bacteria increased with increasing flow rates (Edwards 1997).

As far as nutrients and their relation to *E. coli* there has not been many reports in the literature. Total suspend solid were strongly correlated to indicator bacteria when flow rates were the highest (Pettibone 1996). In another study, a statistical analysis showed that there was a significant correlations between levels of nitrate as well as levels of phosphate with the indicator bacteria faecal coliform (Daby 2002).

In a recent study, model systems were used to determine the persistence of *E. coli* O157 in river water, cattle faeces, and soil cores (Maule 2000). Survival of the organism was found to be greatest in soil cores containing rooted grass. Under these conditions viable numbers were shown to decline from approximately 10^8 /g soil to between 10^6 and 10^7 /g soil after 130 days. When the organism was inoculated into cattle faeces it remained detectable at high levels for more than 50 days. In contrast the organism survived much less readily in cattle slurry and river water where it fell in numbers from more than 10^6 /ml to undetectable levels in 10 and 27 days, respectively (Maule 2000).

The survival characteristics of a mixture of five nalidixic acid-resistant *E. coli* O157:H7 strains innoculated at 10^3 CFU/ml in filtered and autoclaved municipal water, in reservoir water, and in water from two recreational lakes were determined for a period of 91 days and stored at three different temperatures of 8, 15, or 25 degrees C. Greatest

survival was in filtered autoclaved municipal water and least in lake water. Regardless of the water source, survival was greatest at 8 degrees C and least at 25 degrees (Wang 1998).

In study *E. coli O157* was investigated to determine if it would multiply in a medium containing 5% NaCl and in sterilized marine water. Results indicate that *E. coli O157* could survive in unsterilized marine water for at least 15 days. On the basis of these results, it was postulated that *E. coli O157* may survive in natural marine water (Miyagi 2002).

Environmental survival of *Escherichia coli O157* may play an important role in the persistence and dissemination of this organism on farms. The survival of culturable and infectious *E. coli O157* was studied using microcosms simulating cattle water troughs. Culturable *E. coli O157* survived for at least 245 days in the microcosm sediments (LeJeune 2001). In a study aimed to investigate the survival characteristics of *Escherichia coli O157:H7* in farm water, and in sterile distilled municipal water reported that the organism survived in farm water for over 31 days and in the distilled water for 17 days (McGee 2002).

The survival characteristics of *Escherichia coli O157:H7* in private drinking water wells were investigated to assess the potential for human exposure. A non-toxigenic, chromosomally lux-marked strain of *E. coli O157:H7* was inoculated into well water from four different sites in the North East of Scotland. These waters differed significantly in their heavy metal contents as well as nutrient and bacterial grazer concentrations. Grazing and other biological factors were studied using filtered (3 and 0.2

mum) and autoclaved water. The survival of *E. coli* O157:H7 was primarily decreased by elevated copper concentrations (Artz 2002)

Escherichia coli O157:H7 was inoculated at final concentrations of 10^3 or 10^6 /ml into natural non-carbonated mineral water (MW), sterile natural mineral water (SMW) and sterile distilled deionized water (SDDW) and stored at 15 degrees C for 10 weeks. Samples were examined every 7 d for the presence of *E. coli* O157:H7. There was a significant difference in the survival of *E. coli* O157:H7 (10^3 /ml inoculum) between the MW and the SDDW and between the MW and the SMW with the pathogen surviving longest in the MW samples. In contrast, at 10^6 /ml, no significant differences in the survival of *E. coli* O157:H7 were observed between the water types (Kerr M 1999).

In addition studies have reported that *E. coli* survives and even grows in freshwater and marine sediment (Gerba 1976; Hood 1982; LaLiberte 1982). Sediments may contain 100 to 1000 times as many fecal indicator bacteria as the overlaying water (Ashbolt 1993; Van Donsel 1971). *E. coli* survived over 28 and as long as 68 days in sediment (Davies 1995).

Agricultural Animals and Activities

Animal Feeding Operations (AFOs) are agricultural operations where animals are kept and raised in confined situations. AFOs generally congregate animals, feed, manure, and production operations on a small land area. Feed is brought to the animals rather than the animals grazing or otherwise seeking feed in pastures. Animal waste and wastewater can enter water bodies from spills or breaks of waste storage structures (due to accidents or excessive rain), and non-agricultural application of manure to cropland. AFOs that

meet the regulatory definition of a Concentrated Animal Feeding Operation (CAFO) have the potential of being regulated under the NPDES permitting program. A facility is an AFO if animals are stabled/confined, or fed/maintained, for 45 days or more within any 12-month period, and the facility does not produce any crops, vegetation or forage growth (40 CFR 122.23(b)(1)).

Table 2-2. Definition of a Concentrated Animal Feeding Operation (CAFO)

A CAFO is an AFO which:

- Has more than 1,000 animal units (AU), or
- Has 301 to 1,000 AU and wastes are discharged through man-made conveyance or directly into US waters, or
- Is designated a CAFO by the permitting authority on a case-by-case basis

Animal units ("AUs") are defined in EPA's current regulations at 40 CFR 122 and vary by animal type. An AU is equivalent to one beef cow or 2.5 mature swine or up to 100 chickens. Winter-feeding of animals on pasture or rangeland is not normally considered an AFO.

USDA reports that there were 1.2 million livestock and poultry operations in the United States in 1997 (USDA 1999). This number includes all operations that raise beef or dairy cattle, hogs, chickens (broilers or layers), and turkeys, and includes both confinement and non-confinement (i.e., grazing and ranged) production. Of these, EPA estimates that there are about 376,000 AFOs that raise or house animals in confinement, as defined by the existing regulations.

AFOs (including CAFOs) produce and manage large amounts of animal waste, most in the form of manure. USDA estimates that 291 billion pounds (132 million metric tons) of "as excreted" manure were generated in 1997 from major livestock and poultry

operations (USDA 1999). The scale of this unprecedented outpouring of animal waste is staggering: 130 times the waste generated by humans in this country each year (US Senate Comm. Report 1997).

Recent trends across the U.S. livestock and poultry sectors are marked by a decline in the number of operations due to ongoing consolidation in the animal production industry (MacDonald 2000; McBride 1997). Increasingly, larger, more industrialized, and highly specialized operations now account for a greater share of all animal production. This concentrates more animals, and thus more manure and wastewater, in a single location, and raises the potential for significant environmental damages unless manure is properly handled. USDA reports that there were 1.1 million livestock and poultry farms in the United States in 1997, about 50 percent fewer than the 2.3 million farms reported in 1974 (USDA 1999 and 1976). Since the 1970s, the combined forces of population growth and re-location of operations closer to consumer markets and processing sectors have resulted in more AFOs located near densely populated areas. Surface waters in these areas face additional stresses from urban runoff and other point sources. The proximity of large AFOs to human populations thus increases the potential for human health impacts and ecological damage if manure and wastewater at AFOs is improperly discharged.

The most important animal species in terms of as a vector for human infection is cattle. High rates of colonization of *stx*-positive *E. coli* have been found in bovine herds in many countries (Burens 1995; Clarke 1994; Griffin 1991; Hancock 1994; Wells 1991). These rates are as high as 60% but are more typically in the range of 10 to 25%. *Stx*-producing *E. coli* strains are usually isolated from healthy animals but may be associated

with an initial episode of diarrhea in young animals followed by asymptomatic colonization. The isolation rates of *O157:H7* are much lower than those of non-*O157:H7* serotypes. Surveys of U.S. dairy and beef cattle have found *E. coli O157:H7* in 0 to 2.8% of animals, with the highest isolation rates reported from younger rather than older animals (Hancock 1994; Wells 1991). In 1986, *E. coli O157* was recovered from healthy dairy cows, suggesting that dairy and beef herds could serve as a reservoir (Martin 1986). Subsequent studies have confirmed that *E. coli O157* and other EHEC strains are commonly found in beef and dairy cattle (Martin 1986) as well as animals associated with farm as well as sheep, pigs, goats and chickens (Ogden 2002; Kariuki 1999; Griffin 1991; Strachan 2001).

Environmental survival of *Escherichia coli O157* may play an important role in the persistence and dissemination of this organism on farms. The survival of culturable and infectious *E. coli O157* was studied using microcosms simulating cattle mater troughs. *E. coli O157* strains surviving more than 6 months in contaminated microcosms were infectious to a group of 10-week-old calves (LeJeune 2001). Fecal excretion of *E. coli O157* by these calves persisted for 87 days after challenge. Water trough sediments contaminated with feces from cattle excreting *E. coli O157* may serve as a long-term reservoir of this organism on farms and a source of infection for cattle.

E. coli has been identified in cattle on all continents with the exception of Antarctica. In Asia, the pathogen has been identified in Chinese and Japanese cattle (Zhou 2002; Elliot 2001; Dundas 2000; Tanaka 2000) In Europe *E. coli O157* has found in cattle in the Netherlands, Norway, Finland, Britian, Germany, France, Chezsolvokia, (Beutin 2000; Chalmers 2000; Geue 2002; Heuvelink 1999; Heuvelink 1998; Jones 1999;

Lahti 2001; Osek 1999; Osek 2002; Vermozy-Rozand 2002; Vold 2001). In North America, *E. coli* O157 has been identified in cattle and other farm animals in the United States and Canada, as well as South America (Bielaszewska 2000; Hancock 2001; Hoar 2001; Joseph 2002; Laegreid 1999; License 2001; Mariani-Karkdjian 1999; McClure 2000; Mead 1998; Padhye 1992; Philips 1999; Notario 2000; Rasmussen 2001; Samadpour 2002; Sargeant 2000; Verweyen 2000; Wang 1996; Whipp 1994). *E. coli* O157:H7 is least prevalent in Australia (Elliot 2001; Fegan 2002).

Other Animal Reservoirs

Some studies suggest that elk, deer, ducks, birds and other wild animals may also be a source of *E. coli* O157 (Niemi 1991; Wallace 1997; Feldman 2002; Olson 2002; Samadpour 2002; Rice 2003). Companion animals including cats, dogs, reptiles, and birds have been reported to have *E. coli* and/or *E. coli* O157:H7 in their feces (Beutin 1993; Wallace 1997; Synge 2000; Hancock 2000; Enriquez 2001). In addition fecal samples from 300 zoo animals have been analyzed and only six animals, a horse and five species of primates, were positive for *E. coli* O157:H7 (Bauwens 2000).

Causal Connection of Illness and Agriculture

In 1999, an *E. coli* outbreak occurred at the Washington County Fair in New York State. This outbreak was possibly the largest waterborne outbreak of *E. coli* O157:H7 in U.S. history. It took the lives of two fair attendees and sent 71 others to the hospital. An investigation identified 781 persons with confirmed or suspected illness related to this outbreak. The outbreak is thought to have been caused by contamination of the Fair's

Well 6 by either a dormitory septic system or manure runoff from the nearby Youth Cattle Barn (Bopp 2003; Ackman 1997). More recently, in May 2000, an outbreak of *E. coli* O157:H7 in Walkerton, Ontario resulted in at least seven deaths and 1,000 cases of intestinal problems; public health officials theorize that one possible cause was floodwaters washing manure contaminated with *E. coli* into the town's drinking water well; an investigation is currently underway (Brooke 2000). An outbreak of *E. coli* O157:H7 was reported in Walkerton, Ontario, Canada from well water potentially contaminated by manure runoff (Kluger 1998). Cow manure has specifically been implicated as a causative factor in the high bacteria levels and ensuing swimming restrictions on Tainter Lake, Wisconsin (Behm 1989). Among the many outbreaks reports, studies have been published from outbreaks in Scotland (Coia 1998; License 2001), Missouri (Swerdlow 1992) and Idaho (Vane Every 1995). This is only a small sample of such reports. Investigations in Ontario on well water from a farm has shown a relationship between the indicator in the well water and illness (Jackson 1998; Raina 1999). Finally, studies have been published on the persistence of *E. coli* in a farm environment and the risks of contact and the relation with illness (Rahn 1997; Jones 1999; O'Brien 2001; Locking 2001; Ogden 2002; Strachan 2002).

Human Sewage

Population growth in coastal areas is increasing at a rate double that of population growth worldwide. It is estimated that billions of gallons of treated and untreated wastewater are discharged daily into the world's coastal waters. In developing nations, 90% of untreated sewage from urban areas is dumped into streams and oceans (Crossette

1996). In addition, runoff from heavy rains can worsen water quality. Increased bacterial, viral, and toxin contamination may be associated with watershed pollution, loss of wetlands (which naturally filter out pollutants), and overfishing (which decreases predation). Heavy loadings of organic and inorganic nutrients change the ecological balance, stimulating nuisance organisms (Burkholder 1997) and in some cases affecting the virulence of indigenous species (Bates 1991).

Combined sewer systems are sewers that are designed to collect rainwater runoff, domestic sewage, and industrial wastewater in the same pipe. Most of the time, combined sewer systems transport all of their wastewater to a sewage treatment plant, where it is treated and then discharged to a water body. During periods of heavy rainfall or snowmelt, however, the wastewater volume in a combined sewer system can exceed the capacity of the sewer system or treatment plant. For this reason, combined sewer systems are designed to overflow occasionally and discharge excess wastewater directly to nearby streams, rivers, or other water bodies. These overflows, called combined sewer overflows (CSOs), contain not only storm water but also untreated human and industrial waste, toxic materials, and debris. They are a major water pollution concern for the approximately 772 cities in the U.S. that have combined sewer systems. CSOs may be thought of as a type of “urban wet weather” discharge. This means that, like sanitary sewer overflows (SSOs) and storm water discharges, they are discharges from a municipality's wastewater conveyance infrastructure that are caused by precipitation events such as rainfall or heavy snowmelt. Properly designed, operated, and maintained sanitary sewer systems are meant to collect and transport all of the sewage that flows into them to a publicly owned treatment works (POTW). However, occasional unintentional

discharges of raw sewage from municipal sanitary sewers occur in almost every system, also known as SSOs. SSOs have a variety of causes, including but not limited to severe weather, improper system operation and maintenance, and vandalism. EPA estimates that there are at least 40,000 SSOs each year (USEPA 2002d). The untreated sewage from these overflows can contaminate our waters, causing serious water quality problems. It can also back-up into basements, causing property damage and threatening public health. CSOs and wet weather SSOs contain a mixture of raw sewage, industrial wastewater and storm water, and have resulted in beach closings, shellfish bed closings, and aesthetic problems.

Causal Connection of Illness and Sewage

The possible transmission of infectious disease via primary contact with domestic sewage has been the subject of many published epidemiological studies (including: Stevenson 1953; Moore 1959; Cabelli 1982; Seyfield 1985; Fattel 1987; Ferley 1989; Cheunk 1990; Balarajan 1991; Alexander 1992; Fleisher 1993; Corbett 1993, Fleisher 1996; Butler 1997; Haile 1999; Prieto 2001; Bonadonna 2002; Dwight 2002). In addition, CSOs have been identified as a source of bacteria including *E. coli* (Burm 1966; Burm 1967). In a study of recreational water, it was reported that 34.5% of the gastroenteritis infection were directly linked to domestic sewage in the swimming waters and there was illness even though that water was within the EPA levels as acceptable (Fleisher 1998).

Tools for *E. coli* Risk Assessment

Modeling of the Watershed

Global Information Systems (GIS) have been around since the 1960s but this was mainly a research tool used by the federal government and large research universities. However, this has changed and GIS is available to a much larger audience and has become easier to use. This change was made possible by other technology trends such as increased computer power and graphics in personal computers, improved and robust databases, and increased computer savvy in our current society. Now, GIS is a tool not only for scientists at NASA or a major research institution but rather, is also a tool used by municipal planners on a local level and a skill learned by many at the undergraduate level at many colleges across the country. In addition, the general public can access environmental and watershed data in easy to use GIS formats through the EPA webpages.

GIS combines layers of information about a place to give the researcher a better understanding of that place. What layers are combined depends on the researchers purpose such as: finding the best location of a new store, analyzing environmental damage, viewing similar crimes in a city to detect a pattern, or understanding land use in a watershed. Many examples of watershed GIS data exist on the web such as: the Red Cedar hosted by Michigan State University (MSU 2002), and the Kalamazoo River hosted by Western Michigan University (WMU 2002). The USEPA hosts the “Surf your Watershed” system (USEPA 2002) on which any who accesses this system can find GIS information on any watershed in the United States. These watershed GIS databases, like most, are based on USGS topological data (USGS 2000) to define the area of the

watershed and micro-watersheds with it. In addition, these GIS databases have layers that include waterbodies, roads, cities, and in some cases land use coverage data.

There are many examples of GIS used as a tool in the analysis of watersheds. Moreover, GIS has been used to model the activities and systems of a watershed. Non-point pollution in a watershed has been a natural to model using GIS tools. BASINS designed by the EPA (USEPA 2002b) STREAM (Spatial Tools for River basins, Environment and Analysis of Management options) (Schepel 1998), and SIMPLE (Spatially Integrated Models for Phosphorus Loading and Erosion) (Kornecki 1999) are examples of popular models that have been designed for the evaluation of non-point pollution. Agricultural land-use has been studied for non-point run-off. AGNPS (Agricultural Non-Point Source) is a model designed by the USDA (Grunwald 2000, USDA 2002) for use in determining the impact of agricultural activities on a watershed. AGNPS has been used to determine the agricultural impact on coastal and estuarine ecosystems (Choi 1999) and has been modified to integrate ARC/INFO databases in order to evaluate non point source problem areas (Liao 1997). In addition, models have been designed to study impact of the use of buffer strip on the water quality (Tim 1994). GIS tools have been used to evaluate non-point pollution of surface waters with phosphorus and nitrogen (Carpenter 1998, Robinson 1993). Additional GIS methods have been employed to help understand sediment loading from agricultural land use (Rudra 1999).

Of course agricultural lands have not been the only areas studied. Urban systems also have an impact on a watershed and GIS tools have been employed to study these impacts. Storm-water management systems and their impacts have been modeled using

GIS (Shamsi 1996). Estimations of mass loading from these types of systems evaluated (Wong 1997, Adamus 1995).

Larger scale studies have used the entire watershed in order to determine problem areas and what corrective actions could be implemented. The optimization the mix of Best Management Practices (BMP) to reduce the loading on a waterbody has been the goals of some research (Sample 2001, Wang 2000). GIS has been used to manage the ecosystems of a watershed (Crawford 1998).

The impact of land use on a watershed is an obvious use of GIS tools but only recently have studies on this subject have been published. Correlation between water quality using conductivity as the measure and urban land use was identified (Wang 1997). Other assessments of water quality and land use have determined with out surprise that land use does impact water quality (Wang 2001, Bhaduri 2000). There is very little in the literature on using GIS in a watershed to determine risk from microbial pathogens based on land use. There are published results investigating septic systems as potential pollutant but this study used nitrate as its measure (Stark 1999). In Ontario geographic distribution of *E. coli* O157:H7 infection and was compared to cattle population (Michel 1999). The results indicate that cattle density had a positive and significant association with the incidence of reported cases. GIS has been used to model and predict pathogen loading from livestock (Fraser 1998).

The U.S. Environmental Protection Agency's water programs and their counterparts in states and pollution control agencies are increasingly emphasizing watershed and water quality-based assessment and integrated analysis of point and nonpoint sources. Better Assessment Science Integrating point and Non-point Sources

(BASINS) is a system developed to meet the needs of such agencies. It integrates a geographic information system (GIS), national watershed and meteorologic data, and state-of-the-art environmental assessment and modeling tools into one convenient package. Originally released in September 1996, with a second release in 1998 BASINS, addresses three objectives: (1) to facilitate examination of environmental information, (2) to provide an integrated watershed and modeling framework, and (3) to support analysis of point and non-point source management alternatives (USEPA 2002b) BASINS supports the development of total maximum daily loads (TMDLs), which require a watershed-based approach that integrates both point and nonpoint sources. It can support the analysis of a variety of pollutants at multiple scales, using tools that range from simple to sophisticated.

The Bacterial Indicator Tool is a spreadsheet that estimates the bacteria contribution from multiple sources. Output from the tool is used as input to the water quality model in BASINS. The tool estimates the monthly accumulation rate of fecal coliform bacteria on four land uses (cropland, forested, built-up, and pastureland), as well as the asymptotic limit for the accumulation should no washoff occur. The tool also estimates the direct input of fecal coliform bacteria to streams from grazing agricultural animals and failing septic systems (USEPA 2002b).

E. coli Detection Methods

The original mTEC Agar enumeration method (Dufour 1981) for *E. coli* was introduced by EPA in 1986 (USEPA, 1986b). A revised method was developed in 1998

by the EPA and has been designated as the modified mTEC method. Both the mTEC and modified mTEC Agar methods use the membrane filter procedure. The two membrane filter methods provide a direct count of *E. coli* in water based on the development of colonies that grow on the surface of the membrane filter (Difco Labs, Detroit MI).

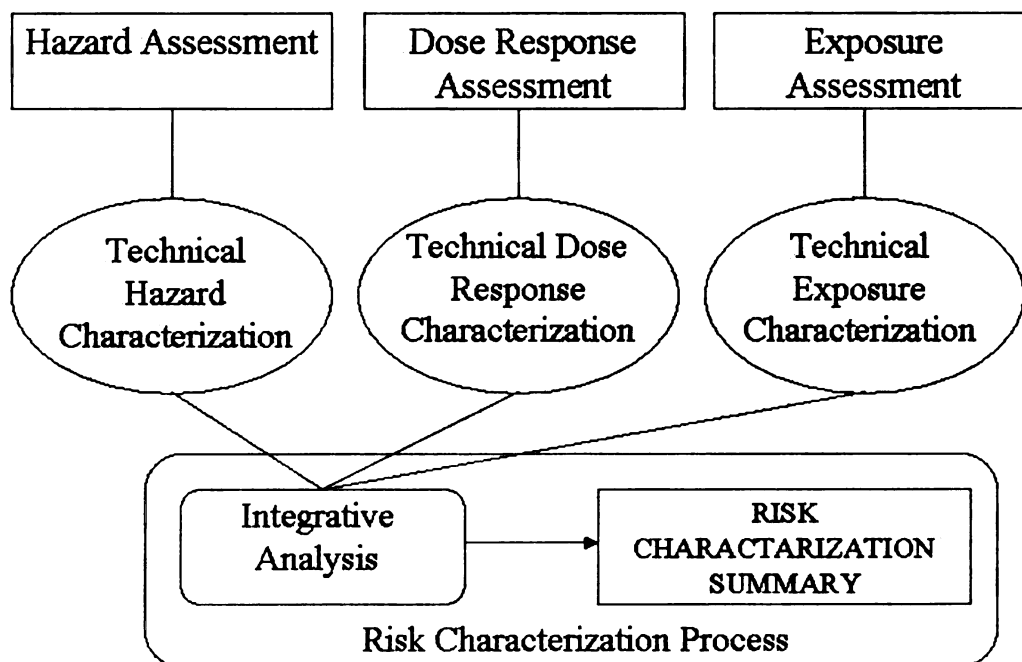
The PCR is a rapid and reliable tool for the molecular-based diagnosis of a variety of infectious diseases (Fredricks 1999). PCR analysis for screening drinking water and environmental samples has been reported (Tsen 1998; Campbell 2001) and has been utilized to identify *E. coli* in primary water specimens (Fricker 1994; Juck 1996; Noble 2001), stool specimens (Paton 1993; Yavzori 1998; Sinton 1998), and outbreaks (Huerta 2000; Lodge 2002). In contrast, isolation of *E. coli* O157:H7 from water and other environmental samples is laborious. Culture is problematic due to large numbers of other flora that either overgrow or mimic the non-sorbitol-fermenting *E. coli* O157:H7 (de Boer 2000). PCR methods are preferred to the traditional culture assays because the *E. coli* O157:H7 grows poorly or not at all at 44.5°C (Doyle 1984). Recently, immunomagnetic separation (IMS) has helped improve recovery by providing an antibody-based concentration procedure that uses magnetic beads coated with antibody against *E. coli* O157. Although many report the usefulness of IMS for testing artificially contaminated samples, few reports have documented the use of IMS with naturally occurring, epidemiologically linked specimens (Cubbon 1996; Wright 1994). Furthermore, there are no reports documenting the use of IMS in support of a waterborne outbreak investigation. Pulsed-field gel electrophoresis (PFGE) is useful for subtyping *E. coli* O157:H7 isolates during outbreak investigations (Akers 1998; Ammon 1999; Bender 1997). PFGE is reproducible and has sufficient discriminatory power to allow detection of minor genetic

variations among isolates (Willshaw 1997; Gouveia 1998). New technologies such as biosensors have been studied for use with pathogenic bacteria (Stokes 2001; Leonard 2003; Lang MJ in process). Several reviews on detection methods have published (including: Grif 1998; Baker 1999; Lejeune 2001; Theron 2002; Leonard 2003)

Risk Assessment Models

The following model (Figure 2-1) is for risk assessment, which was developed by

Figure 2-1. Risk Assessment Model (EPA 2000).



the National Academy of Science and since adopted by the EPA and is the standard for assessing risk in the environment (USEPA 2000). The standard model for risk that is used for a chemical contaminant or a carcinogenic material can not be used for a microbial. Both chemical compounds and carcinogenic materials have a defined amount of material that is plugged into the risk assessment model. This defined amount will only change if one or more the following conditions exist: the contaminant of concern is continuing to enter the environment, or natural attenuation is breaking down the contaminant of concern, or the contaminant of concern is being removed by remediation. Any of these condition the rate of increase or decrease of the contaminant of concern can be calculated. Therefore, the amount of the contaminant of concern can be determined fairly accurately. Based on these accurate calculations, models of mass transfer and transport, as well as, the risk assessment are useable tools. However, microbial substances such as *E. coli* are living material. Microbes live, multiply, or die based on the environmental conditions and nutrients that are available. Microbes can be passed or transmitted from person to person or from animals to people. In addition to the many vectors from which a microbe can enter a person or host, once in the host a small amount of microbes, which would not cause illness, can multiply into thousands or more if the conditions are right and then cause infection. None of these factors that are unique to microbes are considered in standard risk assessment models and such models will not accurately predict the risk from a microbe.

Models have been introduced to determine the risk from *E. coli* in water (Gofti 1999; Gale 2001; Balbus 2002). Models for estimating the incidence from of swimming related illness have been presented (Wymer 2002). Other models for estimating risk have

included long-term exposure and microbial densities (Haas 1996; Pinsky 2000). Still other models have concentrated on the vector pathway (Strachan 2001). Risk assessment models using complicated mathematical equations, Monte Carlo simulations, and calculus have been devised to mathematically describe the unique factors of microbes such as secondary infection and microbe survival (Eisenberg 1996; Chick 2001). For such complicated models, software will need to be developed that is user friendly, in order to have a wide acceptance of these models. Finally dose-response models have attempted to determine infectious doses of pathogenic microbes (Holcomb 1999; Teunis 1999; Haas 2000). All of these dose-response models conclude that more data and studies are needed and that statistical fit of the models to all data sets does not exist.

Epidemiological Evidence

Indicator Bacteria

A causal connection between fecal coliform and gastrointestinal sickness was first identified in 1953. (Stevenson 1953). In the late 1970s, in a landmark prospective cohort study, reported a linear relationship between the incidence of gastroenteritis among swimmers and marine bacterial counts (Cabelli 1982). Between 1973 and 1978, participants were recruited at beaches from three U.S. locations--New York, Lake Pontchartrain, Louisiana, and Boston, Massachusetts--and were contacted by telephone days after going to the beach. Swimming status was self-selected, not randomly assigned, and symptoms were self-reported. The mean proportion of swimmers with gastrointestinal symptoms was 6.8% versus 4.6% in non-swimmers. When enterococcal concentrations were above 1/100 ml, relative risk increased linearly, reaching 4.0 with

concentrations of 1,000/100 ml ($p < 0.001$). In addition, the frequency of gastrointestinal symptoms was inversely related to the distance from known sources of municipal wastewater (Cabelli 1982).

E. coli is an important cause of bacterial waterborne infection in untreated and recreational water (Cabelli 1982). Infection can be life-threatening, especially in the young and in the elderly. It can cause bloody diarrhea and, if not treated promptly, can result in kidney failure and death. In particular, *E. coli* O157:H7 is emerging as the second most important cause of bacterial waterborne disease after *Shigella* species, which is associated with human feces. *E. coli* O157:H7 was unknown until 1982, when it was associated with a multistate outbreak of hemorrhagic colitis (Riley 1983; Tarr 1985; Mead 1999).

There has been a large body of literature involving the epidemiology of illness from the use of recreational water (including: Schroeder 1968; Rosenbarg 1977; Favero 1985; Seyfield 1985; Fattel 1987; Ferley 1989; Cheunk 1990; Balarajan 1991; Jones 1991; Alexander 1992; Corbett 1993; Kay 1994; Fleisher 1998; Haile 1999; Lopez-Pila 2000; Prieto 2001; Dwight 2002). These studies have been conducted all over the globe including Africa, the Mediterranean, South America, New Zealand, China, and Australia (Cheung 1991; Vonschimming 1992; Corbett 1993; Harding 1993; Butler 1997; McBride 1998; Mourino-Perez 1999; Bonadonna 2002; Dionisio 2002; Daby 2002). An excellent review of this body of literature has been published which included 37 studies. This study concluded that a causal dose-related relationship between gastrointestinal symptoms and recreational water quality measured by bacterial indicator counts (Pruss 1998). Athletes, including tri-athletes, white-water canoeists, and surfers, have been

studied and these studies also show a causal dose-related relationship between gastrointestinal symptoms and recreational water quality measured by bacterial indicator counts (Fewtrell 1992; Harrington 1993; Medema 1995; Medema 1997; van Asperen 1998).

The indicator bacteria to determine risk for recreational use in freshwater is *E. coli* (EPA 1989, WHO 1998, EEC1994). There has been debate on the use and accuracy of indicator bacteria level to determine the risk of the use of a waterbody (Haas 2001; Griffin 2001; Muggleston 2001; Leclerc 2001; Efstratiou 2001; Edberg 2000; Figeras 1997). There is evidence of interference of other serotypes that cause inaccuracies in reported *E. coli* levels using the traditional EPA mandated plate culture methods (McLellan 2001). Studies have investigated the original EPA risk analysis and determined bias errors under-estimate the risk (Fleisher 1991; Fleisher 1990). However another study reports contrary results and that the bias in this original studies have bias that over-estimates the risk (Wymer 2002). Other measures such as beta glucosidase levels (an enzyme that *E. coli* produces), RNA bacteriophages, amino acid sequences as determined by PCR, and a matrix of indicators have been studied for use as replacements to the current indicators (Lee 1997; Lopez-Pila 2000; Conboy 2001). The underlying barrier to implementing these newer methods which have been shown to be more accurate, is that the current EPA mandated method is very inexpensive. The cost of equipment, materials and training that is needed for these new methods is too expensive for cash strapped health department and will not implement unless it mandated by the EPA.

E.coli O157:H7

In contrast to the numerous studies, cases and reports of health effects from exposure to indicator bacteria in recreational-use water, there are a limited number of such findings in literature in regards to pathogenic *E. coli O157:H7*. One of the reasons for this that *E. coli O157:H7* was first identified in 1982 (Riley 1983; Tarr PI 1985) and food was typically identified as the transmission route for illness (Griffin 1991, Philips 1999, Coia 1998). In addition it is very difficult to identify in surface water and was first identified in water in 1989 (McGowan 1989). Since *E. coli O157:H7* may have infection dose of as low as 10 cells (Mead 1999; Philips 1999) this may be impossible to detect. That being said there are a few epidemiological studies that report *E. coli O157:H7* as the pathogen that caused illness in swimmers.

The first case of illness caused by *E. coli O157:H7* was reported in 1991 and occurred in a lake near Portland, Oregon. In a case control study 21 persons were identified with *E. coli O157:H7* infections by stool or serum samples and 7 of these persons required hospitalization (Keene 1994, Van Ess 1999). All of the affected persons were children with the median age of six years old and all illnesses were not associated with food or beverage but all of the affected persons reported they had been swimming. It was concluded in the case control studies that swimming was strongly associated with infection ($p < 0.015$) and the affected persons were more likely than controls to have reported swallowing water while swimming (Keene 1994). The reported levels of the indicator bacteria revealed substantial fecal contamination during the time period of the study, in addition, *E. coli O157:H7* was identified in the lake. Even though as many as 10,000 visitors a weekend would use the park in the lake was located, no studies or

calculations to determine prevalence, odds ratio, or relative risk were carried out during the 7 week study period. A rough estimate of the incidence of illness based on seven weeks of weekend visitors would be less than 0.03% (Keene 1994).

In 1993, six children were identified with illness caused by *E. coli* O157:H7 in a 1.5-mile radius in southwest London. One of the children later died from the illness. The affected children had play in a paddling pool in which levels of *E. coli* were identified but *E. coli* O157:H7 was not identified in these pools (Brewster 1994; Hildebrand 1996).

In 1993, 4 children, aged 1.5 to 3.5 years, all living in one town in the Netherlands, were admitted to our hospital with the diagnosis hemolytic uremic syndrome within one week. All 4 patients bathed in the same, shallow, recreational lake within a period of 5 days. *E. coli* O157:H7 was demonstrated in the fecal samples of 2 index patients (Cransberg 1996).

In 1994, an *E. coli* O157:H7 outbreak was reported in Dutchess County, New York. There were 12 confirmed cases of illness and all of the affected persons were under the age of 14 years old. The case control study concluded that the illness was not associated with food or beverage but with swimming and the affected children were more likely than the controls to have ingested lake water (Ackman 1997). *E. coli* O157:H7 was confirmed in affected children by bacterial subtyping. Samples taken from the lake both before and after the outbreak were analyzed for *E. coli* and all results from these samples were below 70cfu/100ml which is considered safe for full body in the water (Ackman 1997).

In 1995, a cluster 12 confirmed cases in children of *E. coli* O157:H7 induced illness was reported in northern Illinois (JAMA 1996). All of the affected children had

been swimming in a lake in a state park. The case control study concluded that the illness was not associated with food or beverage but with swimming (Mudgett 1998). In addition the case control study determined that taking water into the mouth, swallowing water, and time spent swimming all were risk factors for illness. Water samples that were tested during the study showed elevated levels of *E. coli* but were not tested for *E. coli O157:H7*. Water samples and sediment samples tested after the exposure did not reveal any *E. coli O157:H7* in any of the nearly 100 samples (Mudgett 1998).

In 1996, in Georgia it was reported that a two-year girl who was attending a party was diagnosed with *E. coli O157:H7* that was confirmed by cultured analysis. Upon further investigation 18 persons out of a total of 51 people who attended the party, developed gastrointestinal illness and 10 of these met the case definition of *E. coli O157:H7* infection as identified by elevated IgM or IgG levels (Friedman 1999). Swimming in the pool significantly increased the risk of *E. coli O157:H7* illness and no other exposures were identified with illness. Although the pool had little to no chlorine in it, no *E. coli O157:H7* was identified in the pool.

In 1997, five cases of *E. coli O157:H7* induced infection were identified in Finland. The affected children were between the ages of 3 and 8 years old, all had swum in a swallow, and had ingested lake water (Paunio 1999). An additional eight secondary cases were reported and all of these cases were either family members or caretakers of the affected children. The secondary cases were transmitted via person to person from the affected children. All cases were confirmed for *O157:H7* via the analysis of stool samples. Samples of the lake water did not yield any *E. coli O157:H7*.

In 1998, the CDC reported that 42 confirmed cases of *E. coli* O157:H7 infection nationwide and of that number, only 7 cases were connected to swimming pool in Marietta Georgia (CDC 1999). Of the 7 swimming related illnesses unfortunately one of the illnesses resulted in a death. In 1999, an outbreak of *E. coli* O157:H7 infection was reported involving children at a day care in California. Seven cases were identified and in a cohort study the cause was determined as swallowing water in a freshwater lake (Feldman 2002).

Also in 1999, it was reported that 36 patients developed *E. coli* O157:H7 infection in the State of Washington. Of the 36 patients, 28 were swimmers and 8 patients were infected via person-to-person transmission (Samadpour 2002). Although *E. coli* levels were within acceptable levels for swimming, O157:H7 was identified in the lake and is suggested to have come from a duck. This shows that the indicator organism, *E. coli*, may have limitations in determining the real pathogenic risk of the water that is to be used for swimming. The researchers used a PCR enriched method to detect the pathogen. This method was used instead of the traditional culture assays because the *E. coli* O157:H7 grow poorly or not at all at 44.5°C (Doyle 1984).

In all these reports or studies that have identified *E. coli* O157:H7 illness related to swimming, only one identified *E. coli* O157:H7 in the water that was the exposure. In addition, prevalence, risk, and incidence could not be calculated. Although *E. coli* O157:H7 infection from swimming is very rare and the reports indicate that children are at much higher risk than adults. This higher risk may be from weaker immune systems of children as compared to adults but more likely it is due to the facts that children are more likely to ingest water while swimming and that children are more likely than adults to

defecate while swimming. One study estimated that about 8 percent of U.S. outbreaks of *Escherichia coli* O157:H7 between the years 1982 and 1996 among children occurred as a result of swimming (Griffin 1991). All of these studies suggest that the source of the *E. coli* O157:H7 came from a person who was swimming and left behind the pathogen. The use of sealed rubber swimming trunks on children maybe have the greatest impact in lowering the risk of *E. coli* O157:H7 infections when swimming.

Public Policy to Reduce Risk in Recreational Water

There has world-wide debate in regards to public policy and public safety for recreational-use water (Figueras 1997; Bluemnthal 2000; Henrickson 2001; Efstratiou 2001). This review will focus on domestic (United States) public policy and leave the discussion of EEC and WHO policy to the above-mentioned articles.

One of the major goals of the Clean Water Act of 1972 and its amendments (CWA) is to ensure that U.S. waters are safe for fishing and swimming (33 U.S.C.A. §§ 1251 to 1387). The use of water quality indicators that accurately reflect "safety" is essential for this goal. The CWA mandates the use of indicator organisms to determine safe levels and CWA section 303(d) requires the reporting of "impaired waters." In addition, section 303, establishes the water quality standards and Total Maximum Daily Load (TMDL) programs. The TMDL program is required by the rules that amended the CWA issued in 1985 and then subsequently amended in 1992 (USEPA 2002).

TMDL is a calculation of the maximum amount of a pollutant that a waterbody can receive while still meeting water quality standards, and then determines an allocation of that amount to the pollutant's sources. Water quality standards are set by States,

Territories, and Tribes and must meet or exceed the Federal standards set forth in the CWA. These entities identify the uses for each waterbody, for example, drinking water supply, contact recreation (swimming), and aquatic life support (fishing), and the scientific criteria to support that use. A TMDL is the sum of the allowable loads of a single pollutant from all contributing point and non-point sources. The calculation must include a margin of safety to ensure that the waterbody can be used for the purposes the State has designated. Additionally, the calculation must also account for seasonal variation in water quality.

The indicator organism mandated for the monitoring of pathogens in fresh water is *E. coli*. If the level of this organism is consistently elevated from the standard, then the waterbody is declared an “impaired water” as defined in CWA 303 (d) and a TMDL is required to be developed and implemented for that waterbody. As of 2001, there are 5,512 reported “impaired waters” due to pathogens (over 13% all impairments reported and this is the second highest impairment behind sediment which is first at just below 14%) (USEPA 2001).

The Beach Environmental Assessment Closure and Health (BEACH) Act was signed into law in October 2000 and amended the CWA (USEPA 2002c). This legislation directs the EPA Administrator to conduct studies to review health risks; to develop indicators to detect the presence of pathogens; to offer guidance for state-to-state application of the revised water quality criteria; to publish and revise regulations requiring the monitoring of coastal waters; to provide technical assistance to states for uniform assessment and monitoring procedures; and to establish a national coastal

recreation water pollution occurrence database and a listing of communities complying with regulations published pursuant to this legislation. In addition, the Beach Act authorizes \$30 million annually for grants that would help states, local governments and Indian tribes to monitor beach waters and notify the public when beach water exceeds the established criteria. Moreover, some of these grants are designated for the development of improved detection of pathogens in water, both freshwater and marine water.

It is mandated that consistent national health standards for beach water be established by 2004. Currently, the EPA has determined that *E. coli* level is the best indicator of risk to swimmers in fresh water. An *E. coli* concentration of less than 126 CFU/100ml, calculated as a geometric mean over 30 days, is considered to be safe for full body contact recreational use of water (USEPA 1986). If a waterbody has a designated use of full body contact recreational use and the *E. coli* levels are consistently elevated from the above described standard, then the waterbody is declared a “impaired water” as defined in CWA 303 (d) and a TMDL is required to be developed and implemented for that waterbody. Protocol for developing pathogen TMDL EPA Office of Water January 2001

Many county health departments in the State of Michigan routinely collect water samples at beaches to determine if the water is safe for swimming. Samples are generally taken one foot below the surface in water that is between three and six feet in depth. The analysis is performed in a laboratory using standard methods. *E. coli* bacteria are counted and judged against standards established by state rules. Results from the method are

available after approximately 28 hours; so the results from this analysis are reported the following day.

County health departments take a minimum of three samples each time a beach area is monitored. The daily geometric mean calculated from these samples must be below 300 *E. coli* per 100 milliliters for the water to be considered safe for swimming. One or two of the samples may be above 300, but if the daily geometric mean is below 300, the beach is not in violation of the water quality standard. A minimum of five sampling events (consisting of at least three samples per event) must be collected within a 30-day period for the results to be considered a reliable indication of water quality. After 30 days, a geometric mean is calculated for all the individual samples collected within that time frame. This 30-day geometric mean must be below 130 *E. coli* per 100 ml for the water to be considered safe for swimming. Although the method yields results that are very accurate, the results are not reported in less than 24 hrs. Officials close beaches based on old information that may or may not be the actual condition of the water at the time of closure and leave prior groups exposed.

EPA in both its 1992 National Water Quality Inventory and its Report to Congress noted that pollution from wet weather discharges is cited by many states as the leading cause of water quality impairment. Based on their reports and other assessments, the EPA has concluded that wet weather discharges from both point and nonpoint discharges are one of the largest threats remaining to water quality, aquatic life, and human health that exist today. Areas of needed research and interest include but are not limited to:

- Development of technologies for preventing toxic substances and pollutants from entering the downstream storm or combined sewer/drainage systems.
- Development of monitoring methodologies to measure the characteristics and impacts of wet weather flows.
- Development of high-rate and high-efficiency WWTP treatment technologies.

While not subject to secondary treatment requirements, CSOs must nevertheless meet water quality-based and technology based standards under NPDES permits to comply with the Clean Water Act. Based upon U.S. EPA's 1989 CSO strategy and 1994 National CSO Policy, CSO communities are required to implement nine minimum control technologies, and develop a long-term CSO control plan to meet water quality standards. The nine minimum controls are generally met through management of the existing CSS, while the long term controls will involve capital improvements such as the retention and treatment, or sewer separation.

EPA's CSO Control Policy, published April 19, 1994, is the national framework for control of CSOs. The Policy provides guidance on how communities with combined sewer systems can meet Clean Water Act goals in as flexible and cost-effective a manner as possible. EPA's Report to Congress on implementation of the CSO Control Policy assesses the progress made by EPA, states, and municipalities in implementing and enforcing the CSO Control Policy. "Wet weather discharges" refers collectively to point source discharges that result from precipitation events, such as rainfall and snowmelt. Wet weather discharges include storm water runoff, combined sewer overflows (CSOs),

and wet weather sanitary sewer overflows (SSOs). Storm water runoff accumulates pollutants such as oil and grease, chemicals, nutrients, metals, and bacteria as it travels across land. CSOs and wet weather SSOs contain a mixture of raw sewage, industrial wastewater and storm water, and have resulted in beach closings, shellfish bed closings, and aesthetic problems.

Properly designed, operated, and maintained sanitary sewer systems are meant to collect and transport all of the sewage that flows into them to a publicly owned treatment works (POTW). However, occasional unintentional discharges of raw sewage from municipal sanitary sewers occur in almost every system. These types of discharges are called sanitary sewer overflows (SSOs). SSOs have a variety of causes, including but not limited to severe weather, improper system operation and maintenance, and vandalism. EPA estimates that there are at least 40,000 SSOs each year. The untreated sewage from these overflows can contaminate our waters, causing serious water quality problems. It can also back-up into basements, causing property damage and threatening public health.

Table 2-3. The Nine Minimum Controls for Combined Sewer Overflows

1. Proper operation and regular maintenance programs for the sewer system and the CSOs
2. Maximum use of the collection system for storage
3. Review and modification of pretreatment requirements to assure CSO impacts are minimized
4. Maximization of flow to the publicly owned treatment works for treatment
5. Prohibition of CSOs during dry weather
6. Control of solid and floatable materials in CSOs
7. Pollution prevention
8. Public notification to ensure that the public receives adequate notification of CSO occurrences and CSO impacts
9. Monitoring to effectively characterize CSO impacts and the efficacy of CSO controls

Since 1994, the USEPA has enforced these stringent new rules concerning wet weather issues. Under the NPDES permit program, there are three program areas that address each of the wet weather discharges described above. NPDES requirements from runoff from concentrated animal feeding operations (CAFOs) are described in an earlier section of this paper. These programs share a range of cross-cutting issues and affect a similar group of stakeholders. EPA believes that wet weather discharges should be addressed in a coordinated and comprehensive fashion to reduce the threat to water quality, reduce redundant pollution control costs, and provide State and local governments with greater flexibility to solve wet weather discharge problems. To identify and address cross-cutting issues and promote coordination, EPA established the Urban Wet Weather Flows Federal Advisory Committee in 1995.

EPA is proposing to clarify and expand permit requirements for 19,000 municipal sanitary sewer collection systems in order to reduce SSOs. The proposed SSO Rule will help communities improve some of the Nation's most valuable infrastructure - our wastewater collection systems - by requiring facilities to develop and implement new capacity, management, operations, maintenance and public notification programs.

The nation's federal and state regulatory systems for protecting environmental health have failed to keep pace with the rapid growth of factory farms. When Congress passed the Clean Water Act (CWA) (33 U.S.C.A. Sections 1251 to 1387) 30 years ago, it had the foresight to identify feedlots as an industrial source of pollution and to require that feedlots be regulated as strictly as other industries. However, EPA has failed to

enforce these statutory requirements and the implementation of the regulations has been pockmarked with loopholes. According to a 1995 General Accounting Office Report, in 1992 only 30 percent of the 6,600 farms that were large enough to be subject to federal permit requirements actually obtained a permit under the Clean Water Act (US GAO 1995). To a greater or lesser extent, states have attempted to step into the void created by an ineffective federal approach. Unfortunately, the states as a whole have also been ineffective in regulating AFOs (USGAO 1995). Pennsylvania, Colorado and Alabama have no permitting program, though programs are in the works. Illinois regulates only livestock operations with animal waste lagoons but not those with underground manure storage tanks, which are now the norm in Illinois. Some of these have leaked. California's Central Valley issues permits only after an operation is caught polluting (NRDC 1999) EPA's proposed regulatory changes affect the existing NPDES provisions and the existing ELG for "feedlots." The NPDES provisions define and establish permit requirements for CAFOs and the ELG establish the technology-based effluent discharge standard that is applied to CAFOs. Both of these existing regulations were originally promulgated in the 1970s. The EPA proposed revision of the CAFO regulations would affect operations that confine cattle and calves, milking cows, hogs and pigs, and poultry, including broilers, egg laying chickens, and turkeys. Businesses that contract out the raising or finishing phase of production might also be affected by the proposed co-permitting requirements in the proposed CAFO regulations. Affected businesses may include meat packing plants and poultry processing firms. The EPA has proposed two alternatives on defining CAFOs that will be regulated. The two co-proposed alternatives include the "*two-tier structure*" that would define as CAFOs all AFOs with more than

500 AU and the “*three-tier structure*” that would define as CAFOs all AFOs with more than 1,000 AU and any operation with more than 300 AU, if they meet certain “risk-based” conditions, as defined in the in the proposed rules (USEPA 2002). EPA estimates that both proposed alternative structures would regulate about 12,660 operations with more than 1,000 AU, accounting for operations with more than a single animal type. The two-tier structure would also regulate an additional 12,880 operations with between 500 and 1,000 AU, for a total of 25,540 operations. Under the three-tier structure, an estimated 39,330 operations would be subject to the proposed regulations (10 percent of all AFOs), estimated as the total number of animal confinement operations with more than 300 AU (US EPA 2001). These have completed the comment period and the final rules will be promulgated in the near future.

Conclusions

Ever since fecal contamination of water was determined a human health risk, there has always been a great deal of concern regarding the level of coliform bacteria counts in water. Many bodies of water throughout the world are considered to have counts above acceptable levels. The sources of these *E. coli* are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow. Physical factors, such as seasonal variability, rainfall, river flow, nutrient and the survival of bacteria in water, have an impact on *E. coli* levels in a waterbody. Tools exist to assist in determining the risk to swimmers of bacteria illness in a watershed. Although risk assessment models have been

studied, an accurate dose-response model for illness to swimmers of bacteria illness in a watershed is not available.

There has a large body of literature involving the epidemiology of illness from the use of recreational water. Elevated *E. coli* levels have been identified as the cause of the illness and *E. coli* levels have been mandated as the indicator bacteria for the determination of water safety for swimmers. The validity of *E. coli* as an indicator of risk has been debated and *E. coli* may not be an accurate indicator of pathogenic risk.

In contrast to the numerous studies, cases and reports of health effects from exposure to indicator bacteria in recreational-use water, there are a limited number of such findings in literature in regards to pathogenic *E. coli* O157:H7. In all these reports or studies that have identified *E. coli* O157:H7 illness related to swimming, only one identified *E. coli* O157:H7 in the water that was the exposure. Although *E. coli* O157:H7 infection from swimming is very rare and the reports indicate that children are at much higher risk than adults. This higher risk may be from weaker immune systems of children as compared to adults but more likely it is due to the facts that children are more likely to ingest water while swimming and that children are more likely than adults to defecate while swimming. The use of sealed rubber swimming trunks on children maybe have the greatest impact in lowering the risk of *E. coli* O157:H7 infections when swimming.

Public policy in both the United States and around the world has addressed the risk to swimmers of illness from bacteria in water. Levels of indicator bacteria that are acceptable as to the risk for swimmers have been established by USEPA, EEC, and WHO. In addition to these policies, laws and guidelines have been established to decrease the microbial input in a waterbody from sewage sources and agricultural

facilities. Many of these laws and guidelines are being reviewed and revised in order to improve the public health of swimmers.

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Chapter 3

The Effect of Rainfall, River Flow and other Meteorological Aspects on *E. coli* Levels in the Red Cedar River

Introduction

Ever since fecal contamination of water was determined a human health risk, there has always been a great deal of concern regarding the level of coliform bacteria counts in water. Many bodies of water throughout the world are considered to have counts above acceptable levels. The sources of these coliforms are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow (EPA 2000). The indicator organism for fecal contamination in fresh water is *Escherichia coli* (*E.coli*) (EPA 2003, WHO 2003). Physical characteristics of the watershed maybe risk factors that affect the concentration levels of *E.coli*. This study will examine the effect of the seasons, the flow rate of the river, rainfall, air temperature, water temperature, intensity of the sun, humidity, soil moisture, ground temperature, and wind speed as factors related to concentration levels of *E.coli*.

The seasonal variation of *E.coli* concentration levels have been studied and reported in the literature. Over a twelve-year period (1984-1995) a study in Scotland determined that seasonality was a factor with the highest amount of hospital admissions for illness due to *E.coli* in July and August (Douglas 1997). It should be noted that this study was not limited to recreational use of water and showed the seasonal pattern only for patients under 15 years old. In contrast, a study in Florida showed that concentration of fecal indicator organisms were the highest during the late fall and early winter months,

which corresponds to the wet, weather months in Florida (Lipp 2001). In addition the study determined that the levels of fecal indicators were significantly associated with rainfall, stream flow, and temperature. Moreover, a study of two streams in Arkansas over a three year period concluded that concentrations of indicator bacteria increased with increasing flow rates and seasonal effects were observed on the indicators with the highest levels occurring during the summer months (Edwards 1997). Furthermore, in a study of a dairy herd *E. coli* O157:H7 was found in 4.3% of the herd and *E. coli* concentration levels peaked during the May to July timeframe but was not found in the herd from November to May (Mechie 1997). However, a study in northwest England reported that bacteria indicators showed no seasonal variations over a two-year period (Obiri-Danso and Jones 1999).

Other studies support the association of the levels of fecal indicators in a water body and rainfall events. In a study that examined 99 samples from three tributaries that contributed to different drinking water reservoirs showed that *E.coli* levels along with other bacteriological parameters increased considerably during extreme rainfall events (Kistemann 2002). In a similar study on Delaware River, increased concentrations of *Giardia*, *Cryptosporidium* and other microorganisms were associated with rainfall (Atherholt 1998). In a retrospective study on waterborne disease outbreaks in the U.S. for 1948 to 1994, analyzed 548 reported outbreaks as documented by the USEPA database. The results showed that 51% of waterborne disease outbreaks were preceded by precipitation events above the 90th percentile and 67% by events above the 80th percentile (Curriero 2001). This study concluded that there is a statistically significant association between high intensity rainfall events and waterborne disease outbreaks.

Other studies also conclude that *E.coli* levels increase with rainfall (Briski F 2000; Ferguson 1996; Atherholt 1998; Pettibone and Irvine 1996; Niemi and Niemi 1991; Mallin 2001; Noble 2003). Recent study by Rose (2001) suggests that an increase in rainfall or snowmelt increases the impact of diseases caused by microbiologic agents.

An increase in the flow of a river has been also related to an elevation in the concentration levels of *E.coli* in the river. Early studies in Idaho and Oregon found that fecal *E.coli* concentrations were higher during period of high flow than during period of lower flow (Stephenson and Street 1978; Tiedemann 1988). These finding were confirmed in a study of two rivers in Arkansas that concluded that concentrations of indicator bacteria increased with increasing flow rates (Edwards 1997).

The survival characteristics of a mixture of five *E. coli* 0157:H7 strains innoculated at 10^3 CFU/ml in filtered and autoclaved municipal water, in reservoir water, and in water from two recreational lakes were determined for a period of 91 days and stored at three different temperatures of 8, 15, or 25 degrees C. Greatest survival was in filtered autoclaved municipal water and least in lake water. Regardless of the water source, survival was greatest at 8 degrees C and least at 25 degrees (Wang 1998). According to the United States Food and Drug Administration (FDA) *E. coli* grows between the temperatures of 2.5°C and 45°C (USFDA 1992).

An objective of this study is to determine if there is a seasonal effect on the concentration levels of *E.coli* in the Red Cedar River watershed. Another objective is to determine if there is relationship between the flow rate of the river and concentration levels of *E.coli*. In addition, an objective of this study is to determine the statistical significance of concentration levels of *E.coli* as related to the watershed physical

characteristics of rainfall, air temperature, water temperature, intensity of the sun, humidity, soil moisture, ground temperature, wind speed, duck population, as well as river flow. Finally, an objective of this study is to use the statistical data to model risk factors so that water safety can be determined by the changes in physical characteristics which may be determinable in real time. It is hypothesized, based on the results reported in the literature, that the model risk factors are directly related to rainfall and associated factors.

Methods and Materials

Data

The location of the study site is on the Michigan State University (MSU) campus in East Lansing, Michigan. Water samples from the Red cedar River were collected weekly during the 32 weeks from April to November over a three-year period. The water samples were taken from the Farm Lane Bridge, which crosses the Red Cedar River in the center of campus. These were collected by the MSU university physician's office and sent to the Michigan Department of Environmental Quality (MDEQ) for the determination of *E. coli* concentration levels in water. The detection method for the analysis of *E. coli* used by MDEQ was EPA Method 1103.1, which is a mTEC Agar enumeration method introduced by EPA in 1986 and modified in 1998 (Dufour 1981; USEPA, 1986b; USEPA 1998). The river flow measurements were taken from the United States Geological Service (USGS) gauging station that is located about 300 yards down river from the Farm Lane bridge.



Figure 3-1. View of the Farm Lane Bridge over the Red Cedar River from the USGS Gauging Station on the Campus of Michigan State University.

The weather data were collected from the archives of the MSU horticulture research weather station located on campus about two miles south of the Farm Land Bridge. Data that were collected and used in this study included air temperature, humidity, soil moisture at two inch depth, soil temperature at two inch depth, solar radiation, wind speed, and precipitation. Finally, water temperature data and duck count data were collected by Jo Latmore, Ph.D. candidate at MSU as part of a separate project and were only available for a portion of this study.

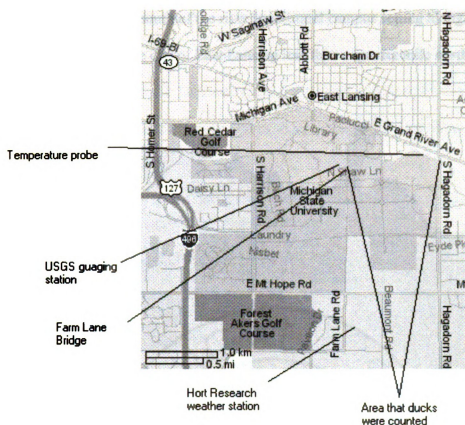


Figure 3-2. Locations of where measurements were taken for this study.

The Red Cedar River arises in Cedar Lake in the south-central Lower peninsula of Michigan and flows about 45 miles (80 kilometers) to its confluence with the Grand River in the city of Lansing. It has 12 tributaries and drains a total area of about 472 square miles (22,000 hectares). The river provides mid-Michigan residents with numerous recreational opportunities that include angling, canoeing, kayaking, photography and bird watching. The river also serves as a source of water for the irrigation of crops throughout the watershed, as well as, drainage for agricultural land. The weekly samples were taken from the 5,400-acre MSU campus with agricultural

operations two miles south of the river, traditional campus setting along the river, and the urban landscape of the city of East Lansing less than a quarter mile north of the river.



Figure 3-3. The Red Cedar River Watershed with The Michigan State University campus outlined in red.

Statistical Analysis

For seasonal effects, correlations were analyzed between the three years of data. The first step in the statistical analysis included assessment of the correlations between the *E.coli* levels and the physical and meteorological measurements. All the data was collected at the same frequency as the water samples for the *E.coli* concentration measurements, weekly over the 32 weeks from April to November over the three-year

period with the exception of the water temperature and duck counts. For all data the number of samples (n) is equal to 96 except for water temperature where the number of samples is equal to 33 and duck count where the number of samples is 40. Because of the lack of normality in the distribution of *E. coli*, precipitation and flow rate data, Spearman's correlation coefficient was used in all the correlations.

Once the physical and meteorological variables that significantly correlated with *E. coli* were identified based on the correlation analysis, they were included in a logistic regression with *E. coli* level exceeding a certain thresholds as a dependent variable. Two different logistic regressions were run with different thresholds, one threshold value at the *E. coli* level of 300cfu/100ml, which is the highest acceptable full body contact level, and another at the *E. coli* level of 1000cfu/100ml, which is the highest acceptable partial body contact level. Statistical analysis was completed using SPSS version 11 software package, as well as, SAS version 6.1.

Results and Discussion

Seasonal Effects

The plot in Figure 3-4 shows the weekly levels of *E. coli* with each year of data overlaid so that the weeks of the each year line up. Note that the peak levels for each year occur on different weeks. Statistical analysis on this data set shows that there is no correlation either using parametric (Pearson) or non-parametric (Spearman)

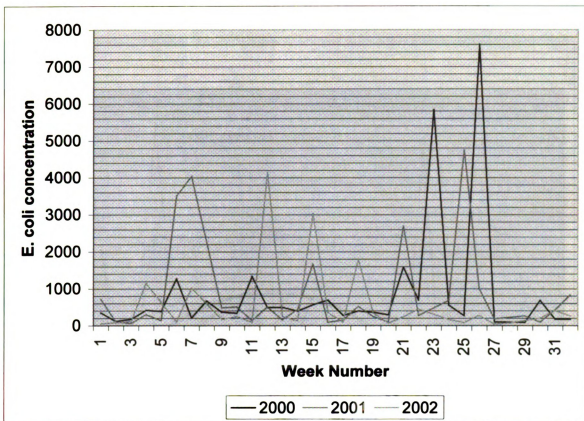


Figure 3-4. *E. coli* Concentration from April to November in the Red Cedar River

correlation coefficients between the three years of data. Based on these analyses, it is concluded that there is no observed seasonal effect for *E. coli* levels from the Farm Lane Bridge over the three-year period from 2000 to 2002.

The plot in Figure 3-5 shows the weekly value for the flow rate at the time that the sampling was done for the *E. coli* analysis. In contrast to the *E. coli* levels, the flow rate correlation using Spearman's Rho, between the three years is statistically significant at least $p < 0.05$ or better. From this analysis, it can be concluded that there is an observed seasonal variation in the flow rate of the Red Cedar River at the Farm Lane Bridge.

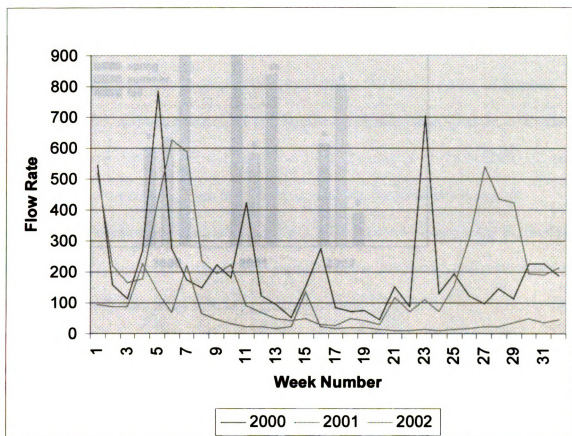
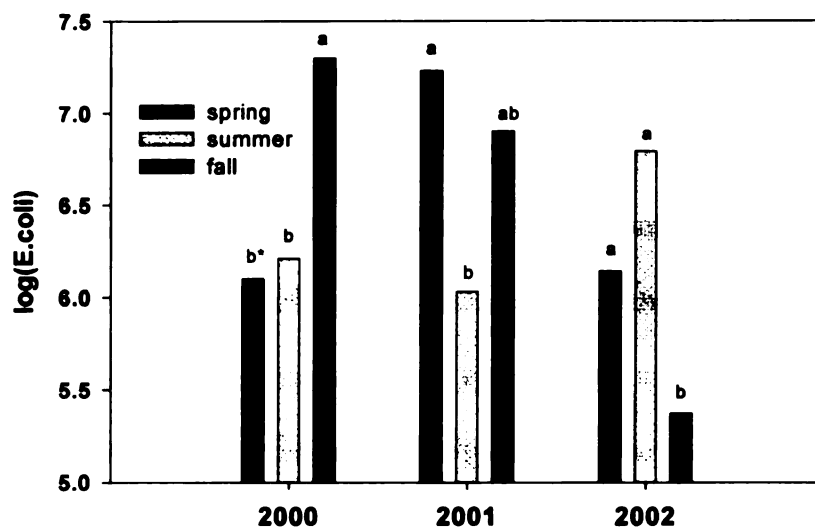
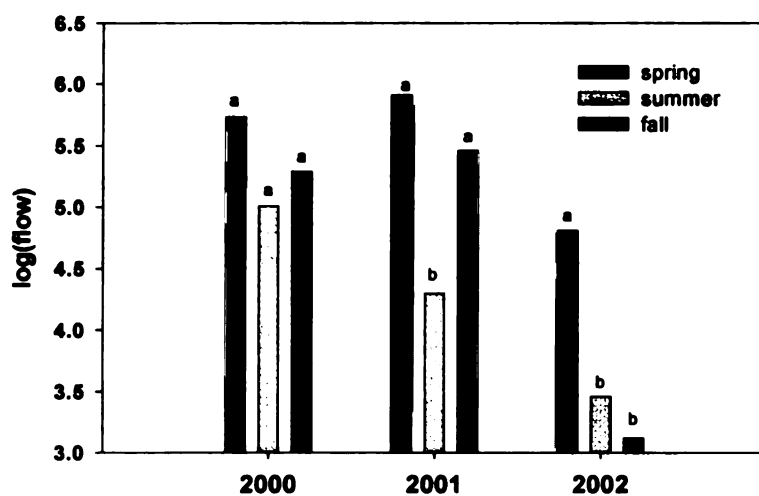


Figure 3-5. Flow Rate from April to November in the Red Cedar River



* means within the same year followed by the same letter are not significantly different ($p < 0.05$)

Figure 3-6. Mean Values of the Log-transformed *E.coli* Concentrations in Spring, Summer and Fall of the Studied Years.



* means within the same year followed by the same letter are not significantly different ($p < 0.05$)

Figure 3-7. Mean Values of the Log-transformed Flow Rates in Spring, Summer and Fall of the Studied Years.

There was no overall seasonal effect in the studied three years of the *E.coli* concentration ($p < 0.1$). However, the interaction between year and season was statistically significant indicating that season effects were different in different years. Indeed, in 2000 average *E.coli* concentration was significantly higher in fall than in spring or summer ($p < 0.1$). In 2001, spring concentration was significantly greater than summer, while there was no difference between fall and summer or fall and spring ($p < 0.05$). In 2002, fall had significantly lower concentration than spring or summer, the latter two being not significantly different from each other ($p < 0.01$). The observed inconsistency in seasonal effects indicates that in the studied area occurrence of the increased *E.coli* concentrations cannot be directly associated with any particular season.

Year-to-Year Observations

Shown in Figure 3-8 are the mean values along with error bars indicating the 95% confidence level for *E.coli* and flow for each year. Note that as flow varies so do *E.coli* levels. In the year 2002 the mean flow rate dropped by about 75% from the previous years and the mean *E.coli* level for the year 2002 dropped over 40% in comparison to the previous years.

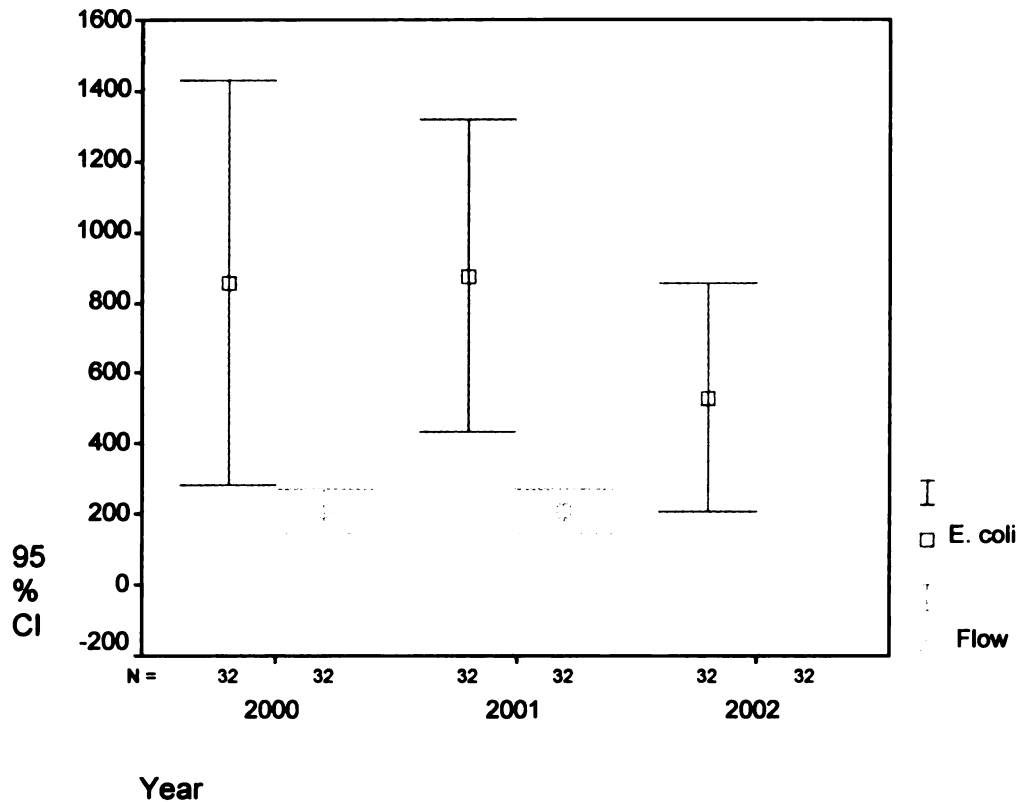


Figure 3-8. Yearly Mean and Standard Deviation of *E. coli* Levels and Flow

Shown in Figure 3-9 are the mean values for *E.coli*, flow (shown as bars) and rainfall (overlaid as lines) for each year. The rainfall data were collected over the day of *E.coli* sampling along with the two days previous to sampling and data was calculated and reported as a cumulative total over a 24, 48, or 72 hour period. Not surprisingly rainfall is associated with the *E. coli* level as does the flow rate. In the year 2002 the mean cumulative rainfall for a 72-hour period dropped by about 65% from the year 2000 and the mean *E. coli* level for the year 2002 dropped about 38% in comparison to the year 2000. In the year 2002 the mean flow rate dropped by about 75% from the year 2000. It makes common sense that the flow rate is lower for dry years and the year 2002 was a drought year with no rain for an 11-week period from June through August.

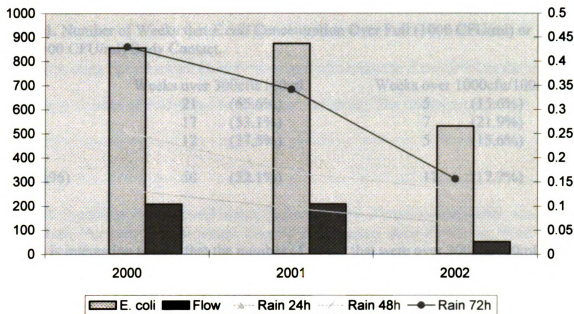


Figure 3-9. Yearly Mean of *E. coli* Levels, Flow, and Rainfall.

Michigan law (Michigan Public Health Code, PA 368 of 1978) based on EPA guidelines (EPA 2003) mandates that the *E. coli* concentration of 300cfu/100ml is the highest acceptable full body contact level, which is considered a person putting their head under water and would include swimming and water skiing. The law also mandates that *E. coli* concentration of 1000cfu/100ml is the highest acceptable partial body contact level which is considered putting a body part other than ones head into the water and would include fishing, canoeing, kayaking or boating. Table 3-1 shows the number weeks for each that the *E. coli* concentration was exceeding the regulated levels.

Table 3-1. Number of Weeks that *E.coli* Concentration Over Full (1000 CFU/ml) or Partial (300 CFU/ml) Body Contact.

Year	Weeks over 300cfu/100ml		Weeks over 1000cfu/100ml	
2000	21	(65.6%)	5	(15.6%)
2001	17	(53.1%)	7	(21.9%)
2002	12	(37.5%)	5	(15.6%)
Total (n=96)	50	(52.1%)	17	(17.7%)

It is interesting to note that the number of weeks that were over 300cfu/100ml decreased from the year 2000 to 2002 in a similar trend to that of rainfall and flow. This may indicates that the *E.coli* concentration at this level is influenced by rainfall and flow. In contrast, the number of weeks that were over 1000cfu/100ml do not follow these trend and indicating that there maybe other facts other than rainfall and flow rate that influence occurrence of the very high concentrations of *E.coli*.

Effect of All Individual Factors

Rainfall and flow were not the only data that was collected to be compared to *E.coli* concentration. Data that was collected and used in this study included air temperature, humidity, soil moisture at two inch depth, soil temperature at two inch depth, solar radiation, wind speed, precipitation, water temperature data and duck count. All the data was collected at the same frequency as the water samples for the *E.coli* concentration measurements, weekly over the 32 weeks from April to November over a three-year period with the exception of the water temperature and duck counts. For all data the number of samples (n) is equal to 96 except for water temperature where the

number of samples is equal to 33 and duck count where the number of samples is 40. For the impact of each physical and meteorological measurement on the *E.coli* levels, correlations using Spearman's rho analysis were run keeping the *E.coli* level as the independent variable and all others as dependant variables. The results are shown in Table 3-2.

Table 3-2. Statistical Analysis of *E. coli* Concentration vs. River Flow, Rainfall, Air Temperature, Humidity, Soil Moisture, Ground Temperature, Solar Radiation, Wind Speed, Water Temperature, and Duck Count. (n = 96)

Statistical Formula	Flow	Rain 24 hrs	Rain 48 hrs.	Rain 72 hrs.
Spearman's rho	0.368**	0.349**	0.510**	0.512**
Statistical Formula	Temp hi	Temp low	Hum hi	Hum lo
Spearman's rho	0.079	0.222*	0.233*	0.330**
Statistical Formula	Soil mst hi	Soil mst low	Grnd tmp hi	Grnd tmp lo
Spearman's rho	0.361**	0.262*	0.157	0.166
Statistical Formula	Solar	Windspd	Water tmp^a	Ducks^b
Spearman's rho	-0.016	0.035	-0.129	0.010

* Statistically significant at $p < 0.05$; ** Statistically significant at $p < 0.01$.

a Water temperature had n = 33

b Duck count had n = 40

From the statistical analysis, river flow, rain, humidity and soil moisture were significantly correlated to *E.coli* concentrations. This group of factors are all related to each other since when it rains humidity increases, soil moisture increases and river flow increases. It is not surprising that all the variables from this group are significantly correlated with *E.coli* levels. Ducks have been shown to be a source of *E.coli* and have been shown as a causation of illness to swimmers (Ackman 1997; Samadpour 2002). From this study there is no correlation between *E. coli* concentration and the number of

ducks in the river upstream from the sampling point. In general, temperature, whether air, soil or water, did not correlate to *E.coli* levels with the exception of the low temperature of the day that the sample was collected. Solar radiation and wind speed were not significantly correlated with *E.coli* concentrations.

Correlograms were calculated for each variable in each year. They indicated that there was no significant spatial correlation between the variables. Hence, it is assumed that the *E.coli*, 72-hour total rainfall (rain72), and air temperature measurements collected at weekly intervals are independent. Analysis of the partial Spearman rank correlation coefficients with rain72 controlled produced no significant correlations between *E.coli* and any of the variables directly or indirectly related to amounts of cumulative 72 hr precipitation, including flow, 24-hour total rainfall (rain24), 48-hour total rainfall (rain48), humidity and soil moisture. The variables that had significant effect on the *E.coli* when the amounts of cumulative 72-hour precipitation have been controlled for were air and soil temperature. Because of this observation and also because of the discussed above absence of the of the increase in *E.coli* in spring when the overall flow is higher than in the other seasons, we may conclude that although flow is significantly positively correlated with *E.coli*, the correlation is rather spurious since the amounts of cumulative precipitation in past 48 or 72 hours is the driving force of both increase in flow and in *E.coli* concentration. Higher correlation between rain72 with *E.coli* than those between flow and *E.coli* can also be partially explained by the fact that high intensive rains not only produce high flow but also increase the runoff from the watershed carrying soil, manure and other potential sources of *E.coli*. High flow, on the

other hand, may result from a sequence of relatively mild rains that did not increase amount of runoff sediment.

Once correlations were identified, the variables that were significantly correlated were then analyzed using a logistic regression to determine the risk factors of each variable. Since river flow, rain, humidity and soil moisture were significantly correlated to *E. coli* concentration they were used as the factors in the logistic regression. Two different logistic regressions were run with different thresholds, one threshold value at the *E. coli* concentration of 300cfu/100ml the highest acceptable full body contact level and 1000cfu/100ml the highest acceptable partial body contact level.

The logistic regression model for describing the probability of *E.coli*>300 as a function of rain72:

$$\log\left(\frac{\pi_{>300}(Rain_{72})}{1 - \pi_{>300}(Rain_{72})}\right) = -0.79 + 1.6Rain_{72}$$

The observed probabilities of *E.coli*>300 and those predicted by the model are shown in Figure 3-10. The rain72 data were divided in several classes such that there is a sufficient (>5) number of events with *E.coli*>300 in each class. The observed probabilities were calculated by dividing the number of occurrences of *E.coli* >300 observed in each rain72 class by the total number of *E.coli* observations in this class.

Positive slope of the logistic regression equation ($p < 0.001$) indicates that the probability of *E.coli* levels above 300 increases as rain72 increases. The lowest probability is observed at zero rain72 and is equal to approximately 30%. The probability of *E.coli*>300 reaches 95% for rain72 values exceed 2.4 cm. For every 1 cm increase in rain72 values the odds of observing *E.coli* concentrations >300 increase on average $e^{1.6} =$

5 times. The 95% confidence interval for the logistic regression coefficient for rain72 is equal to 0.71 to 2.47, hence we may conclude that the odds of *E.coli* exceeding 300 are increasing at least twofold and at most twelve-fold for each 1 cm increase in rain72. This result is supported by requirements in southern California to post health warnings on beaches following all storms in which the rainfall is 1.25 cm or higher (Noble 2003).

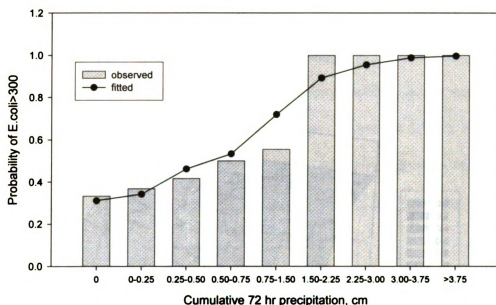


Figure 3-10. Probability of *E.coli* >300 cfu/100ml Resulting from Logistic Regression Based on Cumulative 72 Hour Rainfall.

The only two variables that were statistically significant in the logistic regression for *E.coli* >300 were rain72 ($p < 0.001$) and tempo ($p < 0.05$):

$$\log \left(\frac{\pi_{>300}(Rain_{72})}{1 - \pi_{>300}(Rain_{72})} \right) = -1.57 + 1.79Rain_{72} + 0.082Temp_{low}$$

Plot of the predicted probability of *E.coli*>300 as a function of rain72 and low temperature of the day of sampling (templo) is shown in Figure 3-11. At zero rain72, the probability of *E.coli*>300 is equal to ~11% if the templo is -6.5 °C, however, the probability increases to 50% as the templo increases to 18 °C. At lower templo (<0°C) the 95% probability of *E.coli* concentration>300 is reached at approximately 1.3 cm rain72, however at templo>15°C degrees the 95% probability of *E.coli* >300 occurs at 0.25 cm rain72. The 95% confidence intervals for the logistic regression coefficients for rain72 and templo are equal to 0.80-2.77 and 0.01-0.15, respectively.

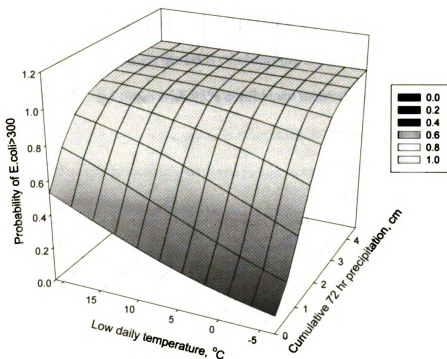


Figure 3-11. Probability of *E.coli* >300 cfm/100ml Resulting from Logistic Regression Based on Cumulative 72 Hour Rainfall and Low Air Temperature.

Since only 17 observations existed with *E.coli*>1000, the number of independent variables that could be used in the regression equation had to be limited to 3 (Stokes 2000). Hence, the logistic regression for >1000 was conducted using several combinations of three independent variables. The only variable that was statistically significant in the logistic regression was rain72 (p<0.01):

$$\log\left(\frac{\pi_{>1000}(Rain_{72})}{1 - \pi_{>1000}(Rain_{72})}\right) = -2.55 + 0.91Rain_{72}$$

Plots of the observed probabilities of *E.coli* >1000 calculated for grouped rain72 data are shown in Figure 3-12. The lowest probability is observed at zero rain72 and is equal to approximately 7%. The highest probability predicted by the model for the observed data was equal to 82% at the highest rain72 value of 4.5 cm. For every 1 cm increase in rain72 values the odds of observing *E.coli* concentrations >1000 increase on average $e^{0.91} = 2.5$ times with 95% confidence interval 1.6 to 3.9 times.

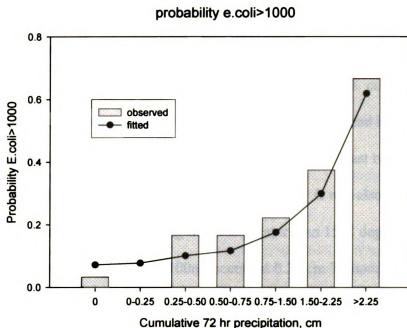


Figure 3-12. Probability of *E.coli* >1000 cfm/100ml Resulting from Logistic Regression Based on Cumulative 72 Hour Rainfall.

Conclusions

Based on these analyses, it is concluded that there is no observed seasonal effect for *E. coli* levels from the Farm Lane Bridge over the three-year period from 2000 to 2002. From the statistical analysis using Spearman's rho, river flow, rain, humidity, low temperature of the sampling day and soil moisture were significantly ($p < 0.05$) correlated to *E. coli* concentrations.

Other studies support the association of the levels of fecal indicators in a water body and rainfall events and the results presented in this study support these findings. In addition, previous studies have shown that increased river flow has been related to increases in the concentration levels of *E. coli* in a river and the results presented in this study also support these findings. From this study there is no correlation between *E. coli*

concentration and the number of ducks in the river upstream from the sampling point.

Solar radiation and wind speed did not correlate to *E. coli* concentrations.

Using governmental guidelines for maximum *E. coli* level for the safe use of water for recreation, statistical models were designed and it was concluded that the odds of *E. coli* exceeding 300 cfu/100ml are increasing at least twofold and at most twelve-fold for each 1 cm increase in 72-hour total rainfall. It was also concluded that if the low temperature of the sampling day was greater than 15°C degrees the 95% probability of *E. coli* exceeding 300 cfu/100ml occurs at 0.25 cm 72-hour total rainfall.

The results that have been presented indicate that programs to control run off from riparian land during a rain event will have the greatest impact on lowering risk of sickness to swimmers from fecal contamination.

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Chapter 4

The Effect of Rainfall, Nutrient Levels, and Land-use on *E. coli* Levels in the Red Cedar River

Introduction

Escherichia coli (*E. coli*) is the type species of the genus *Escherichia*, which contains mostly motile rod-like gram-negative bacilli within the family *Enterobacteriaceae* and the tribe *Escherichia*. *E. coli* bacteria live in the digestive systems of humans and other warm-blooded animals. *E. coli* can be found in the fecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls (Hancock 2001; Niemis and Niemis 1991).

There are a variety of sources that contribute bacteria and other pathogens to the surface water. These sources include illicit waste connections to storm sewers or roadside ditches, septic systems, combined and sanitary sewer overflows, storm (rain) runoff, wild domestic animal waste, and agriculture runoff. Each of these sources is related to a type of land-use and the type of land-use may have an impact on the amount of *E. coli* and other nutrients that are discharged into nearby surface water.

Population growth in coastal areas is increasing at a rate double that of population growth worldwide. It is estimated that billions of gallons of treated and untreated wastewater are discharged daily into the world's coastal waters. In developing nations, 90% of untreated sewage from urban areas is dumped into streams and oceans (Crossette 1996). Coastal ecosystems are under increasing stress from a variety of human activities that cause increased pollution, floral and faunal changes (Vitousek 1997; Epstein 1998).

E. coli sources have been linked to land use, mainly agricultural operations. Cow manure has specifically been implicated as a causative factor in the high bacteria levels and ensuing swimming restrictions on Tainter Lake, Wisconsin (Behm 1989). Among the many outbreaks reported, studies have been published from outbreaks in Scotland (Coia 1998; License 2001), Missouri (Swerdlow 1992) and Idaho (Vane Every 1995), all of which were associated with agricultural activities.

In contrast, studies have shown that residential development has had effect of elevated *E. coli* levels (Frenzel and Couvillion 2002; Smith 2001; Mallin 2000). It has been suggested that impervious surface may have be the cause for the elevated *E. coli* levels in the urban areas (Mallin 2000). However, a study of an urban area to determine sources of fecal contamination in river determined that the source was domesticated animals and wildlife (Murry 2001). In a study of pristine lands in the fiords of Scandinavia, it has been shown that fecal concentration can be above safe levels (Niemis and Niemis 1991).

There are many examples of GIS used as a tool in the analysis of watersheds. Moreover, GIS has been used to model the activities and systems of a watershed. Non-point pollution in a watershed has been a natural to model using GIS tools. BASINS designed by the EPA (USEPA 2002b) STREAM (Spatial Tools for River basins, Environment and Analysis of Management options) (Schepel 1998), and SIMPLE (Spatially Integrated Models for Phosphorus Loading and Erosion) (Kornecki 1999) are examples of popular models that have designed for the evaluation of non-point pollution. Agricultural land-use has been studied for non-point run-off. AGNPS (Agricultural Non-Point Source) is a model designed by the USDA (Grunwald 2000, USDA 2002) for use in

determining the impact of agricultural activities on a watershed. AGNPS has been used to determine the agricultural impact on coastal and estuarine ecosystems (Choi 1999) and has been modified to integrate ARC/INFO databases in order to evaluate non point source problem areas (Liao 1997). In addition, models have been designed to study impact of the use of buffer strip on the water quality (Tim 1994). GIS tools have been used to evaluate non-point pollution of surface waters with phosphorus and nitrogen (Carpenter 1998, Robinson 1993). Additional GIS methods have been employed to help understand sediment loading from agricultural land use (Rudra 1999).

Of course agricultural lands have not been the only areas studied. Urban systems also have an impact on a watershed and GIS tools have been employed to study these impacts. Storm-water management systems and their impacts have been modeled using GIS (Shamsi 1996). Estimations of mass loading from these types of systems evaluated (Wong 1997, Adamus 1995). Larger scale studies have used the entire watershed in order to determine problem areas and what corrective actions could be implemented. The optimization the mix of Best Management Practices (BMP) to reduce the loading on a waterbody has been the goals of some research (Sample 2001, Wang 2000). GIS has been used to manage the ecosystems of a watershed (Crawford 1998).

The impact of land use on a watershed is an obvious use of GIS tools but only recently studies on this subject have been published. Correlation between water quality using conductivity as the measure and urban land use was identified (Wang 1997). Other assessments of water quality and land use have determined with out surprise that land use does impact water quality (Wang 2001, Bhaduri 2000). There is very little in the literature on using GIS in a watershed to determine risk from microbial pathogens based

on land use. There are published results investigating septic systems as potential pollutant but this study used nitrate as its measure (Stark 1999). In Ontario geographic distribution of *E. coli* O157:H7 infection and was compared to cattle population (Michel 1999). The results indicate that cattle density had a positive and significant association with the incidence of reported cases. GIS has been used to model and predict pathogen loading from livestock (Fraser 1998).

The relationship of nutrients levels to *E. coli* concentrations in surface water has rarely been reported in the literature. Total suspended solid were strongly correlated to indicator bacteria when flow rates were the highest (Pettibone and Irvine 1996). In another study, a statistical analysis showed that there was a significant correlations between levels of nitrate as well as levels of phosphate with the indicator bacteria fecal coliform (Daby 2002). Heavy loadings of organic and inorganic nutrients can change the ecological balance, stimulating nuisance organisms (Burkholder 1997) and in some cases affecting the virulence of indigenous species (Bates 1991).

The objective of this study is determine if the type of land-use around a sampling point is associated with elevated *E. coli* concentration levels. Another objective of this study is to determine if the type of land-use around a sampling point is associated with elevated levels of nutrients. In addition, an objective of this study is determine if there is any association between the concentration levels of nutrients and the concentration levels of *E. coli*. It is hypothesized that agricultural land-use has the highest discharges of *E. coli* and nutrients and therefore has a greater negative impact to the Red Cedar River than other types of land-use.

Materials and Methods

The Red Cedar River arises in Cedar Lake in the south-central Lower peninsula of Michigan and flows about 45 miles (80 kilometers) to its confluence with the Grand River in the city of Lansing. It has 12 tributaries and drains a total area of about 472 square miles (22,000 hectares). The river provides mid-Michigan residents with numerous recreational opportunities that include angling, canoeing, kayaking, photography and bird watching. The river also serves as a source of water for the irrigation of crops throughout the watershed, as well as, drainage for agricultural land.



Figure 4-1. The Red Cedar River Watershed with The Michigan State University campus.

A data set that included 17 sampling points was studied over 13 sampling dates over a 32-week period in 2001. The water samples for this multiple sampling point study

were collected by the Ingham County Health Department and the Livingston County Health Department depending on the county in which the sampling point was located. All water samples were sent to the MDEQ for the determination of *E. coli* levels using EPA method 1103.1 (Dufour 1981; USEPA, 1985; USEPA 1986; USEPA 2003). In this multiple sa outside lab were the standard methods that are recognized by the State of Michigan for such water samples for analyzing these nutrients. In addition, rainfall data from three different sites that include East Lansing, Williamston, and Howell so that the rainfall data better represents each of the sampling sites. In addition, the 17 sampling points were part of a larger 38 sampling point studies that collected *E. coli* data weekly for 20 consecutive weeks (July to November) in 2000 and the 17 sampling point study, the dataset includes results for the following nutrients: ammonia nitrate, total phosphorus, and total suspended solids. The nutrient results were generated by an outside lab from the water samples that were split with the MDEQ. The methods that were used by the for 32 consecutive weeks (April to November) in 2001.

The land use was determined by using the GIS program ArcView 3.1, (ESRI Redland CA) with a National Land Cover Data (NLCD) layer for the watershed. Each sampling point was located on the watershed map and a one-mile radius buffer was created around the point. The land use was calculated on the semi circle of the buffer that was upstream of the sampling point. If the land use for the calculated buffer was less than 85% of one land use as categorized by the NLCD then the land use for that sampling point was determined to be a mixed use. The land use variable for this analysis were either urban, mixed, or agriculture. For the impact of each nutrient and rainfall measurement, as well as, land use data on the *E. coli* levels, statistical analysis were

conducted using the Spearman's rho keeping the *E. coli* levels as the independent variable with rainfall, ammonia nitrate, total phosphorus, and total suspended solids set as dependant variables. Statistical analysis was completed using SPSS version 11 software package.

Results and Discussion

The graphical illustration shown in Figure 4-2 depicts the mean *E. coli* concentration for the 17 sampling points. This represents 17 sampling points that were studied over 13 sampling dates over a 32-week period in 2001 and has 221 *E. coli* samples in the statistical analysis. From the analysis shown in Figure 4-2, mixed land use has the highest impact on elevated *E. coli* levels and is followed by urban land use and agricultural land use has the lowest impact on elevated *E. coli* levels. This difference is significantly different at $p < 0.05$.

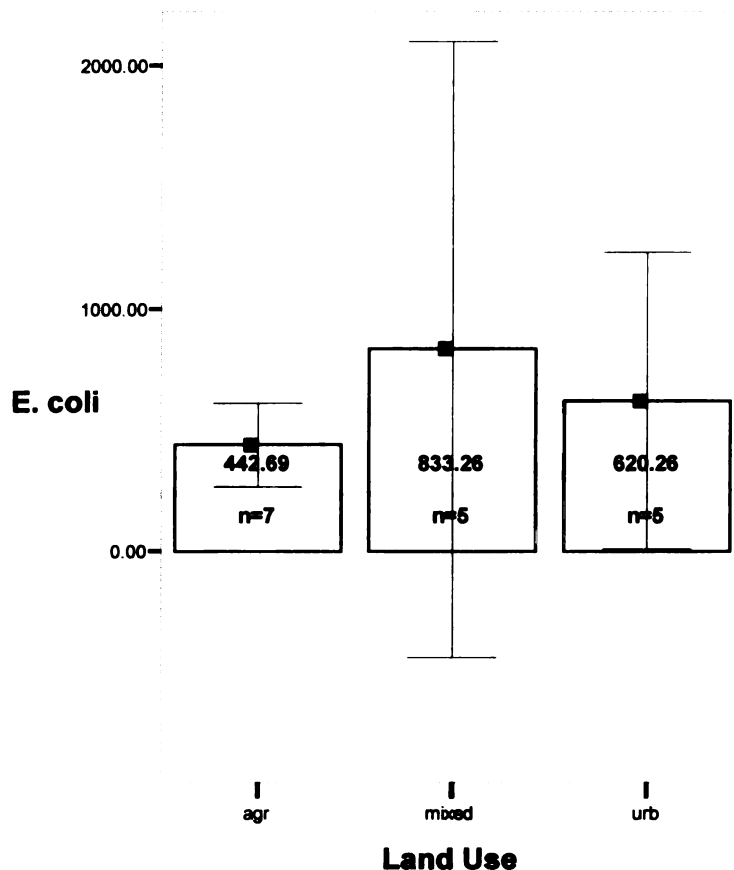


Figure 4-2. Means *E. coli* vs. Land use for 17 Selected Sampling Points
 * vertical bars represent plus or minus one standard error of the mean.

The graphical illustration shown in Figure 4-3 depicts the mean ammonia nitrate concentration for the 17 sampling points. This represents 17 sampling points that were studied over 13 sampling dates over a 32-week period in 2001 and has 221 ammonia nitrate samples in the statistical analysis. From the analysis shown in Figure 4-3, mixed land use has the highest impact on elevated ammonia nitrate levels and urban land use and agricultural land use had similar low impact on elevated ammonia nitrate levels. This difference is significantly different at $p < 0.05$.

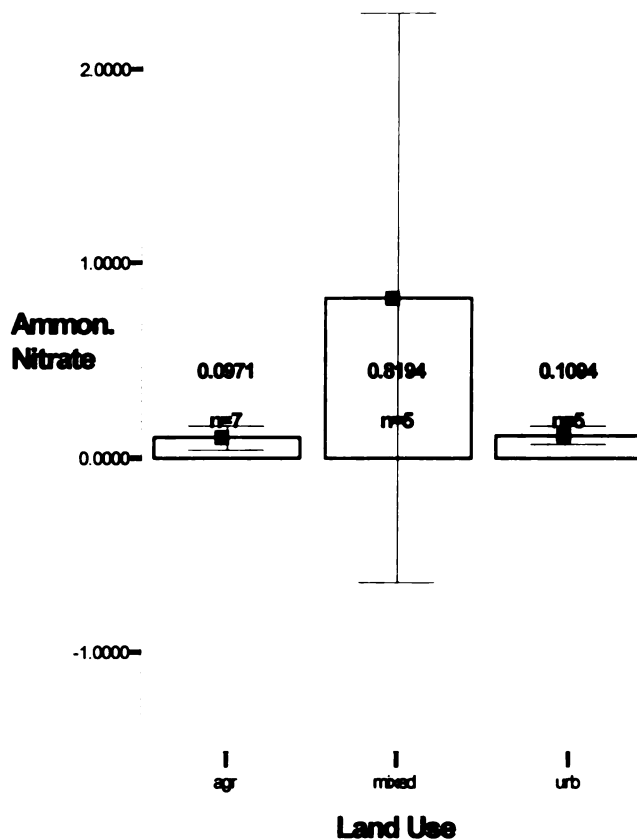


Figure 4-3. Mean Ammonia Nitrate vs. Land use for 17 Select Sampling Points

* vertical bars represent plus or minus one standard error of the mean.

The graphical illustration shown in Figure 4-4 depicts the mean total phosphorus concentration for the 17 sampling points. This represents 17 sampling points that were studied over 13 sampling dates over a 32-week period in 2001 and has 221 total phosphorus samples in the statistical analysis. From the analysis shown in Figure 4-4, mixed land use has the highest impact on elevated total phosphorus levels and is followed by urban land use and agricultural land use has the lowest impact on elevated total phosphorus levels. This difference is not significantly different.

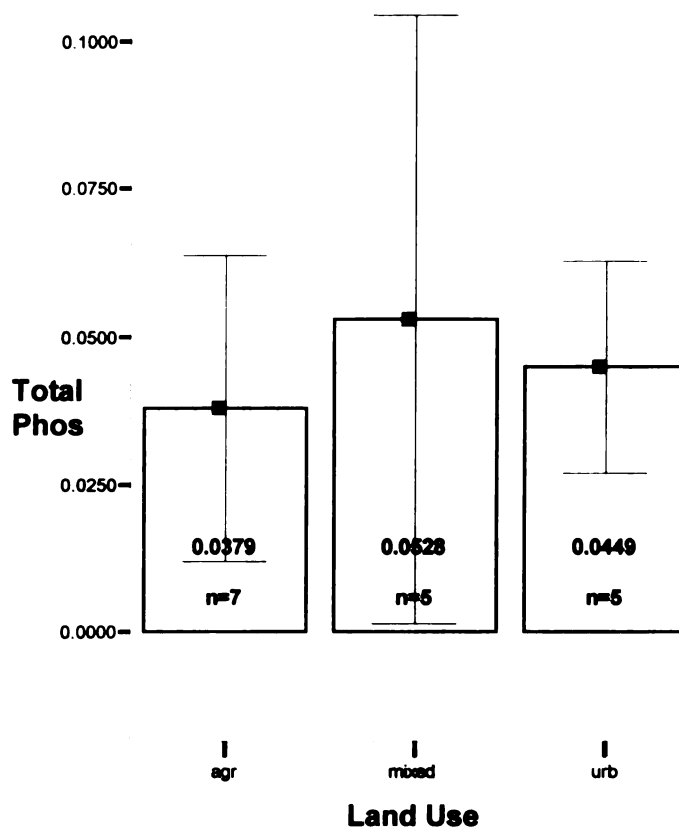


Figure 4-4. Mean Total Phosphorus vs. Land use for 17 Select Sampling Points
 * vertical bars represent plus or minus one standard error of the mean.

The graphical illustration shown in Figure 4-5 depicts the mean total suspended solids concentration for the 17 sampling points. This represents 17 sampling points that were studied over 13 sampling dates over a 32-week period in 2001 and has 221 total suspended solid samples in the statistical analysis. From the analysis shown in Figure 4-5, agricultural land use has the highest impact on elevated total suspended solids levels

and is followed by urban land use and mixed land use has the lowest impact on elevated total suspended solids levels. This difference is significantly different at $p < 0.05$.

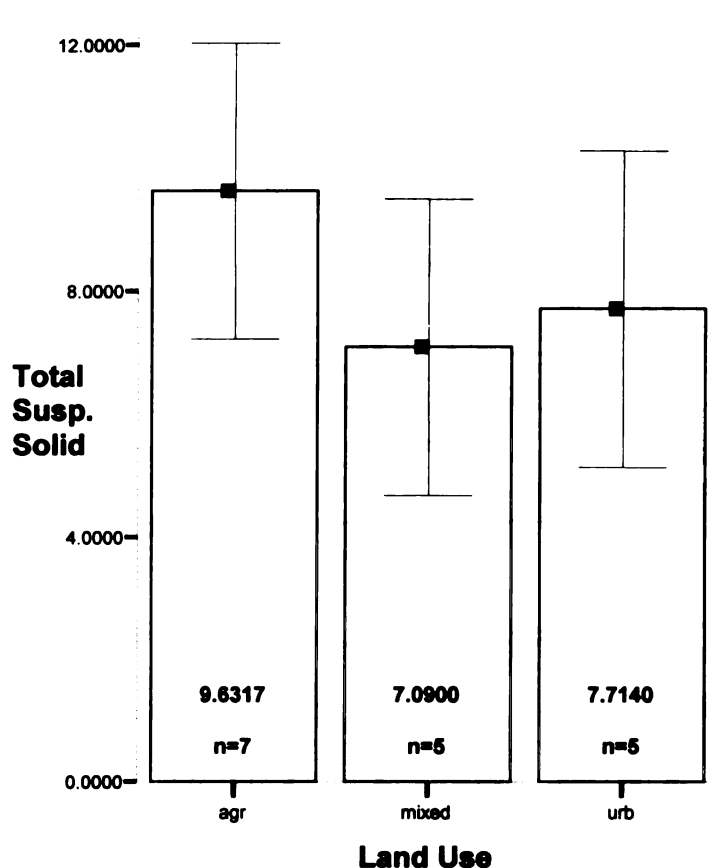


Figure 4-5. Mean Total Suspended Solids vs. Land use for 17 Select Sampling Points
* vertical bars represent plus or minus one standard error of the mean.

The dataset included not only the results for ammonia nitrate, total phosphorus, and total suspended solids but also rainfall. The rainfall data was collected over the day of sampling along with the two days previous to sampling and data was calculated and

reported as a cumulative total over a 24, 48, or 72 hour period. This dataset represents 17 sampling points that were studied over 13 sampling dates over a 32-week period in 2001. For the impact of each rainfall and nutrient measurement on the *E. coli* levels, statistical analysis were conducted using the Spearman's rho keeping the *E. coli* levels as the independent variable with rainfall, ammonia nitrate, total phosphorus, and total suspended solids set as dependant variables. The results are shown in Table 4-1.

Table 4-1. Statistical Analysis of *E. coli* Concentration vs. Rainfall, Ammonia Nitrate, Total Phosphorus, and Total Suspended Solids (n = 221).

Statistical Formula	Rain 24 hrs	Rain 48 hrs.	Rain 72 hrs.
Spearman's rho	0.067	-0.012	0.084

Statistical Formula	Am. Nitrate	Total Phos.	TSS
Spearman's rho	0.274**	0.033	0.169*

* Statistically significant at $p < 0.05$; ** Statistically significant at $p < 0.01$.

From the statistical analysis, ammonia nitrate was significantly correlated to *E. coli* concentrations. The total suspended solids were significantly correlated to *E. coli* concentrations. From this data, elevations in these nutrients do have a correlation with elevated *E. coli* concentrations in the Red Cedar River.

It is interesting to note that rainfall did not show any correlation using either statistical analysis. This is contrary to other reported studies including a study during the same time period on the Red Cedar River (Lang 2003). Other studies support the association of the levels of fecal indicators in a water body and rainfall events. In a study that examined 99 samples from three tributaries that contributed to different drinking water reservoirs showed that *E. coli* levels along with other bacteriological parameters

increased considerably during extreme rainfall events (Kistemann 2002). In a related study on Delaware River, increased concentrations of *Giardia*, *Cryptosporidium* and other microorganisms were associated with rainfall (Atherholt 1998). In a retrospective study on waterborne disease outbreaks in the U.S. for 1948 to 1994, analyzed 548 reported outbreaks as documented by the USEPA database. The results from this study showed that 51% of waterborne disease outbreaks were preceded by precipitation events above the 90th percentile and 67% by events above the 80th percentile (Curriero 2001). This study concluded that there is a statistically significance association between rainfall and waterborne disease outbreaks. Other studies also conclude that *E. coli* levels increase with rainfall (Briski 2000; Ferguson 1996; Atherholt 1998; Pettibone and Irvine 1996; Niemi and Niemi 1991; Mallin 2001; Noble 2003). A recent study suggests that an increase in rainfall or snowmelt increases the impact of diseases caused by microbiologic agents (Rose 2001). In reviewing the data from this study, the rainfall data was collected from three different sites that include East Lansing, Williamston, and Howell so that the rainfall data better represents each of the sampling sites. These rainfall collection sites were not at the 17 sampling sites and probably did not represent the real rainfall that occurred at the 17 sampling sites during the time period that the river water samples were collected.

An expanded dataset that included the sampling sites to include all 38 sampling sites over the two-year period was evaluated to determine the impact of land use on *E. coli* concentrations. The graphical illustration shown in Figure 4-6 depicts the mean *E. coli* concentration for the 38 sampling points. This represents the 38 sampling points that were collected *E. coli* data weekly for 20 consecutive weeks (July to November) in 2000

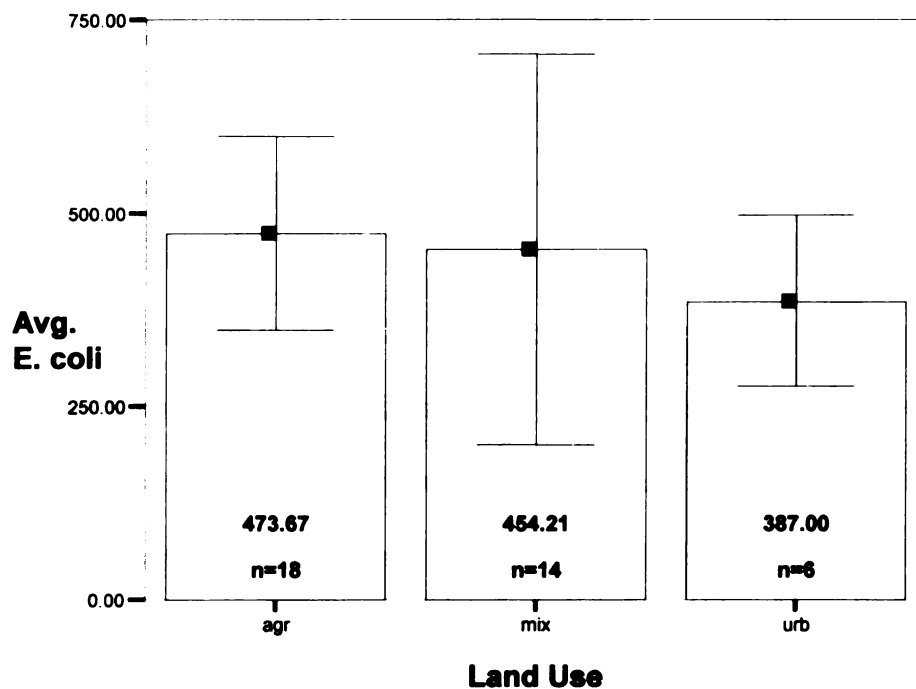


Figure 4-6. Mean *E. coli* Levels vs. Land use for all 38 Sampling Points
 * vertical bars represent plus or minus one standard error of the mean.

and for 32 consecutive weeks (April to November) in 2001 and has 1976 *E. coli* samples in the statistical analysis. From the analysis shown in Figure 4-6, agricultural land use has the highest impact on elevated *E. coli* levels and is followed by mixed land use and urban land use has the lowest impact on elevated *E. coli* levels but this is not significantly different. This result is in contrast to the results shown in Figure 4-2 in which agricultural land use had the lowest impact on elevated *E. coli* concentrations.

Conclusions

Based on the results from this study, it is concluded that elevations of nutrients do have an association with elevations in the levels of *E. coli* concentration. The concentration levels of ammonia nitrate were significantly correlated to *E. coli* concentrations and the levels of total suspended solids were significantly correlated to *E. coli* concentrations.

These results indicate that if the locations of the sources of nutrients that enter into nearby surface water are identified then proper best management practices (BMP) can be implement at these locations and the implemented BMP will have an impact on controlling nutrients into the surface water as well as having an effect to lower the *E. coli* concentration in the surface water.

However, in this study rainfall does not show correlation with *E. coli* levels and this is contradictory to what has been reported by others. This contradiction maybe explained by the fact that the rainfall data was collected from three different sites that include East Lansing, Williamston, and Howell so that the rainfall data better represents the sampling site. These rainfall collection sites were not at the 17 sampling sites and probably did not represent the real rainfall that occurred at the 17 sampling sites during the time period that the river water samples were collected.

The impact of land use on *E. coli* concentrations was investigated but the results did not reveal with any certainty as to which type of land-use has the greatest impact on elevated *E. coli* concentrations. In the small sample that was analyzed, agricultural land use had the greatest impact on elevated total suspended solids. However, mixed land use had the greatest impact on elevated ammonia nitrates. Larger sample sets need to be

studied to determine if the type of land-use does have an impact the concentration levels of *E. coli* and other nutrients in nearby surface water.

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Chapter 5

A Linkage between Technology and Policy in Evaluating the Risk of Microbial Safety in Recreational-use Water

Introduction

Ever since fecal contamination of water was determined as a human health risk, there has always been a great deal of concern regarding the level of coliform bacteria counts in water. Many bodies of water throughout the world are considered to have bacteria counts above acceptable levels. The sources of these bacteria are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow.

The United States Public Health Services (USPHS) conducted a series of studies in the 1940s and 1950s that showed a causal connection between fecal coliform and gastrointestinal sickness exists (Stevenson 1953). In the late 1970s, the EPA conducted a landmark prospective cohort study and reported a linear relationship between the incidence of gastroenteritis among swimmers and marine bacterial counts (Cabelli 1982). Between 1973 and 1978, participants were recruited at beaches from three U.S. locations (New York; Lake Pontchartrain, Louisiana; Boston, Massachusetts) and were contacted by telephone days after going to the beach. Swimming status was self-selected, not randomly assigned, and symptoms were self-reported. The mean proportion of swimmers with gastrointestinal symptoms was 6.8% versus 4.6% in non-swimmers (Cabelli 1982). When bacterial concentrations were above 1/100 ml, relative risk increased linearly, reaching 4.0 with concentrations of 1,000/100 ml ($p < 0.001$). In addition, the frequency

of gastrointestinal symptoms was inversely related to the distance from known sources of municipal wastewater (Cabelli 1982). Since these studies, a large body of literature involving the epidemiology of illness from the use of recreational water has been published (Examples include studies by: Seyfried 1985; Van Asperen 1998; Henrickson 2001; Prieto 2001)

Beach closings and illness after exposure to marine water may be increasing in frequency (Harvell 1991). There were over 12,000 coastal beach closings and advisories in the United States from 1988 to 1994 (an increase of 400% over that period), with over 75% of the closings due to microbial contamination (Barton 1995). A recent report compiled by the National Resources Defense Council (NRDC), which surveyed more than 200 waterfront communities, found that during 1999 there were at least 6,160 beach-use days of closings and advisories at beaches (NRDC 2000). According to the most current report, the NRDC's twelfth annual beach report, at least 13,410 closings and advisories were issued across the country in 2001, a 19 percent jump over the previous year (NRDC 2002).

Public Policy to Reduce Risk in Recreational Water

One of the major goals of the Clean Water Act of 1972 and its amendments (CWA) is to ensure that U.S. waters are safe for fishing and swimming (33 U.S.C.A. §§ 1251 to 1387). The use of water quality indicators that accurately reflect "safety" is essential for this goal. The CWA mandates the use of indicator organisms to determine safe levels and CWA section 303(d) requires the reporting of "impaired waters." In addition, section 303, establishes the water quality standards and Total Maximum Daily

Load (TMDL) programs. The TMDL program is required by the rules that amended the CWA issued in 1985 and then subsequently amended in 1992 (USEPA 2002).

TMDL is a calculation of the maximum amount of a pollutant that a waterbody can receive while still meeting water quality standards, and then determines an allocation of that amount to the pollutant's sources. Water quality standards are set by States, Territories, and Tribes and must meet or exceed the Federal standards set forth in the CWA. These entities identify the uses for each waterbody, for example, drinking water supply, contact recreation (swimming), and aquatic life support (fishing), and the scientific criteria to support that use. A TMDL is the sum of the allowable loads of a single pollutant from all contributing point and non-point sources. The calculation must include a margin of safety to ensure that the waterbody can be used for the purposes the State has designated. Additionally, the calculation must also account for seasonal variation in water quality.

The indicator organism mandated for the monitoring of pathogens in fresh water is *E. coli*. If the level of this organism is consistently elevated from the standard, then the waterbody is declared an "impaired water" as defined in CWA 303 (d) and a TMDL is required to be developed and implemented for that waterbody. As of 2001, there are 5,512 reported "impaired waters" due to pathogens (over 13% of all impairments reported and this is the second highest impairment behind sediment which is just below 14%) (USEPA 2001).

The Beach Environmental Assessment Closure and Health (BEACH) Act was signed into law in October 2000 and amended the CWA (USEPA 2002b). This

legislation directs the EPA Administrator to conduct studies to review health risks; to develop indicators to detect the presence of pathogens; to offer guidance for state-to-state application of the revised water quality criteria; to publish and revise regulations requiring the monitoring of coastal waters; to provide technical assistance to states for uniform assessment and monitoring procedures; and to establish a national coastal recreation water pollution occurrence database and a listing of communities complying with regulations published pursuant to this legislation. In addition, the Beach Act authorizes \$30 million annually for grants that would help states, local governments and Indian tribes to monitor beach waters and notify the public when beach water exceeds the established criteria. Moreover, some of these grants are designated for the development of improved detection of pathogens in water, both freshwater and marine water.

It is mandated that consistent national health standards for beach water be established by 2004. Currently, the EPA has determined that *E. coli* level is the best indicator of risk to swimmers in fresh water. An *E. coli* concentration of less than 126 CFU/100ml, calculated as a geometric mean over 30 days, is considered to be safe for full body contact recreational use of water (USEPA 1986). If a waterbody has a designated use of full body contact recreational use and the *E. coli* levels are consistently elevated from the above described standard, then the waterbody is declared an “impaired water” as defined in CWA 303 (d) and a TMDL is required to be developed and implemented for that waterbody.

Many county health departments in the State of Michigan routinely collect water samples at beaches to determine if the water is safe for swimming. Samples are generally

taken one foot below the surface in water that is between three and six feet in depth. The analysis is performed in a laboratory using standard methods. *E. coli* bacteria are counted and judged against standards established by state rules. Results from the method are available after approximately 28 hours; so the results from this analysis are reported the following day.

County health departments take a minimum of three samples each time a beach area is monitored. The daily geometric mean calculated from these samples must be below 300 *E. coli* per 100 milliliters for the water to be considered safe for swimming. One or two of the samples may be above 300, but if the daily geometric mean is below 300, the beach is not in violation of the water quality standard. A minimum of five sampling events (consisting of at least three samples per event) must be collected within a 30-day period for the results to be considered a reliable indication of water quality. After 30 days, a geometric mean is calculated for all the individual samples collected within that time frame. This 30-day geometric mean must be below 130 *E. coli* per 100 ml for the water to be considered safe for swimming. Although the method yields results that are very accurate, the results are not reported in less than 24 hrs. Officials close beaches based on old information that may or may not be the actual condition of the water at the time of closure and leave prior groups exposed.

E.coli O157:H7

In the early 1980s, *E. coli* O157:H7 was recognized as a pathogen (Riley 1982). The toxicity to humans from this pathogen has been reported to be as low as 10 cells (Phillips 1999). There are an estimated 73,000 cases of *E. coli* O157 infections per year

in the United States, of which approximately 62,000 are food-borne and 11,000 are waterborne (Mead 1999). These estimates consider waterborne cases from ingestion of water as food not from recreational use of a waterbody. Examples of waterborne cases include Walkerton, Ontario where the town's water supply was contaminated by *E. coli* O157:H7 (not included in the estimations because it happened in Canada in the year 2000). The identified cases of *E. coli* O157 infections from the use of recreational water are limited to a few epidemiological studies (Mudgett 1998; Friedman 1999; Olsen 2002).

Since the current accepted analysis for *E. coli* and *E. coli* O157:H7 takes days, officials close beaches based old information that may or may not be the actual condition of the water at the time of the closure and thus leaving prior groups exposed. What is needed is a rapid method that can give information on the beach conditions so that officials can make decisions on closure before the public is exposed to the water. This is supported by a study which conducted on beaches in Lake Michigan over a 12-year period concluded that current protocol is inadequate for predicting risk at the time of beach closure (Whitman 1999). The biosensor in this study that is evaluated and compared to the existing methods produces results in about 10 minutes and is inexpensive. It is believed that the use of such a device would increase the public's safety in the use of recreational water.

Biosensors

A biosensor is an analytical device that integrates biological sensing elements with a transducer (Turner 1998; Ivnitski 1999). The general function of a biosensor is to

convert biological events into a quantifying electrical response (Cahn 1993). Electrochemical immunoassays are biosensors made up of antibodies as biological sensing elements attached to an electrochemical transducer (Zhang 2000). The performance of a biosensor based on polyaniline as an electrochemical transducer in measuring an immunological reaction has been evaluated (Muhammad-Tahir and Alocilja, 2002). Polyaniline in particular, has been one of the most extensively investigated conducting polymers, due to its excellent stability in liquid form, promising electronic properties (Syed 1991) and strong biomolecular interactions (Imisides 1996).

Objectives

The main focus of this project is to improve the safety of recreational-use water through better communication of the actual risk. The specific objectives are: 1) to determine if rain or river flow has an influence on the concentration of *E. coli* and *E. coli* O157:H7; 2) to evaluate if total *E. coli* measured maybe used as an indicator of pathogenic *E. coli* O157:H7 contamination; 3) to evaluate if a biosensor maybe employed to measure quickly the presence of *E. coli* and *E. coli* O157:H7.

Materials and Methods

The Project Site

The Ingham County Health Department (ICHHD), Michigan Department of Environmental Quality (MDEQ), Michigan Department of Community Health (MDCH), and Michigan State University (MSU) collaborated on a study to determine the amount of *E. coli* and pathogenic *E. coli* O157:H7 in the Red Cedar River. For four weeks in April

and May of 2002, four sites were sampled (Hagadorn, Farm Lane, Kalamazoo Street, and Putman Street; See Figure 5-1).

The samples were split with one going to MDEQ for total *E. coli* analysis and then the cultures were given to MDCH for the determination of *E. coli* O157:H7. The Biosensor Lab in the Agricultural Engineering Department at MSU analyzed the other split sample. The Biosensor Lab used a recently invented biosensor built specifically for the rapid analysis *E. coli* O157:H7.

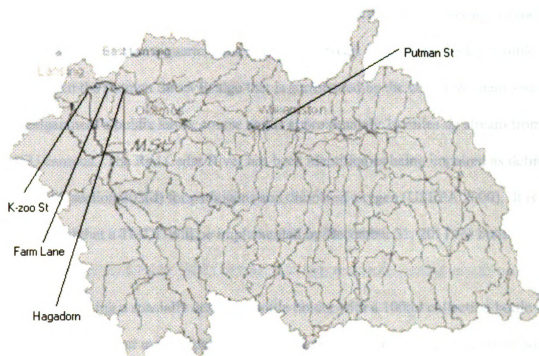


Figure 5-1. Red Cedar River Watershed with Locations of Sampling Sites

The Red Cedar River arises in Cedar Lake in the south-central Lower peninsula of Michigan and flows about 45 miles (80 kilometers) to its confluence with the Grand River in the city of Lansing. It has 12 tributaries and drains a total area of about 472

square miles (22,000 hectares). The river provides mid-Michigan residents with numerous recreational opportunities that include angling, canoeing, kayaking, photography and bird watching. The river also serves as a source of water for the irrigation of crops throughout the watershed. In terms of aesthetics, hundreds of thousands enjoy a walk along the Red Cedar River on the campus of MSU each year. Three of the weekly samples (Hagadorn, Farm Lane, and Kalamazoo Street) were taken from the 5,400 acre MSU campus which has agricultural operations two miles south of the river, traditional campus setting along the river, and the urban landscape of the city of East Lansing less than a quarter mile north of the river. The fourth weekly sample was taken from the Putman Street Bridge that is surrounded by the city of Williamston, Michigan near a public kayak course and is approximately 20 miles up stream from the MSU campus. The Red Cedar River has been identified as being impaired as defined in the CWA section 303(d) for pathogens and dissolved oxygen (USEPA 2000). It is anticipated that a TMDL will be implemented by December 31, 2011 for both impairments.

Lowering a specially designed bottle holder with a 100ml collection bottle into the Red Cedar River made it possible for the sample to be collected. The bottle holder allowed the technician to take the sample from any bridge spanning the Red Cedar. The sampling bottle was lowered from the center of the bridge until it was approximately two feet below the surface of the river and then retrieved full of river water. Four bottles were collected at each site. After the collected samples were properly labeled, they were put in a cooler filled with ice. Three of the four 100ml samples were dropped off to the

MSU biosensor lab for analysis and the remaining 100ml sample for each site was delivered to the MDEQ for analysis.

Weather Related Data Collection

Along with the variation of the concentration of *E. coli* during the study, there were variations in rainfall and river flow for the different sampling events. The National Weather Service (NWS) reported rainfall from a station in Williamston, Michigan where the Putman Street site is located. The Horticulture Research Station reported rainfall for the MSU campus on the campus. The United States Geological Services (USGS) provided the river flow data from USGS staging stations in Williamston and on the MSU campus.

E. coli Materials and Methods (EPA Method 1103.1)

The original mTEC Agar enumeration method (Dufour *et al.*, 1981) for *E. coli* was introduced by EPA in 1986 (USEPA, 1986b). A revised method was developed in 1998 by the EPA and has been designated as the modified mTEC method. Both the mTEC and modified mTEC Agar methods use the membrane filter procedure. The two membrane filter methods provide a direct count of *E. coli* in water based on the development of colonies that grow on the surface of the membrane filter (Difco Labs, Detroit MI). The MDEQ used the revised method for the analysis in this study.

A water sample is filtered through the membrane, which retains the bacteria. After filtration, the membrane containing the bacteria is placed on a selective and differential modified mTEC Agar medium, incubated at $35\pm0.5^{\circ}\text{C}$ for 2 h to resuscitate the injured or

stressed bacteria, and then incubated at $44.5 \pm 0.2^\circ\text{C}$ for 22 h. After 20 minutes, Red colonies on modified mTEC medium are counted with the aid of a fluorescent lamp and a glass lens (2–5x magnification) or stereoscopic microscope. The Enumerated colonies are forwarded to MDCH for *E. coli* O157:H7 biochemical confirmation and serotyping. This procedure adds a few more days before results are finally reported.

Biosensor Material and Methods

The biosensor used in this analysis consists of two parts: The immunosensor and the electronic data collection system. The immunosensor is comprised of four different pads: sample application, conjugate, detection, and absorption. The system was constructed as shown in Figure 5-2. The cellulose membrane was used (5 x 10 mm) for the sample application pad, fiber glass membrane for the conjugate pad (5 x 10mm), nitrocellulose (NC) membrane (5 x 20 mm) for the capture pad, and cellulose membrane (5 x 30 mm) for the absorption pad. Silver electrodes were fabricated on the NC membrane to electrically connect the immunosensor with the electronic data collection system consisting of a copper wafer and an ohmmeter linked to a computer.

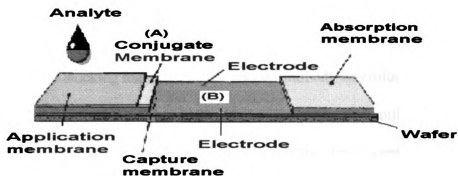


Figure 5-2. Schematic diagram of the immunosensor. Conjugate pad for polyaniline-labeled antibody absorption (A). Detection pad coated on each side with silver electrodes (B). Gap between electrodes is the site for antibody immobilization

Aniline, glutaraldehyde, N, N Dimethylformamide (DMF), Tween-20, lithium chloride, tris buffer, phosphate buffer saline (PBS) were purchased from Sigma-Aldrich (Missouri). Anti- *E. coli* O157:H7 was obtained from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Nitrocellulose (NC) membrane with a flow rate of 160 sec per 4 cm, cellulose membrane, and fiberglass membrane grade G6 were purchased from Millipore (Massachusetts). Micro-tip silver pen was purchased from Chemotronics (Georgia). Other reagents used were of analytical grade. All chemicals and diluents were prepared with doubly deionized water with conductivity below 0.1 μ S/cm. Lyophilized affinity purified polyclonal antibodies of *E. coli* O157:H7 were purchased, and stored at 4°C until rehydrated with phosphate buffer (pH 7.0). Two different concentrations of antibodies were prepared: 500 μ g/ml of antibody was used on the capture pad, and 150 μ g/ml was used for conjugating with polyaniline and coated on the conjugate pad.

A water-soluble polyaniline was synthesized by following a standard procedure of oxidative polymerization of aniline monomer in the presence of ammonium persulfate

(Kim, 2000). A mixture of the antibody and polyaniline was left to react for 30 minutes. The conjugate was then precipitated by centrifugation (13000 rpm for 3 min) using 0.1M Tris buffer as the blocking reagent. The conjugated antibody was diluted in 0.01M LiCl and stored at 4°C before use. When ready, 10µl of the conjugated antibody was applied on the conjugate pad and left to dry. Cellulose membranes for absorption and sample application pads were treated with distilled water three times to remove dirt and surface residuals. The membranes were left to dry and stored inside a petri dish to maintain a clean surface.

Affinity purified antibodies were immobilized on the NC membrane by the following steps. First, the NC membrane was saturated in 10% (v/v) methanol in water for 45 min and left to dry. The membrane was then treated in 0.5% (v/v) glutaraldehyde for 1 hour. After drying, 2.5µl of 0.5mg/ml of antibody was pipetted on the membrane, and incubated at 37°C for one hour. Inactivation of residual functional groups and blocking was carried out simultaneously by incubating the membrane with 0.1M tris buffer, pH 7.6, containing 0.1% Tween-20 for 45 min. The membrane was left in the air to dry.

All prepared membranes were arranged in the order mentioned in Figure 2 and attached onto an etched copper plate using double-sided tape. The prepared biosensor was stored at 4°C before its use. The prepared biosensor was connected to a multimeter and a computer for signal measurement. To begin detection, 0.1 ml of the water sample was pipetted onto the application pad. The generated signal was captured using a multimeter (BK multimeter, MA) in the form of resistance. The biosensor was calibrated using the uninoculated distilled water.

Two tests were performed in this study to assess the performance of the biosensor. Sensitivity testing was conducted to evaluate the detection limit of the biosensor. In this testing, a distilled water sample inoculated with a varying concentration of *E. coli* O157:H7 was used.

The second test involved with the water samples collected from various sources along the Red Cedar River: Kalamazoo Street, Farm Lane, Hagadorn, and Putman Street. One hundred ml of each sample was filtered through a bio-filter membrane (BIOPath, Florida). The membrane filter was transferred to a test tube containing 10 ml of 0.1% peptone water. The test tube was vortexed to release the trapped cells from the membrane. Then, 0.1 ml of each water sample was pipetted onto the application pad of the biosensor. Three replicates were done for each treatment.

Results and Discussion

Pertinent data collected from the four sampling sites shown in Figure 1 are listed in the Table 5-1. The data in Table 5-1 includes *E. coli* concentration in colony forming units (CFU) per 100ml as reported by the MDEQ and *E. coli* O157:H7 concentration in CFU per 100ml as reported by the MDCH. In addition, rainfall data that was calculated in the 24-hour period, 48-hour period, and 72-hour period prior to the collection of the water samples that was analyzed for *the E. coli* and *E. coli* O157:H7 concentrations is included in Table 5-1. The Red Cedar River flow rate in cubic feet per second of water moved is also reported in Table 5-1 for the dates of sampling.

Table 5-1. *E. coli* Concentration, Rainfall, River Flow, and *E. coli* O157:H7 Concentration from the Sampling Sites in the Red Cedar River Watershed.

Kalamazoo Street

Date	E.coli (CFU/100ml)	Rain 24 hrs. (inches)	Rain 48 hrs. (inches)	Rain 72 hrs. (inches)	Flow (ft ³ /sec)	O157:H7 (CFU/100ml)
4/15/2002	190	0	0	0.03	387	0
4/22/2002	60	0.15	0.15	0.17	232	0
4/30/2002	250	0	0.2	0.53	216	0
5/07/2002	110	0.17	0.17	0.17	229	0
5/14/2002	720	0.03	0.2	1.09	456	0

Farm Lane

Date	E.coli (CFU/100ml)	Rain 24 hrs. (inches)	Rain 48 hrs. (inches)	Rain 72 hrs. (inches)	Flow (ft ³ /sec)	O157:H7 (CFU/100ml)
4/15/2002	173	0	0	0.03	387	0
4/22/2002	47	0.15	0.15	0.17	232	0
5/07/2002	107	0.17	0.17	0.17	229	0
5/14/2002	1150	0.03	0.2	1.09	456	0

Hagadorn

Date	E.coli (CFU/100ml)	Rain 24 hrs. (inches)	Rain 48 hrs. (inches)	Rain 72 hrs. (inches)	Flow (ft ³ /sec)	O157:H7 (CFU/100ml)
4/15/2002	90	0	0	0.03	387	0
4/22/2002	80	0.15	0.15	0.17	232	0
5/07/2002	130	0.17	0.17	0.17	229	0
5/14/2002	770	0.03	0.2	1.09	456	0

Putman Street

Date	E.coli (CFU/100ml)	Rain 24 hrs. (inches)	Rain 48 hrs. (inches)	Rain 72 hrs. (inches)	Flow (ft ³ /sec)	O157:H7 (CFU/100ml)
4/16/2002	43	0	0.1	0.1	197	0
4/30/2002	77	0	0.14	0.41	86	0
5/07/2002	133	0.06	0.06	0.06	87	0
5/14/2002	453	0.05	0.13	0.87	227	0

Objective 1: Rain and River Flow influence on *E. coli* Concentration

Along with the variation of the concentration of *E. coli* during the study, there were variations in rainfall and river flow for the different sampling events. The National Weather Service (NWS) reported rainfall from a station in Williamston, Michigan where the Putman Street site is located. The Horticulture Research Station reported rainfall for the MSU campus on the campus. The United States Geological Services (USGS) provided the river flow data from USGS staging stations in Williamston and on the MSU campus. This flow data is provisional at the time of calculations and will not be reviewed and made official until the spring of 2004.

In the statistical analysis that was performed and shown in Table 5-2, rainfall was collected for the 24 hour period of the day of the sample collection and in addition the cumulative total of rainfall for 48 hour period and 72 hour period. The statistical analysis indicates that there is an influence of higher concentration of *E. coli* from both increased rainfall and increased river flow. There are older studies that reported similar findings (Stephenson 1978; Tiedemann 1988). More recently, in a three-year study in Arkansas, it was reported that *E. coli* concentrations increased with increasing flow rates (Edwards 1997). In addition, a study that examined 99 samples from three tributaries that contributed to different drinking water reservoirs showed that *E. coli* levels along with other bacteriological parameters increased considerably during extreme rainfall events (Kistemann 2002). It is interesting to note that the 24 hour period of rainfall is not statistically significant in influencing *E. coli* concentration but this may be explained by the fact that the rainfall total that is reported is from midnight to midnight of a day and the sampling was done in the late morning. Since the rainfall collection includes time

that is after the sampling is done, it might correctly reflect the rainfall impact in the 24-hour period. In both the Pearson's correlation and ANOVA the total rainfall for a 72-hour period was statistically significant at $p < 0.0001$.

Table 5-2. Statistical Analysis of *E. coli* Concentration vs. Rainfall, River Flow, and *E. coli* O157:H7 Concentration.

Statistical Formula	Rain 24 hrs	Rain 48 hrs.	Rain 72 hrs.	Flow	O157:H7
Pearson's Correlation	-.315	.429*	.899**	.674**	NA
ANOVA	.082	.267	14.129**	4.760*	NA

* Statistically significant at $p < 0.05$; ** Statistically significant at $p < 0.01$.
 NA = Statistical analysis not possible since all entries were zero.

Objective 2: *E. coli* as an Indicator of Pathogenic Contamination

The *E. coli* concentrations varied from a very low level of 43 CFU/100ml to a very high level of 1150 CFU/100ml, however, no *E. coli* O157:H7 was detected in any of the samples analyzed. Within the parameters of this study, the results show that the use of total *E. coli* concentration as an indicator of *E. coli* O157:H7 does not provide a reliable risk factor of the pathogenic bacteria to humans using the water. The statistical analysis shown in Table 5-2 indicates that there is no correlation between *E. coli* and *E. coli* O157:H7 concentrations.

This suggests that *E. coli* levels may not be an accurate indicator of the level of pathogenic bacteria in the water. Therefore the use of *E. coli* levels for determining the risk of pathogenic *E. coli* O157:H7 to humans using recreational water may have to be redefined. A study in Italy concluded that concentrations of indicators of fecal contamination, whether within or over the values established by legislation, are not always related to the presence and density of individual pathogenic serotypes

(Bonadonna 2002). In this reported study, for many cases, the values of pathogenic serotype levels were not linear with respect to the levels of the bacterial indicators.

Objective 3: Biosensor Performance

Figure 5-3 illustrates the response of the biosensor tested with inoculated distilled water samples. The resistance drop was determined by calculating the difference between the resistance outputs of the blank and that of the inoculated samples. The difference in measured resistance (resistance drop) was the reduction in resistance due to electron transfer facilitated by the polyaniline-labeled antibody between the electrodes.

In water samples inoculated with *E. coli* O157:H7, the signal was shown to be proportional to the cell concentration between 10^1 and 10^4 CFU/ml. The signal was observed to decrease at concentrations higher than 10^4 CFU/ml (Figure 5-3). Statistical analysis reveals that the sensitivity limit of the biosensor was at $8.3 \pm 0.1 \times 10^1$ CFU/ml.

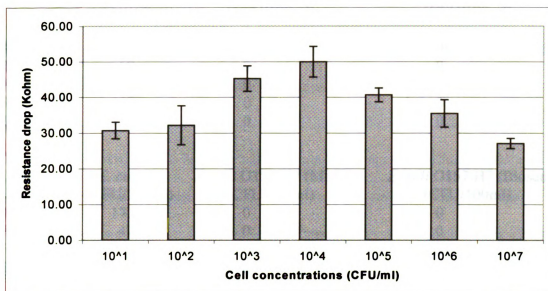


Figure 5-3. Resistance drop of the biosensor tested in distilled water samples inoculated with *E. coli* O157:H7.

The biosensor did not report any false positive during this study and the results were identical to the reported concentrations from traditional methods that use the culturing of the pathogenic bacteria as performed by MDCH shown in Table 3. The levels of *E. coli* O157:H7 remained zero despite a wide variation in the levels of total *E. coli*. The advantage in the biosensor is that it produced results in less than 20 minutes in contrast to the traditional method that produced results in three days.

Table 5-3. *E. coli* O157:H7 Analyzed by Traditional Methods and by the Biosensor.

Kalamazoo Street

Date	<i>E. coli</i> (CFU/100ml)	<i>E. coli</i> O157:H7 (MDCH) (CFU/100ml)	<i>E. coli</i> O157:H7(Biosensor) (CFU/100ml)
4/15/2002	190	0	0
4/22/2002	60	0	0
4/30/2002	250	0	0
5/07/2002	110	0	0
5/14/2002	720	0	0

Farm Lane

Date	<i>E. coli</i> (CFU/100ml)	<i>E. coli</i> O157:H7 (MDCH) (CFU/100ml)	<i>E. coli</i> O157:H7 (Biosensor) (CFU/100ml)
4/15/2002	173	0	0
4/22/2002	47	0	0
5/07/2002	107	0	0
5/14/2002	1150	0	0

Hagadorn

Date	<i>E. coli</i> (CFU/100ml)	<i>E. coli</i> O157:H7 (MDCH) (CFU/100ml)	<i>E. coli</i> O157:H7 (Biosensor) (CFU/100ml)
4/15/2002	90	0	0
4/22/2002	80	0	0
5/07/2002	130	0	0
5/14/2002	770	0	0

Putman Street

Date	<i>E. coli</i> (CFU/100ml)	<i>E. coli</i> O157:H7 (MDCH) (CFU/100ml)	<i>E. coli</i> O157:H7 (Biosensor) (CFU/100ml)
4/16/2002	43	0	0
4/30/2002	77	0	0
5/07/2002	133	0	0
5/14/2002	453	0	0

Additional studies are needed to determine the repeatability and reliability of this device. Additional pathogenic serotypes, as well as, total *E. coli* could be adapted to the biosensor and studies need to be conducted to test such configurations. The biosensor

was only tested in freshwater and results from marine (salt water) conditions are necessary to determine the versatility of this device. Finally, since this study was conducted on a river, a similar study needs to be conducted for beaches located on lakes. With additional studies and implementing the findings from these future studies, in the near future the biosensor may provide the best available method for determining the safety of recreational-use water. Image this: a lifeguard dips a biosensor type device into the lake water. This device tests the water for the levels of many different strains of bacteria. Using the biosensor device is as easy as testing for pH. The results are available in minutes and the lifeguard can determine if the water is safe before the beach is opened to the public.

Conclusions

Methods of measuring pathogens directly are costly and time-consuming. Therefore, indicator organisms such as *E. coli* are used instead of analyzing the pathogens themselves. In many cases the TMDL implementation plan specifies the use of best management practices (BMPs) or a systems of BMPs. Water quality monitoring is an important component of the TMDL implementation plan and is needed to measure the success of both individual activities and the overall water quality of the waterbody. Although the samples were limited, the biosensor has shown promise for water quality analysis both before and after the installation of the BMPs. Since the biosensor analysis is both rapid and inexpensive, it could be an excellent tool for determining the success of installed BMPs non point pathogenic pollution as well as monitoring the TMDL implementation plan's improvement of the water quality.

The *O157:H7* results were identical for all samples by both the standard methods and the biosensor. The biosensor did not report any false positive. *E. coli* O157:H7 was zero despite a wide variation in the levels of total *E. coli*. This suggests that *E. coli* levels may not be an accurate indicator of the level of pathogenic bacteria in the water and therefore the use *E. coli* levels for determining the risk to humans using recreational water may suggest a risk that is greater than the true risk for the use of the water. More data is needed to confirm these findings and also to compare levels of other strains of bacteria to total *E. coli*, which is the defined indicator bacterium for water quality.

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Chapter 6

Conclusions and Recommendations

Ever since fecal contamination of water was determined a human health risk, there has always been a great deal of concern regarding the level of coliform bacteria counts in water. Many bodies of water throughout the world are considered to have counts above acceptable levels. The sources or pathways of these *E. coli* are thought to be fecal contamination from humans, domestic animals and wildlife, as well as runoff from agricultural land, inadequate septic systems or sewer overflow. Physical factors, such as seasonal variability, rainfall, river flow, nutrient and the survival of bacteria in water, have an impact on *E. coli* levels in a waterbody. Tools exist to assist in determining the risk to swimmers of bacteria illness in a watershed. Although risk assessment models have been studied, an accurate dose-response model for illness to swimmers of bacteria illness in a watershed is not available.

There is a large body of literature involving the epidemiology of illness from the use of recreational water. Elevated *E. coli* levels have been identified as the cause of the illness and *E. coli* levels have been mandated as the indicator bacteria for the determination of water safety for swimmers. The validity of *E. coli* as an indicator of risk of fecal contamination has been debated and *E. coli* may not be an accurate indicator of pathogenic risk.

In contrast to the numerous studies, cases and reports of health effects from exposure to indicator bacteria in recreational-use water, there are a limited number of such findings in literature in regards to pathogenic *E. coli* O157:H7. In all these reports

or studies that have identified *E. coli* O157:H7 illness related to swimming, only one identified *E. coli* O157:H7 in the water that was the exposure. Although *E. coli* O157:H7 infection from swimming is very rare, the reports indicate that children are at much higher risk than adults. This higher risk may be from weaker immune systems of children as compared to adults but more likely, it is due to the fact that children are more likely to ingest water while swimming and that children are more likely than adults to defecate while swimming. The use of sealed rubber swimming trunks on children may have the greatest impact in lowering the risk of *E. coli* O157:H7 infections when swimming.

Public policy in both the United States and around the world has addressed the risk to swimmers of illness from bacteria in water. The levels of indicator bacteria that is acceptable as to the risk for swimmers have been established by USEPA, EEC, and WHO. In addition to these policies, laws and guidelines have been established to decrease the microbial input in a waterbody from sewage sources and agricultural facilities. Many of these laws and guidelines are being reviewed and revised in order to improve the public health of swimmers.

Based on the data collected and analyzed in this study, it is concluded that there is no observed seasonal effect for *E. coli* levels from the Farm Lane Bridge over the three-year period from 2000 to 2002. From the statistical analysis using Spearman's rho, river flow, rain, humidity, low temperature of the sampling day and soil moisture were significantly ($p < 0.05$) correlated to *E. coli* concentrations.

Other studies support the association of the levels of fecal indicators in a water body and rainfall events and the results presented in this study support these findings. In addition, previous studies have shown that increased river flow has been related to

increases in the concentration levels of *E. coli* in a river and the results presented in this study also support these findings. From this study there is no correlation between *E. coli* concentration and the number of ducks in the river upstream from the sampling point. Solar radiation and wind speed did not correlate to *E. coli* concentrations.

Using governmental guidelines for maximum *E. coli* level for the safe use of water for recreation, statistical models were designed and it was concluded that the odds of *E. coli* exceeding 300 cfu/100ml are increasing at least twofold and at most twelve-fold for each 1 cm increase in 72-hour total rainfall. It was also concluded that if the low temperature of the sampling day was greater than 15°C degrees the 95% probability of *E. coli* exceeding 300 cfu/100ml occurs at 0.25 cm 72-hour total rainfall.

Based on the results from this study, it is concluded that elevations of nutrients do have an association with elevations in the levels of *E. coli* concentration. The concentration levels of ammonia nitrate were significantly correlated to *E. coli* concentrations and the levels of total suspended solids were significantly correlated to *E. coli* concentrations.

These results indicate that if the locations of the sources of nutrients that enter into nearby surface water are identified then proper best management practices (BMP) can be implement at these locations and the implemented BMP will have an impact on controlling nutrients into the surface water as well as having an effect to lower the *E. coli* concentration in the surface water.

However, in this study rainfall did not show significant positive correlation with *E. coli* levels and this is contradictory to what has been reported by others. This contradiction maybe explained by the fact that the rainfall data was collected from three

different sites that include East Lansing, Williamston, and Howell so that the rainfall data better represents the sampling site. These rainfall collection sites were not at the 17 sampling sites and probably did not represent the real rainfall that occurred at the 17 sampling sites during the time period that the river water samples were collected.

The impact of land use on *E. coli* concentrations was investigated but the results did not reveal with any certainty as to which type of land-use has the greatest impact on elevated *E. coli* concentrations. In the small sample set that was analyzed, agricultural land use had the greatest impact on elevated total suspended solids. However, mixed land use had the greatest impact on elevated ammonia nitrates. Larger sample sets need to be studied to determine if the type of land-use does have an impact the concentration levels of *E. coli* and other nutrients in nearby surface water.

Methods of measuring pathogens directly are costly and time-consuming. Therefore, indicator organisms such as *E. coli* are used instead of analyzing the pathogens themselves. In many cases the TMDL implementation plan specifies the use of BMPs or a systems of BMPs. Water quality monitoring is an important component of the TMDL implementation plan and is needed to measure the success of both individual activities and the overall water quality of the waterbody. The biosensor could be used for water quality analysis both before and after the installation of the BMPs. Since the biosensor analysis is both rapid and inexpensive, it could be an excellent tool for determining the success of installed BMPs non point pathogenic pollution as well as monitoring the TMDL implementation plan's improvement of the water quality.

The *E. coli* O157:H7 results were identical for all samples by both the standard methods and the biosensor. The biosensor did not report any false positives. The levels

of *E. coli* O157:H7 remained constant in relation to variation in the levels of total *E. coli*. This suggests that *E. coli* levels may not be an accurate indicator of the level of pathogenic bacteria in the water and therefore the use *E. coli* levels for determining the risk to humans using recreational water may suggest a risk that is greater than the true risk for the use of the water. Since this collaborative study was done with out funding, it was only operational for a four-week period. More data is needed to confirm these findings and also to compare levels of other strains of bacteria to total *E. coli*, which is the defined indicator bacterium for water quality.

However, there are weaknesses of this study. The biosensor was only tested in freshwater and results from marine (salt water) conditions are necessary to determine the versatility of this device. The sample set is small due to lack of funding for this project. Additional studies are needed to determine the repeatability and reliability of this device. Additional pathogenic serotypes, as well as, total *E. coli* could be adapted to the biosensor and studies need to be conducted to test such configurations. Finally, since this study was conducted on a river, a similar study needs to be conducted for beaches located on lakes. With additional studies and implementing the findings from these future studies, in the near future the biosensor may provide the best available method for determining the safety of recreational use water. Image this; a lifeguard dips a biosensor type device into the lake water. This device tests the water for the levels of many different strains of bacteria. Using the biosensor device is as easy as testing for pH. The results are available in minutes and the lifeguard can determine if the water is safe before the beach is opened to the public.

Appendix

Table A-1. Farm Lane Three Years of Data.

Table A-2. Yearly Mean of *E. coli* Concentration, Flow and Rainfall

Table A-3. Nutrients, *E. coli*, and Land-use Data.

Table A-1. Farm Lane Three Years of Data.

Week	Year	E. coli	thes 300	thes 1000	Flow	Rain 24	Rain 48	Rain 72	temp hi	temp low	humd hi	humd lo
1	2000	350	1	0	546	0	0	0.06	61.6	35.6	99.4	40.1
2	2000	113	0	0	157	0	0	0	69.4	34.9	99.7	29.7
3	2000	170	0	0	113	0	0	0	82.5	64.7	83.3	41.8
4	2000	410	1	0	267	0	0	0.7	54.5	39.5	88.6	34
5	2000	383	1	0	784	0	0.35	1.71	68.3	44.4	100	41.3
6	2000	1273	1	1	275	0	0.59	0.61	71	44.3	98.7	51
7	2000	207	0	0	173	0	0	0.07	67.2	44.1	100.9	45.2
8	2000	670	1	0	149	0.4	0.4	0.4	77.9	61.2	98.3	65.4
9	2000	360	1	0	221	0.04	0.04	0.04	70.2	57	99	50.5
10	2000	333	1	0	180	0.26	0.51	0.51	80.5	65	100.8	56.6
11	2000	1333	1	1	423	0	0.53	1.02	83.1	56.7	101.9	43.8
12	2000	490	1	0	124	0.09	0.09	0.09	81.3	62.9	90.9	64
13	2000	493	1	0	92	0.01	0.36	0.64	79.7	60.2	100.4	51.9
14	2000	393	1	0	53	0	0	0	74.6	51.5	100	43.5
15	2000	560	1	0	152	0.57	0.57	1.77	75.4	66.1	99.9	78.5
16	2000	690	1	0	274	0.89	1.44	1.44	82.6	59.4	101.3	64.5
17	2000	270	0	0	83	0	0	0.04	77.6	55.1	101.2	57.3
18	2000	393	1	0	71	0	0	0.01	69.9	45.4	101.4	39.2
19	2000	357	1	0	74	0	0	0	77.8	61.7	101.6	58.5
20	2000	287	0	0	46	0	0	0	70.2	52.2	97.3	46.2
21	2000	1580	1	1	153	1.5	1.5	1.52	72.9	64.3	100.9	89.6
22	2000	693	1	0	86	0	0	0	78.4	44.8	102	37.2
23	2000	5833	1	1	704	0	1.1	1.59	56.1	45.7	97.4	54.4
24	2000	550	1	0	128	0	0	0	76.1	52.7	88.4	40.6
25	2000	267	0	0	194	0.01	0.01	0.09	43.4	33.2	97.5	43.8
26	2000	7600	1	1	123	0.18	0.19	0.19	68	50.1	101.1	64.5
27	2000	93	0	0	96	0	0	0	68.1	41.8	89.3	43.4
28	2000	90	0	0	145	0	0	0.27	56	27.2	102	37.1
29	2000	85	0	0	113	0	0	0	59.7	31	98.5	33.5
30	2000	683	1	0	226	0	0.04	0.62	41.8	37.6	94.8	76.2
31	2000	170	0	0	225	0	0.01	0.01	37.9	27.4	85.4	55.3
32	2000	170	0	0	188	0.3	0.36	0.36	45.3	38.6	102.2	95.6
1	2001	720	1	0	518	0.42	0.91	1.32	67.6	48.1	99.5	64.2
2	2001	100	0	0	218	0	0	0.01	67	35.9	92.3	21.5
3	2001	57	0	0	164	0	0	0.02	70.3	49	54.5	31.2
4	2001	297	0	0	176	0	0	0.19	66.3	34.2	99.8	27.3
5	2001	133	0	0	429	0.04	0.04	0.04	74.4	57.5	97.2	66.9
6	2001	3500	1	1	625	0.13	0.76	0.93	69.8	49.2	100.5	56.6
7	2001	4033	1	1	586	0	0.59	0.88	61.1	46.1	98.2	69.3
8	2001	2255	1	1	234	0.16	0	0	79.9	54.8	96.9	51.6
9	2001	477	1	0	193	0	0.01	0.84	80.3	58.3	95.3	35.7
10	2001	503	1	0	224	0	0	0.22	80.8	52.5	99.9	30.6
11	2001	133	0	0	91	0	0	0	76.4	49.2	89.5	33.5
12	2001	503	1	0	67	0	0.01	0.01	88.4	66.3	98.2	28.2
13	2001	150	0	0	47	0	0	0	87.7	52.6	96.8	28.2
14	2001	443	1	0	41	0	0.16	0.16	90.7	67	98.9	44.8
15	2001	1667	1	1	49	0.34	0.34	0.34	87.1	62.6	98.9	45.4
16	2001	87	0	0	30	0	0	0	91.2	58.2	99.4	27.4
17	2001	147	0	0	27	0	0	0.02	83.1	58.7	98.8	42.1
18	2001	517	1	0	47	0.1	0.23	0.23	72.3	61.1	98	68.4
19	2001	247	0	0	42	0	0	0	80.2	62.7	98.1	58.7
20	2001	90	0	0	29	0	0	0	77.5	44.3	99.5	41.7
21	2001	2700	1	1	117	0.69	1.09	1.33	77.7	59.8	99.9	73.9
22	2001	270	0	0	70	0	0	0	74.4	51	98.6	45.5
23	2001	475	1	0	110	0.24	0.26	0.81	43.8	38.4	97.7	85.1
24	2001	680	1	0	72	0	0	0	71.5	35.3	100.4	34.9
25	2001	4750	1	1	156	0	0.01	0.76	49.4	27.5	98.9	33.4
26	2001	1000	1	1	308	0.53	0.57	1.24	69.1	46.1	99	57.1
27	2001	180	0	0	540	0	0	0	63.9	41.8	94.4	34.6
28	2001	218.5	0	0	435	0	0	0.18	50.7	25.3	100.2	38.1
29	2001	257	0	0	423	0	0	0.47	58	38.4	86.1	200
30	2001	93	0	0	194	0.25	0.25	0.25	52.7	24	99.1	45.8
31	2001	417	1	0	190	0.14	0.14	0.15	57.4	34.4	96.9	56.5
32	2001	830	1	0	214	0	0.02	0.52	45.9	40.5	86.8	70

Table A-1. Farm Lane Three Years of Data. (continued)

Week	Year	E. coli	thes 300	thes 1000	Flow	Rain 24	Rain 48	Rain 72	temp hi	temp low	humd hi	humd lo
1	2002	47	0	0	92	0.1	0.11	0.2	44.7	32.8	97.7	48.9
2	2002	77	0	0	87	0.21	0.39	0.39	55.9	36.1	100.1	85.5
3	2002	107	0	0	87	0.14	0.14	0.14	65.4	56.4	98.3	51.5
4	2002	1150	1	1	227	0.01	0.81	0.91	51.3	42.4	99.5	54.3
5	2002	623.5	1	0	137	0	0	0.03	46.3	31.4	98.8	39.7
6	2002	97	0	0	68	0	0	0.14	79.1	43.8	97	23.9
7	2002	1023	1	1	220	0.37	0.37	0.37	61.8	46.6	96.8	56.4
8	2002	590	1	0	65	0	0	0	85.5	55	98.9	36.6
9	2002	163	0	0	46	0	0.06	0.15	70.5	52.1	91.4	38.9
10	2002	237	0	0	33	0	0	0	89.8	65.6	93.8	37.6
11	2002	90	0	0	24	0	0	0	92.5	67.9	95.8	41.8
12	2002	4133	1	1	22	0.2	0.2	0.2	90.9	59.4	92.2	31.7
13	2002	240	0	0	16	0	0	0	88.3	55.1	97.1	40.3
14	2002	130	0	0	21	0.01	0.01	0.01	91.2	66	89.5	48.7
15	2002	3033	1	1	134	0.78	1.17	1.23	86.5	69.4	97.3	66.8
16	2002	350	1	0	21	0.01	0.02	0.02	8108	65.7	93.8	50.6
17	2002	90	0	0	16	0	0	0	88.5	62.6	97.8	41.3
18	2002	1797	1	1	18	0.17	0.17	0.17	70.8	54.2	98	63
19	2002	343	1	0	18	0	0	0	82.5	56.8	99.2	39.7
20	2002	63	0	0	12	0	0	0	82.5	64.8	97.1	49
21	2002	233	0	0	9.2	0	0	0	90.5	56.8	98.8	33.5
22	2002	443	1	0	8.6	0	0	0	75.9	42	98.9	30.5
23	2002	303	0	0	12	0	0.14	0.14	65.2	39.7	98.8	32.5
24	2002	163	0	0	9.7	0.03	0.03	0.03	77.8	55.9	93.5	48.2
25	2002	77	0	0	14	0	0.1	0.1	53.9	35	96	32.2
26	2002	273	0	0	15	0	0.12	0.18	56.7	31.1	87.7	28.5
27	2002	37	0	0	22	0	0	0	50.7	24.7	99	39.5
28	2002	67	0	0	21	0	0	0.01	48.1	31.1	87.5	46.2
29	2002	133.5	0	0	34	0	0	0	48.6	25.4	95.9	39.6
30	2002	200	0	0	47	0.02	0.46	0.53	51.5	37.6	96.9	78.5
31	2002	400	1	0	34	0	0	0	40.6	20.2	92.2	51.5
32	2002	260	0	0	46	0	0.01	0.03	40.2	28.7	99.4	55.8

Table A-1. Farm Lane Three Years of Data. (continued)

soil moist 1	soil mos 1 lo	soil moist 2	soil mos 2 lo	soil temp hi	soil temp lo	solar rad	wind speed	duck count	water temp	Week	Year
0.287	0.271	0.579	0.468	60.5	42.9	374.9	13.2			1	2000
0.264	0.238	0.773	0.573	66.4	46.6	644.3	17.9			2	2000
0.205	0.191	0.571	0.492	71.6	61	409.7	23.3			3	2000
0.336	0.321	0.88	0.772	60.4	51.8	598.7	31.7			4	2000
0.355	0.337	0.895	0.73	71.1	53.1	555.2	12.5			5	2000
0.362	0.344	0.928	0.759	75	53.4	677.8	12.5			6	2000
0.365	0.347	0.949	0.789	71.2	56.2	453.8	9.8			7	2000
0.368	0.306	1.237	0.538	75.9	69.7	275.3	19.2			8	2000
0.356	0.347	1.192	0.899	78.5	64.5	346.8	10.5			9	2000
0.379	0.359	1.161	0.936	85.3	67.8	563	14.5			10	2000
0.362	0.351	1.204	0.916	86.1	62.7	592.5	11.2			11	2000
0.332	0.319	1.041	0.867	77.8	64.5	335.2	25.9			12	2000
0.356	0.34	1.205	0.977	81.6	63.6	521.2	13.9			13	2000
0.286	0.272	1.06	0.821	83.2	61	505.4	11.2			14	2000
0.343	0.307		1.588	75	68.5	158.8	16.6			15	2000
0.358	0.33		0.795	76.9	65.7	302.2	22.6			16	2000
0.278	0.273	7.38	0.332	75.9	64.4	309.2	13.2			17	2000
0.267	0.258		0.102	69.6	57.1	620.5	13.9			18	2000
0.292	0.279	7.43	3.372	71.7	66.9	364.1	15.9			19	2000
0.216	0.198	5.803	0.256	70.4	61.8	309.6	23.9			20	2000
0.341	0.191	7.88	4.53	71.1	68.2	49.4	13.5			21	2000
0.301	0.292	6866	0.056	67.8	54.2	462.4	15.9	25		22	2000
0.316	0.307	2.7	1.838	60.7	54.5	364.6	16.6	28		23	2000
0.274	0.269	2.292	1.341	64.3	53.9	386.5	22.6	16		24	2000
0.297	0.289	3.403	2.127	47.4	43.9	225.5	18.6	0		25	2000
0.276	0.258	2.226	1.331	60.6	52.4	164.3	12.5	5		26	2000
0.247	0.239	2.276	1.275	57.9	49.5	282.5	13.9	73		27	2000
0.298	0.29	0.471	0.458	52.2	43.1	304.7	12.2	18		28	2000
0.268	0.261	0.454	0.42	50.9	41.8	247.6	10.2	31		29	2000
0.278	0.275	0.469	0.468	45	43.2	38	12.9	3		30	2000
0.284	0.282	0.469	0.468	38.4	34.8	135	22.3	8		31	2000
0.296	0.286	0.494	0.468	42.5	37.8	41.7	11.2	2		32	2000
0.303	0.285	0.491	0.418	62.2	50.6	302	20.2	1	14.07	1	2001
0.222	0.207	0.386	0.34	62.7	45.3	572.4	15.9	7	13.76	2	2001
0.178	0.168	0.275	0.25	63.9	53	490.6	20.6	0	15.97	3	2001
0.238	0.229	0.375	0.324	69.5	47.2	606.1	16.9	0	14.23	4	2001
0.323	0.322	7.23	0	70.4	60.2	189.8	30.3	1	16.44	5	2001
0.353	0.341	7.63	0.155	81.3	54	488.8	19.6	4	13.92	6	2001
0.356	0.343	7.46	0.062	72	53.3	256.8	19.2	0	12.68	7	2001
	0.282	6.963	0.498	86.1	59.6	397.7	19.9	0	18.04	8	2001
0.287	0.26	6.723	2.567	92.8	60.3	694	20.6	0	20.63	9	2001
0.282	0.255	6.59	2.118	94.6	58.3	695.3	12.5	3	19.33	10	2001
0.191	0.191	6.708	5.056	92.7	64.1	534.2	24.3	0	19.01	11	2001
0.191	0.187	6.447	4.179	104	69.8	653.6	12.9	0	21.63	12	2001
0.18	0.179	5.196	3.585	99.4	64.2	532.7	13.5	0	21.29	13	2001
0.191	0.191	4.035	2.816	102.9	69.7	545.9	12.9	0	25.55	14	2001
0.313	0.187	4.193	2.943	101.4	69.1	493.4	37.7	0	21.79	15	2001
0.191	0.179	3.391	2.318	105.1	69.3	588.1	12.5	0	24.16	16	2001
0.18	0.179	3.407	2.482	97.1	68.3	419	14.9	12	22.96	17	2001
0.315	0.267	4.921	3.94	80.8	64.5	270.9	15.5		19.66	18	2001
0.247	0.215	5.314	4.045	88.8	68.6	314.1	17.2		21.29	19	2001
0.18	0.179	5.078	3.438	91	57.6	524.8	13.9	5	18.68	20	2001
0.376	0.277	6.497	3.89	81.2	65.6	261.8	25.3	5	20.14	21	2001
0.191	0.191	5.998	3.367	75	57.3	309.7	13.5	0	16.6	22	2001
0.381	0.338	5.014	3.506	51.5	45.9	126.1	18.2	4	10.98	23	2001
0.308	0.287	4.805	2.692	65.5	45.4	421.6	9.5		11.91	24	2001
0.336	0.321	5.253	1.193	51	39	311.4	20.9	0	8.19	25	2001
0.402	0.355	4.707	3.466	61.8	51	95.1	27.3	0	13.15	26	2001
0.314	0.306	5.193	1.313	59.2	48.6	197.4	14.9	0	10.21	27	2001
0.347	0.339	1.816	0.206	48.5	35.8	281.6	20.6	0	6.64	28	2001
0.341	0.332	5.281	0.638	51.2	41.9	254.7	22.3	0	8.19	29	2001
0.371	0.299	0.785	0	47.9	34.6	224.4	12.9	0	5.71	30	2001
0.364	0.329	0.956	0.91	52.7	40.1	122.4	14.2	0	10.05	31	2001
0.348	0.343	0.957	0.933	48.4	43.1	118.9	16.6	0	8.19	32	2001

Table A-1. Farm Lane Three Years of Data. (continued)

soil moist 1	soil mos 1	soil moist 2	soil mos 2	soil temp h	soil temp k	solar rad	wind speed	duck count	water temp	Week	Year
0.243	0.227	0.804	0.781	54.7	43.7	202.6	20.6		8.34	1	2002
0.31	0.273	0.758	0.698	51.8	43.3	98.9	30			2	2002
0.281	0.238	0.781	0.745	61.4	54.2	192.8	22.3			3	2002
		0.902	0.865	54.3	49.8	137.2	17.2			4	2002
		0.859	0.821	49.9	43.1	333.7	13.5			5	2002
		0.753	0.703	66.1	50.8	632.5	14.5			6	2002
0.34	0.257	0.695	0.608	62.2	56.9	268.9	22.3			7	2002
0.275	0.256	0.654	0.621	71.2	59.2	641.5	10.5			8	2002
0.342	0.322	1.26	1.224	64.5	67.9	483	21.9			9	2002
0.32	0.29	1.206	1.156	78.8	67.8	552.8	15.9			10	2002
0.304	0.267	1.183	1.137	83.9	71.3	602.9	16.9			11	2002
0.26	0.215	1.167	1.128	83.3	69.9	559	26.3			12	2002
0.297	0.284	1.506	1.444	79.4	65.6	574.1	16.6			13	2002
0.313	0.301	1.558	1.485	80.9	69.1	364.7	19.2			14	2002
0.429	0.354	1.673	1.505	81	72.3	333.9	20.9			15	2002
0.345	0.328	1.529	1.466	80.8	70.2	447.9	18.9			16	2002
0.314	0.295	1.477	1.425	84.3	70.7	548.6	18.6			17	2002
0.361	0.322	1.582	1.502	70.4	64.6	199.3	14.2 x			18	2002
0.394	0.367	1.677	1.609	79.1	64.9	544.3	10.5			19	2002
0.344	0.333	1.595	1.55	77.3	69.3	315.4	19.2			20	2002
0.318	0.295	1.536	1.486	82.2	66.7	452.8	12.9			21	2002
0.275	0.256	1.425	1.356	73.8	57.4	478.8	12.5			22	2002
0.325	3.11	1.386	1.331	64.2	53.1	400.2	15.2			23	2002
0.301	0.286	1.301	1.314	71.6	59.3	302.8	15.9			24	2002
0.329	0.314	1.468	1.427	57.7	49.5	369.9	24.3			25	2002
0.308	0.297	1.434	1.386	56.9	43.3	353.8	13.9			26	2002
0.296	0.286	1.408	1.362	50.1	39.5	232	14.5			27	2002
0.319	0.309	1.505	1.471	48.9	41.1	171.9	15.2			28	2002
0.284	0.275	1.477	1.432	46.7	37.2	202.4	15.2			29	2002
0.35	0.331	1.74	1.698	53	45.1	43.3	14.9			30	2002
0.3	0.294	1.747	1.698	38	32.9	139.6	17.6			31	2002
0.309	0.304	1.789	1.75	39.2	36.5	81.7	15.5			32	2002

Table A-2. Yearly Mean of E. coli Concentration, Flow and Rainfall

Year		E. coli	Flow	Rain 24h	Rain 48h	Rain 72h
2000	Mean	854.656	207.437	.1328	.2528	.4300
	N	32	32	32	32	32
	Std.	1594.15	175.2794	.3194	.4131	.5802
2001	Mean	872.796	208.312	.0950	.1684	.3412
	N	32	32	32	32	32
	Std.	1226.14	180.0730	.1754	.2954	.4322
2002	Mean	530.406	51.140	.0641	.1347	.1556
	N	32	32	32	32	32
	Std.	894.4360	56.8654	.1563	.2599	.2766
Total	Mean	752.619	155.630	.0973	.1853	.3090
	N	96	96	96	96	96
	Std.	1267.25	164.8587	.2282	.3297	.4571

Table A-3. Nutrients, *E. coli*, and Land-use Data.

Site	Week	Date	E. coli	AN	TP	TSS	Rain 24	Rain 48	Rain 72	Land use
LA	1	4/25/2001	97	0.584	0.855	6.53	0	0.31	0.74	Agr
LA	3	5/9/2001	197	0.142	0.091	9.56	0.04	0.72	0.72	Agr
LA	5	5/23/2001	130	0.16	0.013	15.4	0	0.44	0.44	Agr
LA	7	6/6/2001	220	0.063	0.023	13.13	0.02	0.02	0.02	Agr
LA	9	6/20/2001	257	0.067	0.019	9.7	0	0.18	0.18	Agr
LA	13	7/18/2001	280	0.074	0.001	8.6	0.04	0.04	0.04	Agr
LA	15	8/1/2001	550	0.128	0.009	4.76	0	0	0.38	Agr
LA	17	8/15/2001	290	0.047	0.091	4.4	0	0	0	Agr
LA	19	8/29/2001	223	0.071	0.018	6.5	0.06	0.58	0.58	Agr
LA	23	9/25/2001	847	0.172	0.006	8.61	0	0	0.62	Agr
LA	25	10/8/2001	210	1.057	0.026	4.4	0.14	0.14	0.14	Agr
LA	29	11/8/2001	87	0.138	0.033	3.92	0	0	0	Agr
LA	31	11/20/2001	133	0.047	0.016	4	0.13	0.13	0.13	Agr
LB	1	4/25/2001	90	0.013	0.046	3.96	0	0.08	0.34	Mixed
LB	3	5/9/2001	360	0.825	0.021	10.8	0	0.45	0.45	Mixed
LB	5	5/23/2001	460	0.512	0.018	14.8	0	0.35	0.66	Mixed
LB	7	6/6/2001	360	0.072	0.01	13.47	0.03	0.03	0.07	Mixed
LB	9	6/20/2001	340	0.068	0.018	5.4	0	0.12	0.13	Mixed
LB	13	7/18/2001	640	0.116	0.004	10.1	0	0	0	Mixed
LB	15	8/1/2001	880	0.169	0.005	9.18	0	0	1.03	Mixed
LB	17	8/15/2001	2400	0.185	0.094	14.8	0	0	0	Mixed
LB	19	8/29/2001	510	0.097	0.014	8.6	0	0	0.1	Mixed
LB	23	9/25/2001	560	0.073	0.01	7.35	0.09	0.37	0.37	Mixed
LB	25	10/8/2001	350	0.098	0.005	4.8	0	0	0.62	Mixed
LB	29	11/8/2001	160	0.042	0.02	6.26	0	0	0	Mixed
LB	31	11/20/2001	380	0.038	0.01	4.4	0.19	0.19	0.19	Mixed
LE	1	4/25/2001	250	0.015	0.05	4.73	0	0.08	0.34	Agr
LE	3	5/9/2001	170	0.124	0.013	15.2	0	0.45	0.45	Agr
LE	5	5/23/2001	120	0.189	0.008	10.4	0	0.35	0.66	Agr
LE	7	6/6/2001	130	0.101	0.01	12.5	0.03	0.03	0.07	Agr
LE	9	6/20/2001	320	0.151	0.019	22.2	0	0.12	0.13	Agr
LE	13	7/18/2001	480	0.107	0.004	16	0	0	0	Agr
LE	15	8/1/2001	490	0.198	0.019	17.6	0	0	1.03	Agr
LE	17	8/15/2001	640	0.088	0.1	14	0	0	0	Agr
LE	19	8/29/2001	350	0.107	0.025	25.2	0	0	0.1	Agr
LE	23	9/25/2001	280	0.138	0.009	6.8	0.09	0.37	0.37	Agr
LE	25	10/8/2001	210	0.065	0.005	6.7	0	0	0.62	Agr
LE	29	11/8/2001	30	0.037	0.028	2.86	0	0	0	Agr
LE	31	11/20/2001	120	0.035	0.01	3.2	0.19	0.19	0.19	Agr
LG	1	4/25/2001	70	0.035	0.046	6.34	0	0.08	0.34	Mixed
LG	3	5/9/2001	460	0.165	0.006	7.79	0	0.45	0.45	Mixed
LG	5	5/23/2001	200	0.15	0.002	13.4	0	0.35	0.66	Mixed
LG	7	6/6/2001	210	0.115	0.04	9.46	0.03	0.03	0.07	Mixed
LG	9	6/20/2001	310	0.094	0.018	6.2	0	0.12	0.13	Mixed
LG	13	7/18/2001	300	0.123	0.01	11.6	0	0	0	Mixed
LG	15	8/1/2001	440	0.226	0.014	10.7	0	0	1.03	Mixed
LG	17	8/15/2001	900	0.089	0.091	12.9	0	0	0	Mixed
LG	19	8/29/2001	300	0.08	0.019	14	0	0	0.1	Mixed
LG	23	9/25/2001	410	0.119	0.014	14.4	0.09	0.37	0.37	Mixed
LG	25	10/8/2001	230	0.046	0.009	4.8	0	0	0.62	Mixed
LG	29	11/8/2001	20	0.044	0.03	4.12	0	0	0	Mixed
LG	31	11/20/2001	80	0.039	0.016	7.1	0.19	0.19	0.19	Mixed
LI	1	4/25/2001	100	0.016	0.044	4.65	0	0.08	0.34	Agr
LI	3	5/9/2001	280	0.083	0.004	18.7	0	0.45	0.45	Agr
LI	5	5/23/2001	170	0.138	0.01	9.7	0	0.35	0.66	Agr
LI	7	6/6/2001	180	0.076	0.009	10.6	0.03	0.03	0.07	Agr
LI	9	6/20/2001	2800	0.095	0.014	9.2	0	0.12	0.13	Agr
LI	13	7/18/2001	440	0.041	0.005	6.8	0	0	0	Agr
LI	15	8/1/2001	870	0.081	0.008	28.4	0	0	1.03	Agr
LI	17	8/15/2001	3000	0.047	0.091	30.6	0	0	0	Agr
LI	19	8/29/2001	1210	0.079	0.013	6.1	0	0	0.1	Agr
LI	23	9/25/2001	490	0.073	0.005	3.44	0.09	0.37	0.37	Agr
LI	25	10/8/2001	190	0.028	0.054	1.6	0	0	0.62	Agr
LI	29	11/8/2001	90	0.028	0.023	4.44	0	0	0	Agr
LI	31	11/20/2001	160	0.04	0.138	5	0.19	0.19	0.19	Agr

Table A-3. Nutrients, *E. coli*, and Land-use Data. (continued)

Site	Week	Date	E. coli	AN	TP	TSS	Rain 24	Rain 48	Rain 72	Land use
S1	1	4/25/2001	83	0.034	0.053	6.05	0	0.51	0.98	urban
S1	3	5/9/2001	1470	0.107	0.004	6.6	0.04	0.54	0.54	urban
S1	5	5/23/2001	77	0.179	0.039	9.5	0	0.05	0.05	urban
S1	7	6/6/2001	210	0.092	0.018	16.16	0.06	0.06	0.06	urban
S1	9	6/20/2001	200	0.078	0.066	7.27	0	0.78	1	urban
S1	13	7/18/2001	203	0.06	0.004	4.6	0	0	0	urban
S1	15	8/15/2001	430	0.085	0.096	14.5	0	0	0	urban
S1	17	8/29/2001	257	0.05	0.008	6.4	0	0.03	0.03	urban
S1	19	9/25/2001	887	0.068	0.01	19	0.03	0.71	0.71	urban
S1	23	10/8/2001	207	0.022	0.014	3.8	0	0	0.2	urban
S1	25	10/23/2001	490	0.011	0.039	35.9	0.31	0.31	0.31	urban
S1	29	11/6/2001	107	0.042	0.025	4.09	0	0	0	urban
S1	31	11/20/2001	227	0.018	0.029	3.4	0.14	0.14	0.15	urban
S1B	1	4/25/2001	170	0.106	0.123	4.17	0	0.51	0.98	Mixed
S1B	3	5/9/2001	147	0.08	0.028	3.47	0.04	0.54	0.54	Mixed
S1B	5	5/23/2001	163	0.129	0.023	9.8	0	0.05	0.05	Mixed
S1B	7	6/6/2001	127	0.065	0.01	12.25	0.06	0.06	0.06	Mixed
S1B	9	6/20/2001	2237	0.078	0.18	7.6	0	0.78	1	Mixed
S1B	13	7/18/2001	140	0.075	0.001	2.8	0	0	0	Mixed
S1B	15	8/1/2001	187	0.376	0.13	6.5	0	0	0	Mixed
S1B	17	8/15/2001	207	1.43	0.045		0	0.03	0.03	Mixed
S1B	19	9/25/2001	553	0.337	0.011	6.96	0.03	0.71	0.71	Mixed
S1B	23	10/8/2001	560	0.025	0.038	4	0	0	0.2	Mixed
S1B	25	10/23/2001	633	0.209	0.035	9.29	0.31	0.31	0.31	Mixed
S1B	29	11/6/2001	100	0.198	0.044	2.45	0	0	0	Mixed
S1B	31	11/20/2001	247	0.153	0.145	2.1	0.14	0.14	0.15	Mixed
S2B	1	4/25/2001	120	0.012	0.041	6.42	0	0.51	0.98	urban
S2B	3	5/9/2001	380	0.155	0.051	6.33	0.04	0.54	0.54	urban
S2B	5	5/23/2001	90	0.257	0.014	11.3	0	0.05	0.05	urban
S2B	7	6/6/2001	60	0.142	0.005	21.22	0.06	0.06	0.06	urban
S2B	9	6/20/2001	1450	0.293	0.078	8.6	0	0.78	1	urban
S2B	13	7/18/2001	310	0.254	0.001	5.2	0	0	0	urban
S2B	15	8/1/2001	1100	0.084	0.12	8	0	0	0	urban
S2B	17	8/15/2001	180	0.086	0.013		0	0.03	0.03	urban
S2B	19	9/25/2001	370	0.108	0.009	5.78	0.03	0.71	0.71	urban
S2B	23	10/8/2001	80	0.025	0.041	1	0	0	0.2	urban
S2B	25	10/23/2001	220	0.011	0.063	5.5	0.31	0.31	0.31	urban
S2B	29	11/6/2001	60	0.063	0.025	1.56	0	0	0	urban
S2B	31	11/20/2001	390	0.038	0.014	1.5	0.14	0.14	0.15	urban
S5	1	4/25/2001	30	0.023	0.099	15	0	0.31	0.74	Agr
S5	3	5/9/2001	100	0.054	0.025	6.94	0.04	0.72	0.72	Agr
S5	5	5/23/2001	80	0.105	0.015	10.3	0	0.44	0.44	Agr
S5	7	6/6/2001	230	0.038	0.008	6.92	0.02	0.02	0.02	Agr
S5	9	6/20/2001	380	0.065	0.049	3.6	0	0.18	0.18	Agr
S5	13	7/18/2001	520	0.034	0.004	5.1	0.04	0.04	0.04	Agr
S5	15	8/1/2001	420	0.023	0.099	13.2	0	0	0.38	Agr
S5	17	8/15/2001	500	0.032	0.001		0	0	0	Agr
S5	19	9/25/2001	310	0.03	0.009	2.55	0.06	0.58	0.58	Agr
S5	23	10/8/2001	300	0.09	0.009	2	0	0	0.62	Agr
S5	25	10/23/2001	120	0.058	0.025	5.66	0.14	0.14	0.14	Agr
S5	29	11/6/2001	120	0.018	0.046	2.76	0	0	0	Agr
S5	31	11/20/2001	1260	1.122	0.019	3.5	0.13	0.13	0.13	Agr
S8	1	4/25/2001	10	0.012	0.039	2.11	0	0.31	0.74	Agr
S8	3	5/9/2001	225	0.056	0.033	2.45	0.04	0.72	0.72	Agr
S8	5	5/23/2001	60	0.074	0.005	8.6	0	0.44	0.44	Agr
S8	7	6/6/2001	200	0.071	0.009	15.04	0.02	0.02	0.02	Agr
S8	9	6/20/2001	1030	0.097	0.031	11.6	0	0.18	0.18	Agr
S8	13	7/18/2001	2200	0.068		16.3	0.04	0.04	0.04	Agr
S8	15	8/1/2001	3000	0.017			0	0	0.38	Agr
S8	17	8/10/2001		0.049	0.003		0	0	0	Agr
S8	19	9/25/2001	320	0.041	0.009	14.4	0.06	0.58	0.58	Agr
S8	23	10/8/2001	130	0.058	0.008	2.2	0	0	0.62	Agr
S8	25	10/23/2001	180	0.02	0.018	3.64	0.14	0.14	0.14	Agr
S8	29	11/6/2001	70	0.033	0.025	6.46	0	0	0	Agr
S8	31	11/20/2001	120	0.031	0.013	2	0.13	0.13	0.13	Agr

Table A-3. Nutrients, *E. coli*, and Land-use Data. (continued)

Site	Week	Date	E. coli	AN	TP	TSS	Rain 24	Rain 48	Rain 72	Land use
S10	1	4/25/2001	73	0.19	0.093	3.75	0	0.31	0.74	Mixed
S10	3	5/9/2001	3833	0.272	0.1	9.49	0.04	0.72	0.72	Mixed
S10	5	5/23/2001	250	0.159	0.003	9.7	0	0.44	0.44	Mixed
S10	7	6/6/2001	163	0.067	0.035	11.63	0.02	0.02	0.02	Mixed
S10	9	6/20/2001	287	0.115	0.12	14.4	0	0.18	0.18	Mixed
S10	13	7/18/2001	127	0.097	0.005	17	0.04	0.04	0.04	Mixed
S10	15	8/1/2001	210	0.069	0.11	18.7	0	0	0.38	Mixed
S10	17	8/15/2001	147	0.116	0.041		0	0	0	Mixed
S10	19	9/25/2001	10533	0.149	0.014	7.36	0.06	0.58	0.58	Mixed
S10	23	10/8/2001	833	0.065	0.026	5.8	0	0	0.62	Mixed
S10	25	10/23/2001	190	0.049	0.023	5.45	0.14	0.14	0.14	Mixed
S10	29	11/6/2001	153	0.095	0.046	4.67	0	0	0	Mixed
S10	31	11/20/2001	2643	0.069	0.039	4.2	0.13	0.13	0.13	Mixed
S11	1	4/25/2001	110	0.014	0.033	3.52	0	0.31	0.74	urban
S11	3	5/9/2001	170	0.079	0.05	1.41	0.04	0.72	0.72	urban
S11	5	5/23/2001	90	0.111	0.003	12.1	0	0.44	0.44	urban
S11	7	6/6/2001	150	0.079	0.018	8.37	0.02	0.02	0.02	urban
S11	9	6/20/2001	420	0.08	0.031	4.1	0	0.18	0.18	urban
S11	13	7/18/2001	280	0.044	0.005	5.4	0.04	0.04	0.04	urban
S11	15	8/1/2001	310	0.021	0.096	5.1	0	0	0.38	urban
S11	17	8/15/2001	230	0.049	0.008		0	0	0	urban
S11	19	9/25/2001	690	0.033	0.006	2.2	0.06	0.58	0.58	urban
S11	23	10/8/2001	150	0.013	0.008	0.8	0	0	0.62	urban
S11	25	10/23/2001	250	0.012	0.026	19.4	0.14	0.14	0.14	urban
S11	29	11/6/2001	330	0.025	0.028	5.35	0	0	0	urban
S11	31	11/20/2001	230	0.022	0.014	1.8	0.13	0.13	0.13	urban
S13	1	4/25/2001	110	0.01	0.039	4.05	0	0.31	0.74	Agr
S13	3	5/9/2001	540	0.138	0.014	3.92	0.04	0.72	0.72	Agr
S13	5	5/23/2001	320	0.11	0.001	13.7	0	0.44	0.44	Agr
S13	7	6/6/2001	300	0.075	0.023	17.17	0.02	0.02	0.02	Agr
S13	9	6/20/2001	460	0.08	0.05	12.1	0	0.18	0.18	Agr
S13	13	7/18/2001	450	0.04	0.005	7.2	0.04	0.04	0.04	Agr
S13	15	8/1/2001	620	0.048	0.125	8.2	0	0	0.38	Agr
S13	17	8/15/2001	550	0.033	0.014		0	0	0	Agr
S13	19	9/25/2001	380	0.042	0.009	4.18	0.06	0.58	0.58	Agr
S13	23	10/8/2001	700	0.013	0.02	2.2	0	0	0.62	Agr
S13	25	10/23/2001	370	0.039	0.058	20	0.14	0.14	0.14	Agr
S13	29	11/6/2001	130	0.043	0.03	3.15	0	0	0	Agr
S13	31	11/20/2001	120	0.029	0.019	2.3	0.13	0.13	0.13	Agr
S14	1	4/25/2001	80	0.013	0.044	3.31	0	0.31	0.74	Agr
S14	3	5/9/2001	390	0.067	0.026	2.65	0.04	0.72	0.72	Agr
S14	5	5/23/2001	190	0.133	0.001	16.3	0	0.44	0.44	Agr
S14	7	6/6/2001	250	0.064	0.035	16.84	0.02	0.02	0.02	Agr
S14	9	6/20/2001	670	0.061	0.056	15.9	0	0.18	0.18	Agr
S14	13	7/18/2001	660	0.04	0.005	7.2	0.04	0.04	0.04	Agr
S14	15	8/1/2001	460	0.032	0.101	6	0	0	0.38	Agr
S14	17	8/15/2001	580	0.054	0.011		0	0	0	Agr
S14	19	9/25/2001	440	0.069	0.015	4.97	0.06	0.58	0.58	Agr
S14	23	10/8/2001	810	0.012	0.014	4.4	0	0	0.62	Agr
S14	25	10/23/2001	320	0.034	0.028	12.6	0.14	0.14	0.14	Agr
S14	29	11/6/2001	40	0.036	0.033	6.51	0	0	0	Agr
S14	31	11/20/2001	190	0.021	0.013	2.5	0.13	0.13	0.13	Agr
S16	1	4/25/2001	127	0.486	0.11	6.19	0	0.31	0.74	Agr
S16	3	5/9/2001	393	0.219	0.086	10.3	0.04	0.72	0.72	Agr
S16	5	5/23/2001	350	0.19	0.031	17.9	0	0.44	0.44	Agr
S16	7	6/6/2001	273	0.116	0.068	13.6	0.02	0.02	0.02	Agr
S16	9	6/20/2001	223	0.101	0.076	10.6	0	0.18	0.18	Agr
S16	13	7/18/2001	100	0.071	0.001	3.5	0.04	0.04	0.04	Agr
S16	15	8/1/2001	483	0.132	0.106	2.8	0	0	0.38	Agr
S16	17	8/15/2001	310	0.144	0.026		0	0	0	Agr
S16	19	9/25/2001	1007	0.733	0.363	9.07	0.06	0.58	0.58	Agr
S16	23	10/8/2001	443	0.817	0.036	4.2	0	0	0.62	Agr
S16	25	10/23/2001	587	NS		NS	0.14	0.14	0.14	Agr
S16	29	11/6/2001	587	0.179	0.025	3.26	0	0	0	Agr
S16	31	11/20/2001	213	0.133	0.05	3.3	0.13	0.13	0.13	Agr
S17	1	4/25/2001	2500	0.117	0.085	2.32	0	0.31	0.74	Mixed
S17	3	5/9/2001	2000	0.24	0.091	3.49	0.04	0.72	0.72	Mixed
S17	5	5/23/2001	1800	0.15	0.008	3.3	0	0.44	0.44	Mixed
S17	7	6/6/2001	1700	0.229	0.014	2.83	0.02	0.02	0.02	Mixed
S17	9	6/20/2001	410	0.091	0.1	1	0	0.18	0.18	Mixed
S17	13	7/18/2001	20	0.13	0.021	1.4	0.04	0.04	0.04	Mixed
S17	15	8/1/2001	4900	1.816	0.331	3.4	0	0	0.38	Mixed
S17	17	8/15/2001	900	2.467	0.491		0	0	0	Mixed

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