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Module 7: Microorganisms in Urban Surface Waters

Potential Human Health Effects Associated with Microorganisms in Urban Waters

High concentrations of pathogens and indicator organisms found in urban receiving waters are a common cause of concern. Though some question the actual public health risk associated with exposure to these organisms, large amounts of resources are spent attempting to identify and correct their source. This discussion is a summary describing the potential human health effects associated with pathogens and common indicator organisms found in urban waters, as well as a brief discussion of the development of water quality standards for the indicators. This information will enable the reader to more effectively consider the level of risk that may actually be locally present.

Introduction

Urban receiving waters have many beneficial uses, including: stormwater conveyance (flood prevention), biological uses (warm water fishery, aquatic life uses, biological integrity, etc.), non-contact recreation (linear parks, aesthetics, boating, etc.), contact recreation (swimming and fishing), and water supply. Pollutants entering these receiving waters by way of urban stormwater conveyance systems, or wet weather sewage overflows, may adversely impact many of the desired uses. Urban runoff or wet weather flows include not only precipitation and washoff from lawns and landscaped areas, buildings, roadways and parking lots, but often separate sewer overflows or discharges resulting from inflow and infiltration (Lalor and Pitt 1998).

Water Environment & Technology (1996a) reported that the National Water Quality Inventory released by the U.S. Environmental Protection Agency (U.S. EPA) showed only a slight improvement in the attainment of beneficial uses in U.S. receiving waters. Bacteria and nutrients were cited as leading problems, and urban runoff was cited as a leading source of these problems. Bacteria, in particular, are associated with limiting human recreational and drinking water use. Recent epidemiological studies have shown significant health effects associated with pathogens in stormwater contaminated marine swimming areas (Haile, *et al.* 1999). Pathogens found in stormwater from separate storm drainage systems are a significant public health concern, as are pathogenic protozoa associated with likely sewage-contaminated stormwater (Ellis 1985; Oliveiri 1989; Bryan 1999; LeChevallier, *et al.* 1991, 1995, and 1995).

Over the years, numerous studies have investigated microorganisms in stormwater (such as Ellis and Wang 1995, Field, *et al*. 1976, Geldreich 1965, Geldreich, *et al*. 1968, and Olivieri, *et al*. 1977). Probably the most comprehensive early characterization study was the Nationwide Urban Runoff Program (NURP) (EPA 1983) conducted at many locations throughout the United States. Almost 220 NURP monitoring stations had reported about one-half million analyses, including more than 1,600 fecal coliform urban runoff observations from 70 test catchments over a one to three year period. These test catchments ranged in size from less than one acre to more than 10,000 acres. Most of these catchments were of residential land use, but almost all land uses in urban areas were included (commercial, industrial, open space, etc.). The fecal coliform observations had an overall range of ten to 270,000 organisms/100 mL. The average of the site means was about 20,000 fecal coliform organisms/100 mL. The overall range of observations for fecal coliform indicator bacteria in urban waters is therefore very large. However, the typical values observed, and especially the occasional extremely high values, are of great concern when they are compared to existing water quality standards and criteria for recreational use water.

There are several exposure pathways through which contaminated stormwater can cause potential human health problems. These include exposure to stormwater contaminants at swimming and recreational areas affected by stormwater discharges, drinking water supplies contaminated by stormwater discharges, and the consumption of fish and shellfish that have been contaminated by stormwater pollutants.

Isolating the risks associated with stormwater alone can be a difficult task. Watersheds are often very large and the receiving waters are affected by many sewage and industrial point discharges, and upstream agricultural nonpoint discharges, in addition to the local stormwater discharges. Even in waters receiving only stormwater discharges, inappropriate sanitary and other wastewaters may be discharging through the storm drainage system (Pitt, *et al*. 1993). These multiple sources make it especially difficult to identify specific cause and effect relationships associated with stormwater discharges alone. Therefore, much of the human risk assessment associated with stormwater exposure has been determined using theoretical evaluations, which rely on stormwater characteristics and laboratory studies in lieu of actual population studies. However, some site investigations, especially related to swimming beach problems associated with nearby stormwater discharges (Haile 1996 and Haile 1999), have been conducted, and in- stream studies of the fate and transport of pathogens and indicator organisms have recently been carried out (Easton 2000).

Traditionally, indicator bacteria have been used to evaluate potential health risks of contaminated water (Geldreich and Kenner 1969; Geldreich 1976). These indicator bacteria have been used as surrogates for the actual pathogens of concern due to the lack of technology, lack of expertise, and high cost of detecting and/or enumerating the pathogens. Recently, indicator bacteria data used to evaluate health risk due to pathogens have been shown to be inadequate (Kay and Fricker 1997). In particular, the low infectious dose and high persistence of viral and protozoan pathogens confounds the use of indicator bacteria as predictors of health risk (NRC 1994). The relationship is further complicated by the fact that indicator bacteria and pathogens do not share identical sources.

Unfortunately, most microbiological water quality standards are based on indicator bacteria, not pathogens. Recent improvements in technology have enabled detection and enumeration of the pathogens actually generating the health risk. It would seem prudent, therefore, to begin assessing the health risks using these new methods, and subsequently to base the standards, at least partly, on the pathogen measurements (as opposed to indicator) associated assessments of risk.

Pathogens

Water Environment & Technology (1996) reported that the latest National Water Quality Inventory released by the EPA only showed a slight improvement in the attainment of beneficial uses in the nation’s waters. Urban runoff was cited as the leading source of problems in estuaries, with nutrients and bacteria as the leading problems. Problems in rivers and lakes were mostly caused by agricultural runoff, with urban runoff the third ranked source for lakes, and the fourth ranked source for rivers. Bacteria, siltation, and nutrients were the leading problems in the nation’s rivers and lakes.

Pathogens in stormwater are a significant concern potentially affecting human health. The use of indicator bacteria is controversial for stormwater, as well as the assumed time of typical exposure of swimmers to contaminated receiving waters. However, recent epidemiological studies have shown significant health effects associated with stormwater contaminated marine swimming areas. Protozoan pathogens, especially associated with likely sewage-contaminated stormwater, is also of public health concern.

Fecal indicators (i.e., fecal coliforms, fecal streptococcus, *Escherichia coli*, and enterococci) are usually found in elevated concentrations in stormwater runoff, greatly exceeding water quality criteria and standards for primary and secondary contact (MWCOG 1984). This suggests that fecal pathogen levels are also elevated, though significant correlations with fecal coliforms are tenuous (EPA 1986). Dieoff of fecal organisms in receiving waters during summer months is relatively rapid, with 99% dying within 24 to 48 hrs (Burton 1985). However, fecal microorganisms also accumulate in sediments where survival is extended for weeks to months (Burton, *et al*. 1987). Sediment bacteriological analyses conducted in Birmingham, AL, area urban lakes have found elevated pore water concentrations (several hundred to several thousand organisms/100 mL) of *E. coli* and enterococci extending to at least 0.1 m in the sediments. Also, when gently disturbed, the water layer over the sediments is also found to significantly increase in microorganism concentrations (Ontario Ministry of the Environment 1983). In-situ dieoff studies also indicate that bacteria sedimentation may be a more important fate mechanism of stormwater bacteria than dieoff (Easton 2000).

Good correlations between the incidence of gastroenteritis in swimmers and *E. coli* and enterococci concentrations in water have resulted in recreational water criteria that were revised in the mid 1980s (EPA 1986). High fecal microorganism concentrations in stormwaters originate from wastes of wildlife, pets, livestock, septic systems, and combined sewer overflows (CSOs). The ecological effects of these inputs of fecal organisms are unknown; however public health is at risk in swimming areas that receive stormwaters.

Urban Bacteria Sources

The Regional Municipality of Ottawa-Carleton (1972) recognized the importance of rooftop, street surface, and open field runoff in contributing bacteria contaminants to surface waters in the Ottawa area. Gore and Storrie/Proctor and Redfern (1981) also investigated various urban bacteria sources affecting the Rideau River. They examined dry weather continuous coliform sources, the resuspension of contaminated river bottom sediments, exfiltration from sanitary sewers, and bird feces. These sources were all considered in an attempt to explain the relatively high dry weather coliform bacteria concentrations found in the river. They concluded, however, that stormwater runoff is the most probable source for the wet weather and continuing dry weather bacteria Rideau River concentrations. The slow travel time of the river water usually does not allow the river to recover completely from one rainstorm before another begins.

The Regional Municipality of Ottawa-Carleton (1972) noted the early Ottawa activities in correcting stormwater and sanitary sewage cross-connections. Since that time, many combined sewer overflows have also been eliminated from the Rideau River. Loijens (1981) stated that as a result of sewer separation activities, only one overflow remained active by 1981 (Clegg Street). During river surveys in 1978 and 1979 in the vicinity of this outfall, increased bacteria levels were not found. Gore and Storrie/Proctor and Redfern (1981) stated that there was no evidence that combined sewer overflows are causing the elevated fecal coliform bacteria levels in the river. Environment Canada, (1980) however, stated that high, dry weather bacteria density levels, especially when considering the fecal coliform to fecal strep. ratio (however, see discussion later in this module concerning the use of this ratio), constitutes presumptive evidence of low volume sporadic inputs of sanitary sewage from diverse sources into the downstream Rideau River sectors. The case study presented in Appendix A examines some of these issues.

Street surfaces have been identified as potential major sources of urban runoff bacteria. Pitt and Bozeman (1982) found that parking lots, street surfaces, and sidewalks were the major contributors of indicator bacteria in the Coyote Creek watershed in California. Gupta, *et al*. (1981) found high concentrations of fecal coliforms at a highway runoff site in Milwaukee. This site was entirely impervious and located on an elevated bridge deck. The only likely sources of fecal coliforms at this site were atmospheric deposition, bird droppings and possibly feces debris falling from livestock trucks or other vehicles.

Several studies have found that the bacteria in stormwater in residential and light commercial areas were from predominantly nonhuman origins. Geldreich and Kenner (1969) stated that the fecal coliforms in stormwater are from dogs, cats, and rodents in city areas, and from farm animals and wildlife in rural areas. Qureshi and Dutka, (1979) found that there may be an initial flush of animal feces when runoff first develops. However, the most important bacteria source for runoff is the feces bacteria that have been distributed generally in the soils and on the surfaces of the drainage area. An important source may be feces bacteria that are distributed in the soil and not the fresh feces washing off the impervious surfaces.

Some studies have investigated vegetation sources of coliform bacteria. For example, Geldreich (1965) found that the washoff of bacteria from vegetation does not contribute significant bacteria to the runoff. They also found that most of the bacteria on vegetation is of insect origin. Geldreich, *et al*. (1980) found that recreation activities in water bodies also increase the fecal coliform and fecal strep. concentrations. These organisms of intestinal origin will concentrate in areas near the shore or in areas of stratification. Fennell, *et al*. (1974) found that open dumps containing domestic refuse can be a reservoir of Salmonella bacteria that can be spread to nearby water bodies by foraging animals and birds.

When a drainage basin has much of its surface paved, the urban runoff bacteria concentrations can be expected to peak near the beginning of the rainfall event and then decrease as the event continues. Initial high levels of bacteria may be associated with direct flushing of feces from paved surfaces. These feces are from dogs defecating on parking lots and street areas and from birds roosting on rooftops. When a drainage area has a lot of landscaped areas or open land, relatively high bacteria concentrations in the urban runoff may occur throughout the rain event associated with continuous erosion of contaminated soils. However, recent bacteria tests in Tuscaloosa, AL, in a small one acre totally paved and roofed area (near the City Hall) found elevated *E.coli* and enterococci levels throughout a several day storm of several inches in depth. The bacteria were not source limited in this test area that had no obvious locations of contamination or urban wildlife.

Inappropriate Sanitary Sewage Discharges into Urban Streams

Urban stormwater runoff includes waters from many other sources that find their way into storm drainage systems, besides from precipitation. There are cases where pollutant levels in storm drainage are much higher than they would otherwise be because of excessive amounts of contaminants that are introduced into the storm drainage system by various non-stormwater discharges. Additionally, baseflows (during dry weather) are also common in storm drainage systems. Dry-weather flows and wet-weather flows have been monitored during numerous urban runoff studies. These studies have found that discharges observed at outfalls during dry weather were significantly different from wet-weather discharges and may account for the majority of the annual discharges for some pollutants of concern from the storm drainage system.

In many cases, sanitary sewage was an important component (although not necessarily the only component) of the dry weather discharges from the storm drainage systems. From a human health perspective (associated with pathogens), it may not require much raw or poorly treated sewage to cause a receiving water problem. However, at low discharge rates, the DO receiving water levels may be minimally affected. The effect these discharges have on the receiving waters is therefore highly dependent on many site specific factors, including frequency and quantity of sewage discharges and the creek flows. In many urban areas, the receiving waters are small creeks in completely developed watersheds. These creeks are the most at risk from these discharges as dry base flows may be predominately dry weather flows from the drainage systems. In Tokyo (Fujita 1998), for example, numerous instances were found where correcting inappropriate sanitary sewage discharges resulted in the urban streams losing all of their flow. In cities that are adjacent to large receiving waters, these discharges likely have little impact (such as DO impacts from Nashville CSO discharges on the Cumberland River, as studied by Cardozo, *et al*. 1994). The presence of pathogens from raw, or poorly treated sewage, in urban streams, however, obviously presents a potentially serious public health threat. Even if the receiving waters are not designated as water contact recreation, children are often seen playing in small city streams.

There have been a few epidemiology studies published describing the increased health risks associated with contaminated dry weather flows affecting public swimming beaches. The following discussion presents an overview of the development of water quality criteria for water contact recreation, plus the results of a recent epidemiological study that specifically examined human health problems associated with swimming in water affected by stormwater. In most cases, the levels of indicator organisms and pathogens causing increased illness were well within the range found in urban streams.

Microorganisms in Urban Waters

As discussed previously, microorganisms frequently interfere with beneficial uses in urban receiving waters. The use of conventional indicator organisms may be helpful, but investigations of specific pathogens are also possible with new analytical technologies. The following discussion contains some background on the development of water quality standards for indicator organisms, describes some new analytical procedures available, and presents an approach that measures organism "dieoff" *in-situ*, which is important for assessing public health risk associated with water contact in urban receiving waters.

Pathogens in stormwater and urban receiving waters are a significant concern potentially affecting human health. The use of indicator bacteria is controversial for stormwater, as well as the assumed time of typical exposure of swimmers to contaminated receiving waters. However, recent epidemiological studies have shown significant health effects associated with stormwater contaminated marine swimming areas. Protozoan pathogens, especially associated with likely sewage-contaminated stormwater, is also of public health concern.

Human health standards for body contact recreation (and for fish and water consumption) are based on indicator organism monitoring. Traditional monitoring and analysis methods for actual pathogens, with few exceptions, requires an extended laboratory effort, is very costly and not very accurate. Therefore, the use of indicator organisms has become established. Dufour (1984a) presents an excellent overview of the history of indicator bacterial standards and water contact recreation.

Total coliforms were initially used as indicators for monitoring outdoor bathing waters, based on a classification scheme presented by W.J. Scott in 1934. Total coliform bacteria refers to a number of bacteria including *Escherichia*, *Klebsiella*, *Citrobacter*, and *Enterobacter* (DHS 1997). They are able to grow at 35^{o}C and ferment lactose. They are all gram negative asporogenous rods and have been associated with feces of warm blooded animals. They are also present in soil.

The fecal coliform test is not specific for any one coliform type, or groups of types, but instead has an excellent positive correlation for coliform bacteria derived from the intestinal tract of warm blooded animals (Geldreich, *et al*. 1968). The fecal coliform test measures *Escherichia coli* as well as all other coliforms that can ferment lactose at 44.5^{o}C and are found in warm blooded fecal discharges. Geldreich (1976) found that the fecal coliform test represents over 96 percent of the coliforms derived from human feces and from 93 to 98 percent of those discharged in feces from other warm blooded animals, including livestock, poultry, cats, dogs, and rodents. In many urban runoff studies, all of the fecal coliforms were *E. coli* (Quresh and Dutka 1979). *E. coli*, a member of the fecal coliform group, has been used as a better indicator of fresh fecal contamination, compared to fecal coliforms. Table 1 indicates the species and subspecies of the Streptococcus and Enterococcus groups of bacteria that are used as indicators of fecal contamination (DHS 1997).

Table 1. Streptococcus Species used as Indicators of Fecal Contamination

Indicator organism	Enterococcus group	Streptococcus group
Group D antigen		
Streptococcus faecalis	X	X
S. facealis subsp. *liquifaciens*	X	X
S. faecalis subsp. *zymogenes*	X	X
S. faecium	X	X
S. bovis		X
S. equinus		X
Group Q antigen		
S. avium		X

Source: DHS (1997)

Fecal strep. bacteria are all of the intestinal Streptococci bacteria from warm blooded animal feces (Geldreich and Kenner 1969). The types and concentrations of different bacteria biotypes varies for different animal sources. Fecal streptococci bacteria are indicators of fecal contamination. The enterococcus group is a subgroup that is considered a better indication of human fecal contamination. *S. bovis* and *S. equinus* are considered related to feces from non-human warm blooded animals (such as from meat processing facilities, dairy wastes, and feedlot and other agricultural runoff), indicating that enterococcus may be a better indication of human feces contamination. However, *S. facealis* subsp. *liquifaciens* is also associated with vegetation, insects, and some soils (DHS 1997).

The EPA's evaluation of bacteriological data indicated that using the fecal coliform indicator group at the maximum geometric mean of 200 organisms per 100 mL, as recommended in *Quality Criteria for Water* would cause an estimated 8 illness per 1,000 swimmers at freshwater beaches. Additional criteria, using *E. coli* and *enterococci* bacteria analyses, were developed using these currently accepted illness rates. These bacteria are assumed to be more specifically related to poorly treated human sewage than the fecal coliform bacteria indicator. It should be noted that these indicators only relate to gastrointestinal illness, and not other problems associated with waters contaminated with other bacterial or viral pathogens. Common swimming beach problems associated with contamination by stormwater include skin and ear infections caused by *Psuedomonas aeruginosa* and *Shigella*.

Viruses may also be important pathogens in urban runoff. Very small amounts of an infectious virus are capable of producing disease, especially when compared to the large numbers of bacteria organisms required for infection (Berg 1965). The quantity of enterroviruses which must be ingested to produce infections is usually not known (Olivieri, *et al.* 1977b). Viruses are usually detected at low levels in urban receiving waters and storm runoff. They stated that even though the minimum infective doses may be small, the information available indicates that stormwater virus threats to human health is small. Because of the low levels of virus necessary for infection, dilution of viruses after discharges to receiving waters does not significantly reduce their hazard.

Sampling requirements for microorganism evaluations is more challenging than for most constituents, requiring sterile sample containers and tools, plus rapid shipment of the samples to the laboratory and immediate initiation of analyses. Bacteriological analyses are becoming much more simplified with special procedures and methods developed by HACH, Millipore, and IDEXX Corp., for example. Available methods require little more than mixing a freeze-dried "reagent" with a measured amount of sample, pouring the mixture into special incubation trays and sealing them, and finally placing them into incubators for the designated time (usually from 18 to 48 hours).

The IDEXX method for *E. coli*, Colilert-18, is used by many state resources agencies for EPA reporting purposes. It is used for the simultaneous detection, specific identification and confirmation of total coliforms and *E. coli* in water. It is based on IDEXX's patented Defined Substrate Technology® (DST™). It is a most probable number (MPN) method. Colilert-18 utilizes nutrient indicators that produce color and /or fluorescence when metabolized by total coliforms and *E. coli.* When the colilert-18 reagent is added to a sample and incubated, it can detect these bacteria at 1 CFU in 100 mL within 18 hours with as many as 2 million heterotrophic bacteria per 100 mL present. The required apparatus includes the Quanti-tray sealer, an incubator, a 6 watt 365 nm UV light, and a fluorescence comparator. This procedure requires 100 mL of sample which should be analyzed quickly after sampling. Marine water samples must be diluted at least 10 fold with sterile fresh water to reduce the salinity. Quality control includes testing with cultures of *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

The Enterolert procedure, also from IDEXX, is very similar to the Colilert method outlined above. Enterolert is used for the detection of enterococci such as *E. faecium* or *E. faecalis* in fresh and marine water. When the enterolert reagent is added to a sample and incubated, bacteria down to one CFU in a 100 mL sample can be detected within 24 hours. This method also has a quality control procedure that should be conducted on each lot of Enterolert, using test cultures of *Enterococcus faecium*, *Serratia marcescens* (gram -), and *Aerococcus viridans* (gram +).

Urban Water Pathogens and their Public Health Significance

The occurrence of Salmonella biotypes is typically low and their reported density is less than one organism/100mL in stormwater. *Pseudomonas aeruginosa* are frequently encountered at densities greater than ten organisms/100mL, but only after rains. The observed ranges of concentrations and percent isolations of bacterial biotypes vary significantly from site to site and at the same location for different times. Many potentially pathogenic bacteria biotypes may be present in urban runoff. Because of the low probability of ingestion of urban runoff, many of the potential human diseases associated with these biotypes are not likely to occur. The pathogenic organisms of most concern in urban runoff are usually associated with skin infections and body contact in urban receiving waters. The most important biotype causing skin infections would be *Pseudomonas aeruginosa*. This biotype has been detected frequently in most urban runoff studies in concentrations that may cause potential infections. However, there is little information associating the cause and effect of increased Pseudomonas concentrations with increased infections. Shigella may be present in urban runoff and receiving waters. This pathogen, when ingested in low numbers, can cause dysentery.

Salmonella

Salmonella has been reported in some, but not all, urban stormwaters. Qureshi and Dutka (1979) frequently detected Salmonella in southern Ontario stormwaters. They did not find any predictable patterns of Salmonella isolations as they were found throughout the various sampling periods. Olivieri, *et al*. (1977a) found Salmonella frequently in Baltimore runoff, but at relatively low concentrations. Typical concentrations were from five to 300 Salmonella organisms/ten liters. The concentrations of Salmonella were about ten times higher in the stormwater samples than in the urban stream receiving the runoff. They also did not find any marked seasonal variations in Salmonella concentrations. Almost all of the stormwater samples that had fecal coliform concentrations greater that 2000 organisms/100 mL had detectable Salmonella concentrations, while about 25 percent of the samples having fecal coliform concentrations less than 200 organisms/100 mL had detectable Salmonella.

Quite a few urban runoff studies have not detected Salmonella. Schillinger and Stuart (1978) found that Salmonella isolations were not common in a Montana subdivision runoff study and that the isolations did not correlate well with fecal coliform concentrations. Environment Canada (1980) stated that Salmonella were virtually absent from Ottawa storm drainage samples in 1979. They concluded that Salmonella are seldom present in significant numbers in Ottawa urban runoff. The types of Salmonella found in southern Ontario were *S. thompson* and *S. typhimurium var copenhagen* (Qureshi and Dutka 1979).

Olivieri, *et al*. (1977b) stated that the primary human enteric disease producing Salmonella biotypes associated with the ingestion of water include *S. typhi* (typhoid fever), *S. paratyphi* (paratyphoid fever), and Salmonella species (salmonellosis). These biotypes are all rare except for Salmonella. The dose of Salmonella required to produce an infection is quite large (approximately 105 organisms). The salmonellosis health hazard associated with water contact in urban streams is believed to be small because of this relatively large infective dose. If two liters of stormwater having typical Salmonella concentrations (ten Salmonella organisms per ten liters) is ingested, less than 0.001 of the required infective dose would be ingested. However, if a worse case Salmonella stormwater concentration of 10,000 organisms per ten liters occurred, the ingestion of only 10 mL of stormwater may result in an infective dose. They stated that the low concentrations of Salmonella, coupled with the unlikely event of consuming enough stormwater, make the Salmonella health hazard associated with urban runoff small.

Staphylococci

Staphylococcus aureus is an important human pathogen as it can cause boils, carbuncles, abscesses, and impetigo on skin on contact. Olivieri, *et al*. (1977b) stated that the typical concentrations of Staphylococci are not very high in urban streams. They also noted that there was little information available relating the degree of risk of staph. infections with water concentrations. They concluded that *Staph. aureus* appears to be the most potentially hazardous pathogen associated with urban runoff, but there is no evidence available that skin, eye, or ear infections can be caused by the presence of this organism in recreational waters. They concluded that there is little reason for extensive public health concern over recreational waters receiving urban storm runoff containing staph. organisms.

Shigella

Olivieri, *et al*. (1977b) stated that there is circumstantial evidence that Shigella is present in urban runoff and receiving waters and could present a significant health hazard. Shigella species causing bacillary dysentery are one of the primary human enteric disease producing bacteria agents present in water. The infective dose of Shigella necessary to cause dysentery is quite low (ten to 100 organisms). Because of this low required infective dose and the assumed presence of Shigella in urban waters, it may be a significant health hazard associated with urban runoff.

Streptococcus

Streptococcus faecalis and *atypical S. faecalis* are of limited sanitary significance (Geldreich 1976). Streptococcus determinations for urban runoff are most useful for identifying the presence of *S. bovis* and *S. equinus* that are specific indicators of non-human, warm blooded animal pollution. However, it is difficult to interpret fecal strep. data when their concentrations are lower than 100 organisms/100 mL because of the ubiquitious occurrence of *S. faecalis var. liquifaciens*. This biotype is generally the predominant strep. biotype occurring at low fecal strep. concentrations.

Pseudomonas aeruginosa

Pseudomonas is reported to be the most abundant pathogenic bacteria organism in urban runoff and streams (Olivieri, *et al*. (1977b). This pathogen is associated with eye and ear infections and is resistant to antibiotics. They also stated that past studies have failed to show any relationships between *P. aeruginosa* concentrations in bathing waters and ear infections. However, Pseudomonas concentrations in urban runoff are at significantly greater concentrations (about 100 items) than the values associated with past bathing beach studies. Cabelli, *et al*. (1976) stated that *Pseudomonas aeruginosa* is indigenous in about 15 percent of the human population. Swimmer's ear or other Pseudomonas infections may, therefore, be caused by trauma to the ear canals associated with swimming and diving, and not exposure to Pseudomonas in the bathing water.

Environment Canada (1980) stated that there is preliminary evidence of the direct relationship between very low levels of *Pseudomonas aeruginosa* and an increase in incidents of ear infections in swimmers. They stated that a control level for this Pseudomonas biotype of between 23 and 30 organisms/100 mL is being considered. Cabelli, *et al.* (1976) stated that *P. aeruginosa* densities greater than ten organisms/100 mL were frequently associated with fecal coliform levels considerably less than 200 organisms/100 mL. *P. aeruginosa* densities were sometimes very low when the fecal coliform levels were greater than 200 organisms/100 mL. An average estimated *P. aeruginosa* density associated with a fecal coliform concentration of 200 organisms/100 mL is about 12/100 mL. They further stated that *P. aeruginosa* by itself cannot be used as a basis for water standards for the prevention of enteric diseases during recreational uses of surface waters. The determinations of this biotype should be used in conjunction with fecal coliform or other indicator organism concentrations for a specific location. They recommended that bathing beaches that are subject to urban runoff pollution be temporarily closed until the *P. aeruginosa* concentrations return to a baseline concentration.

Campylobacter

Koenraad, *et al*. (1997) investigated the contamination of surface waters by Campylobacter and its associated human health risks. They reported that campylobacteriosis is one of the most frequently occurring acute gastroenteritis diseases in humans. Typical investigations have focused on the consumption of poultry, raw milk, and untreated water as the major sources of this bacterial illness. Koenraad, *et al.* (1997) found that human exposures to Campylobacter contaminated surface waters is likely a more important risk factor than previously considered. In fact, they felt that Campylobacter infections may be more common than Salmonella infections. The incidence of campylobacteriosis due to exposure to contaminated recreational waters has been estimated to be between 1.2 to 170 per 100,000 individuals. The natural habitat of Campylobacter is the intestinal tract of warm-blooded animals (including poultry, pigs, cattle, gulls, geese, pigeons, magpies, rodents, shellfish, and even flies). It does not seem to multiply outside of its host, but it can survive fairly well in aquatic environments. It can remain culturable and infective for more than 2 months under ideal environmental conditions. Besides runoff, treated wastewater effluent is also a major likely source of Campylobacter in surface waters. Sanitary wastewater may contain up to 50,000 MPN of Campylobacter per 100 mL, with 90 to 99% reductions occurring during typical wastewater treatment.

Viruses

It is believed that approximately half of all waterborne diseases are of viral origin. Unfortunately, it is very difficult and time consuming to identify viruses from either environmental samples or sick individuals. When the EPA conducted its extensive epidemiological investigations of freshwater and marine swimming beaches (discussed above) in the 1980's, two viruses common to human gastrointestinal tracts (coliphage and enterovirus) were evaluated as potential pathogen indicators. These two indicators did not show good correlations between their presence and the incidence of gastroenteritis. Viruses tend to survive for slightly longer periods in natural waters than do gram negative bacteria. It is believed that the high correlation observed between gastroenteritis and the presence of enterococci may be because the gram positive enterococci's longer survival more closely mimics viral survival. Therefore, enterococci may serve as a good recreational water indicator for the presence of viral pathogens.

Pfiesteria piscicida

Special human health concerns have also been expressed about *Pfiesteria piscicida*, a marine dinoflagellate that apparently is associated with coastal eutrophication caused by runoff nutrients (Maguire and Walker 1997). Dramatic blooms and resulting fish kills have been associated with increased nutrient loading from manure-laden runoff from large livestock feedlot operations. This organism has gathered much attention in the popular press, usually called the "cell from hell" (Zimmerman 1998). It has been implicated as causing symptoms of nausea, fatigue, memory loss, and skin infections in south Atlantic coastal bay watermen. *Pfiesteria* and *Pfiesteria*-like organisms have also been implicated as the primary cause of many major fish kills and fish disease events in Virginia, Maryland, North Carolina, and Delaware. In August of 1997, hundreds of dead and dying fish were found in the Pocomoke River, near Shelltown, Maryland, in the Chesapeake Bay, prompting the closure of a portion of the river. Subsequent fish kills and confirmed occurrences of *Pfiesteria* led to further closures of the Manokin and Chicamacomico Rivers. The Maryland Department of Health and Mental Hygiene also presented preliminary evidence that adverse public health effects could result from exposure to the toxins released by *Pfiesteria* and *Pfiesteria*-like organisms. The increasing numbers of fish kills of Atlantic menhaden (an oily, non-game fish) motivated Maryland's governor to appoint a Citizens *Pfiesteria* Action Commission. The Commission conveyed a forum of noted scientists to examine the existing information on *Pfiesteria*.

Pfiesteria has a complex life cycle, including at lease 24 flagellated, amoeboid, and encysted stages. Only a few of these stages appear to be toxic, but their complex nature makes them difficult to identify by nonexperts (Maguire and Walker 1997). *Pfiesteria* spends much of its life span in a nontoxic predatory form, feeding on bacteria and algae, or as encysted dormant cells in muddy sediment. Large schools of oily fish (such as the Atlantic menhaden) trigger the encysted cells to emerge and excrete toxins. These toxins make the fish lethargic, so they remain in the area where the toxins attack the fish skin, causing open sores to develop. The *Pfiesteria* then feed on the sloughing fish tissue. Unfortunately, people working in the water during these toxin releases may also be affected (Zimmerman 1998).

Researchers suggest that excessive nutrients (causing eutrophication) increase the algae and other organic matter that the *Pfiesteria* and Atlantic menhaden use for food. The increased concentrations of *Pfiesteria* above natural background levels increase the likelihood of toxic problems. Maguire and Walker (1997) state that other factors apparently are also involved, including stream hydraulics, water temperature, and salinity. They feel that Pfiesteria is only one example of the increasing threats affecting coastal ecosystems that are experiencing increased nutrient levels. Most of the resulting algal blooms only present nuisance conditions, but a small number can result in human health problems (mostly as shellfish poisonings). The increased nutrient discharges are mostly associated with agricultural operations, especially animal wastes from large poultry and swine operations. In the Pocomoke River watershed, the Maryland Department of Natural Resources estimates that about 80% of the phosphorus and 75% of the nitrogen load is from agricultural sources. Urban runoff may also be a causative factor of eutrophication in coastal communities, especially those having small enclosed coastal lagoons or embayments, or in rapidly growing urban areas. Zimmerman (1998) points out that the Chesapeake Bay area is one of the country's most rapidly growing areas, with the population expected to increase by 12 percent in a 15 year period.

Protozoa in Urban Watersheds

States, *et al.* (1997) examined *Cryptosporidium* and *Giardia* in river water serving as Pittsburgh's water supply. They collected monthly samples from the Allegheny and Youghiogheny Rivers for two years. They also sampled a small stream flowing through a diary farm, treated sanitary sewage effluent, and CSOs. Table 2 summarizes their observations. The CSO samples had much greater numbers of the protozoa than any of the other samples collected. No raw sewage samples were obtained, but they were assumed to be very high because of the high CSO sample values. The effluent from the sewage treatment plant was the next highest, at less than half of the CSO values. The diary farm stream was not significantly different from either of the two large rivers. The water treatment process appeared to effectively remove *Giardia*, but some *Cryptosporidium* was found in the filtered water. Sedimentation treatment of the river water seemed to remove some of the protozoa, but that treatment process alone would not be adequate.

Table 2. Observed *Giardia* and *Cryptosporidium* in the Pittsburgh, PA, area (States, *et al.* 1997)

Samples	*Giardia* cysts			*Cryptosporidium* oocysts		
	Number of samples	Occurrence (%)	Geometric mean of detectable samples (#/100 L)	Number of samples	Occurrence (%)	Geometric mean of detectable samples (#/100 L)
CSOs	5	100	28,700	5	80	2,010
Sewage treatment plant effluent	24	83	664	24	33	924
Dairy farm stream	24	55	82	24	82	42
Allegheny River	24	63	34	24	63	31
Settled Allegheny River water	24	8	29	24	29	12
Filtered Allegheny River water	24	0	0	24	21	0.5
Filter backwash water	24	8	59	24	38	328
Youghiogheny River	24	54	118	24	63	58

States, et al. (1997) also reviewed prior Giardia and Cryptosporidium monitoring data, as summarized in Table 3. Raw drinking water supplies were shown to have highly variable levels of these protozoa, typically up to several hundred Giardia cysts and Cryptosporidium oocysts per 100 L and were found in 5 to 50% of the samples evaluated. Conventional water treatment appeared to remove about 90% of the protozoa.

Table 3. Observed *Giardia* and *Cryptosporidium* In Raw Water Supplies and in Treated Water (States, *et al.* 1997)

Samples	*Giardia* cysts			*Cryptosporidium* oocysts		
	Number of samples	Occurrence (%)	Geometric mean of detectable samples (#/100 L)	Number of samples	Occurrence (%)	Geometric mean of detectable samples (#/100 L)
Rivers, streams, and lakes in 17 states (Rose, *et al.* 1991)	257	16	3 (average)	257	55	43 (average)
Drinking water samples (Rose, *et al.* 1991)	36	0	0	36	17	0.5 to 1.7 (range)
Raw surface water supply samples at 72 water treatment plants (LeChevallier and Norton 1995)	262	45	200	262	52	240
Finished drinking water from above plants (LeChevallier and Norton 1995)	262	4.6	2.6	262	13.4	3.3
Raw surface water supply samples at 66 water treatment plants (LeChevallier, *et al.* 1991a)	85	81	277	85	87	270
Filtered drinking water from above plants (LeChevallier, *et al.* 1991b)	83	17	4.5	83	27	1.5
Finished water samples from 33 conventional water treatment plants (Hancock, *et al.* 1996)	55	5 presumptive 2 confirmed	2 to 5 presumptive (range) 2 confirmed	55	7 presumptive	1 to 26 presumptive (range)
Existing data on finished water from 130 U.S. water treatment plants (Rosen, *et al.* 1996)	1237	4.9	na	1237	7.1	na

Protozoa became an important public issue with the 1993 *Cryptosporidium*-caused disease outbreak in Milwaukee when about 400,000 people became ill from drinking contaminated water. Mac Kenzie, *et al.* (1994) prepared an overview of the outbreak, describing the investigation on the causes of the illness and the number of people affected. They point out that *Cryptosporidium*-caused disease in humans was first documented in 1976, but had received little attention and no routine monitoring. *Cryptosporidium* now is being monitored routinely at many areas and is the subject of much research concerning its sources and pathways. At the time of the Milwaukee outbreak, both of the city's water treatment plants (using water from Lake Michigan) were operating within acceptable limits, based on required monitoring. However, at one of the plants (which delivered water to most of the infected people), the treated water experienced a large increase in turbidity (from about 0.3 NTU to about 1.5 NTU) at the time of the outbreak that was not being well monitored (the continuous monitoring equipment was not functioning, and values were only obtained every 8 hours). More than half of the residents receiving water from this plant became ill. The plant had recently changed its coagulant from polyaluminum chloride to alum and equipment to assist in determining the correct chemical dosages was not being used. The finished water had apparently relatively high levels of *Cryptosporidium* because some individuals became ill after only drinking less than 1 L of water. *Cryptosporidium* oocysts have often been found in untreated surface waters, and it was thought that *Cryptosporidium* oocysts entered the water treatment supply before the increase in turbidity was apparent. Mac Kenzie, *et al.* (1994) point out that monitoring in the United Kingdom has uncovered sudden, irregular, community-wide increases in cryptosporidiosis that were likely caused by waterborne transmission. They also stated that the source of the *Cryptosporidium* oocysts was speculative, but could have included cattle feces contamination in the Milwaukee and Menomonee Rivers, slaughterhouse wastes, and human sewage. The rivers were also swelled by high spring rains and snowmelt runoff that may have aided the transport of upstream *Cryptosporidium* oocysts into the lake near the water intakes.

The *Journal of the American Water Works Association* has published numerous articles on protozoa contamination of drinking water supplies. Crockett and Haas (1997) describe a watershed investigation to identify sources of *Giardia* and *Cryptosporidium* in the Philadelphia watershed. They describe the difficulties associated with monitoring *Cryptosporidium* and *Giardia* in surface waters because of low analytical recoveries and the cost of analyses. Large variations in observed protozoa concentrations made it difficult to identify major sources during the preliminary stages of their investigations. They do expect that wastewater treatment plant discharges are a major local source, although animals (especially calves and lambs) are likely significant contributors. Combined sewer overflows had *Giardia* levels similar to raw sewage, but the CSOs were much less than the raw sewage for *Cryptosporidium*. LeChevallier, *et al.* (1997) investigated *Giardia* and *Cryptosporidium* in open reservoirs storing finished drinking water. This gave them an opportunity to observe small increases in oocyst concentrations associated from nonpoint sources of contamination from the highly controlled surrounding area. They observed significantly larger oocyst concentrations at the effluent (median values of 6.0 Giardia/100 L and 14 *Cryptosporidium*/100 L) in the reservoirs than in the influents (median values of 1.6 *Giardia*/100 L and 1.0 *Cryptosporidium*/100 L). No human wastes could influence any of the tested reservoirs and the increases were therefore likely caused by wastes from indigenous animals or birds, either directly contaminating the water, or through runoff from the adjacent wooded areas.

A Management Training Audioconference Seminar on *Cryptosporidium* and Water (MTA 1997) was broadcast in May of 1997 to familiarize state and local agencies about possible *Cryptosporidium* problems that may be evident as a result of the EPA's Information Collection Rule which began in July of 1997. This regulation requires all communities serving more than 100,000 people to monitor their source water for *Cryptosporidium* oocysts. If the source water has more than 10 *Cryptosporidium* oocysts per liter, then the finished water must also be monitored. It is likely that many source waters will be found to be affected by *Cryptosporidium*. They reviewed one study that found the percentage of positive samples of *Cryptosporidium* in lakes, rivers, and springs was about 50 to 60% and about 5% in wells. In contrast, the percentage of samples testing positive for *Giardia* was about 10 to 20% in lakes and rivers, and very low in springs and wells.

Levels of Microorganisms Observed in Urban Runoff and Sanitary Sewage Flows

There have been many studies in the United States and Canada that have examined the bacteria quality of urban runoff. Many of these studies also examined significant factors affecting the bacteria concentrations.

Lager, *et al.* (1977) summarized the results of a study conducted in Tulsa, Oklahoma, which looked at the precipitation and land use factors that affected pollutant concentrations in urban stormwater. The only two factors that had a significant effect on fecal coliform concentrations were the interevent period and the intensity of the rain event. The amount of rain and the street density also affected the total coliform concentrations. Seidler (1979) in a semi-rural watershed in Oregon also found that bacteria concentrations during storm events were more related to the length of the dry period before the storm than the season of the year or the actual amount of precipitation. However, Qureshi and Dutka (1979) found little relationship between intensity and the amount of rain versus the presence of indicator and pathogenic microorganisms in southern Ontario stormwater runoff. Olivieri, *et al.* (1977a) found that the density of fecal coliforms in Baltimore, Maryland, urban runoff appeared to be independent of the instantaneous flows and the length of time since the last rainfall. Gupta, *et al.* (1981) also found that flow did not have a significant effect on the instantaneous bacteria concentrations.

In contrast to these studies, another series of studies found some relationships between bacteria densities and the urban runoff flows. The concentrations of bacteria were normally high during periods of high flows and lower during other times in these studies (Evans and Owens 1972; Casserly and Davis 1979; Pontius 1977; Davis 1979; and Siedler 1979). The concentrations of bacteria in urban stormwater have been found to vary during storms, sometimes as a function of various observed factors and sometimes independent of observed factors. The important factors varied from site to site. Most of these studies consisted of relatively few completely monitored storms but many samples were usually included within the few storms monitored. The variable nature of bacteria deposition, accumulation, transport, and dieoff makes it very difficult to identify consistent influencing factors. The most reasonable approach in characterizing urban runoff bacteria conditions appears to be to study as many storms as possible in the watersheds of concern. Statistical analyses can then be used to help identify probable concentrations and yields.

The Nationwide Urban Runoff Program (NURP) projects (EPA 1983) conducted at many locations throughout the United States obtained urban runoff bacteria conditions for a variety of test sites. Seventy test catchments in the

NURP program monitored urban runoff bacteria quality. These test catchments ranged in size from less than one acre to more than 10,000 acres. Most of these catchments were of residential land use, but almost all land uses in urban areas were included (commercial, industrial, open space, etc.). Table 4 summarizes the total coliform, fecal coliform, and fecal strep. bacteria concentrations available for these catchments. More than 1,600 fecal coliform urban runoff observations are available from the NURP program, with an overall observed range of ten to 270,000 organisms/100 mL. The average of the site means was about 20,000 fecal coliform organisms/100 mL. These data were for samples collected from 1978 to 1981, with most of the data from samples collected in 1980.

Table 4. NURP Urban Catchment Runoff Data (EPA 1983)

		Total Coliforms		Fecal Coliforms		Fecal Strep.	
NURP City, State	Monitoring Site	mean* value (#/100mL)	# of obs.	mean value (#/100mL)	# of obs.	mean value (#/100mL)	# of obs.
Durham, NH	Shop/Save parking lot	7,800	7	480	7	1,100	7
Lake Quinsigamond, MA	Jordan Pond	180,000	33	24,000	25	---	---
	Rt. 9	750,000	17	110,000	17	---	---
	Locust St.	850,000	18	230,000	18	---	---
	Fitz. Brook at Anne St.	340,000	20	33,000	18	---	---
	Coal Mine Bk. at Convent	39,000	19	280	11	---	---
	Tilly Brook	900,000	32	110,000	32	---	---
Long Ialand, NY	Huntington	47,000	23	23,000	23	62,000	23
	Laurel Hollow	16,000	24	6,000	24	76,000	24
	N. of Belmont L.	150,000	16	41,000	16	45,000	16
	N. of Belmont L.	12,000	41	4,300	41	46,000	41
	Massapequa Pond influent	74,000	224	19,000	206	74,000	224
	Maaaspequa Pond effluent	54,000	210	3,800	203	42,000	210
Irondequoit Bay, NY	East Rocheater	---	---	4,800	22	---	---
	Southgate	---	---	8,700	22	---	---
	Cranston Rd.	---	---	4,800	14	---	---
	Baird	---	---	5,500	23	---	---
Wash., D.C.	Stedwick	2,400	1	3	1	3	1
	Stedwick	240,000	1	9,300	1	2,400	1
	Stedwick	240,000	1	46,000	1	2,400	1
	Lakeridge	1,800	4	120	4	120	4
	Lakeridge	1,900	4	70	4	170	3
	Stratton Woods	240,000	1	46,000	1	2,400	1
	Westleigh Rt. 28	1,500	4	720	4	1,200	4
	Fairidge	240,000	1	9,300	1	2,400	1
Winston-Salem, NC	into Tar Branch	---	---	17,000	95	- -	---
	into Burke Branch	---	---	13,000	66	---	- -
Knoxville, TN	Clinton Plaza	---	---	1,600	1	2,000	1
	Union Ave.	---	---	10	1	200	1
Mllwaukee, WI	Wood Center	---	---	16,000	1	9,800	1
	N. Hastings	---	---	26,000	4	85,000	4
	North Burbank	---	---	270,000	4	280,000	4
	State Fair	---	---	36,000	2	37,000	2
	Rustler	---	---	8,900	3	5,700	2
	Post Office	---	---	4,000	1	6,500	1
Austin, TX	NW Austin into Woodhollow Dam	61,000	4	15,000	8	---	---
	from Woodhollow Dam	37,000	2	8,600	10	---	---
	Rolllngwood	---	---	730	1	---	---
Denver, CO	Big Dry Trib.	---	---	6,200	39	---	---
	Asbury Park (inflow to HIG)	- -	---	3,800	9	---	---
	Asbury Park (outflow from HIG)	-	---	31,000	10	---	---
	North Ave. (inflow to HIG)	---	---	2,700	44	---	---
	North Ave. (outflow from HIG)	---	---	5,900	24	---	---
	Cherry Knolls	---	---	6,300	29	- -	---
	116th & Claude	- -	---	25,000	45	- -	---
	Villa Italia	---	---	6,800	34	---	---
Salt Lake City, UT	No. Temple	150,000	1	7,100	12	21,000	11
	8th So.	- -	---	120,000	3	6,000	3

		Total Coliforms		Fecal Coliforms		Fecal Strep.	
NURP City, State	Monitoring Site	mean* value (#/100mL)	# of obs.	mean value (#/100mL)	# of obs.	mean value (#/100mL)	# of obs.
	1300 So	---	---	6,600	5	53,000	5
	1300 So	---	---	480	1	52,000	2
	South	1,200	5	730	9	1,300	9
	90th So.	24,000	4	26,000	6	23,000	6
Rapid City, SD	Meade St.	---	---	94,000	16	---	---
Bellevue, WA	Lake Hills	---	---	3,300	97	---	---
	Surrey Downs	14,000	7	3,300	97	7,300	7
	148th Ave.	---	---	560	68	---	---
Eugene, OR	Polk St.	---	---	31,000	12	---	---
	A-3 at Wallis Rd.	---	---	1,900	25	---	---
	A-3 at Bertelsen	---	---	1,500	25	---	---
	A-2 at Golden Gds.	---	---	190	13	---	---
	Q St.	---	---	10	1	---	---
	72nd St.	---	---	620	6	---	---
	So. Banch Q st.	—-	---	1,700	9	---	---
	N. Branch Q St.	-—	---	56	7	---	---
	Q St. at 2nd St.	-—	---	9,700	23	---	---
	Q St. at Garden Way	---	---	2,100	16	---	---
	Q St. at Skipworth	---	---	230	10	—-	---
	Marcola Rd.	-—	-—	10	1	—-	---
	Springfield Mill Race	—	—	2,400	16	-—	---
	Eugene Mill Race	-—	—-	1,000	7	---	---
Overall number of observations:			724		1,655		620
overall minimum:		1,200		10		120	
overall maximun:		900,000		270,000		280,000	
average of site means:		170,000		22,000		32,000	

* arithmetic mean values

Table 5 summarizes the results from about 25 older studies that monitored coliform bacteria in urban runoff. These represent many stations throughout the United States with some locations in Canada and Europe. The overall NURP reported average fecal coliform concentration was about 2.2 x 10^4 organisms/100 mL, while the average from the other studies was about 3 x 10^4 fecal coliform organism/100 mL. These average concentration values are all surprisingly close. However, the overall observed range is quite high, ranging from not detecting any fecal coliforms to as high as 10 x 10^7 organisms/100 mL.

Table 5. Bacteria Values Reported during Early Urban Stormwater Studies (number of organisms/100 mL, empty cells have no available data) (Pitt 1983)

City Province/State	Site/station/or land use	Total Coliforms				Fecal Coliforms				Fecal Streptococci				Ref*
		Geo. mean	Min.	Max.	# of obs.	Geo. mean	Min.	Max.	# of obs.	Geo. mean	Min.	Max.	# of obs.	
Burlington, Ontario	Aldershot Plaza	2.3 X 10^5	2.9 X 10^3	2.2 X 10^7	8	6.3 X 10^2	1.0 X 10^2	7.5 X 10^3	8	7.5 X 10^2	1.6 X 10^2	5.5 X 10^3	8	19
	Malvern Rd.	3.5 X 10^2	3	5.3 X 10^3	10	57	1	1.8 X 10^3	10	89	13	9.2 X 10^2	10	19
Toronto, Ontario	Brucewood		100	1.1 X 10^4			10	1.9 X 10^4						17
	Guelph – north		<10^4	8.3 X 10^5			<10^3	7.1 X 10^4						17
	Windsor – A		200	1.2 X 10^6			100	2.0 X 10^4						17
	NA	6.1 X 10^5	7.0 X 10^4	3.2 X 10^6	18	1.1 X 10^4	3.0 X 10^3	2.7 X 10^4	18	2.9 X 10^4	1.3 X 10^3	7.1 X 10^4	18	17
	NA	2.0 X 10^4				5.0 X 10^3								24
Milwaukee, WI	Highway site – 794		3.0 X 10^3	6.0 X 10^5			10	>10^5			40	4.3 X 10^3		13
	Highway site - 45		4.5 X 10^3	7.9 X 10^6			4.9 X 10^2	3.0 X 10^5			1.3 X 10^3	3.0 X 10^5		13
Cincinnati, OH	Residential			5.0 X 10^5				8.0 X 10^4				1.0 X 10^5		25
	Residential street					1.4 X 10^3	50	4.7 X 10^4		2.9 X 10^4	2.2 X 10^3	1.5 X 10^5		12
	Suburban bus. area					8.7 X 10^3	2.5 X 10^3	4.0 X 10^4		3.2 X 10^4	1.3 X 10^4	5.6 X 10^4		12
Ann Arbor, MI	NA	2.1 X 10^6	1.2 X 10^5	3.4 X 10^7	22 storms	1.2 X 10^5	7.4 X 10^3	7.5 X 10^5	22 storms	2.1 X 10^5	3.1 X 10^4	6.7 X 10^5	22 storms	6
Harrisburg, PA	Highway site		10^2	1.8 X 10^5			<1	>10^5			6.4 X 10^2	2.0 X 10^5		13
Washington, DC	NA		1.2 X 10^5	3.2 X 10^6			4.0 X 10^4	1.3 X 10^6						14
Baltimore, MD	NA	1.2 X 10^5				2.4 X 10^4				1.7 X 10^5				16
	Stoney Run		5.4 X 10^3	1.6 X 10^6	17		1.3 X 10^3	5.4 X 10^4	17		5.3 X 10^2	3.0 X 10^5	17	11
	Glen Ave.		7.9 X 10^3	1.6 X 10^6	17		1.4 X 10^3	2.3 X 10^5	17		9.2 X 10^3	2.8 X 10^6	17	11
	Howard Park		4.9 X 10^3	2.8 X 10^7	17		2.3 X 10^3	2.9 X 10^6	17		<10^3	1.4 X 10^6	17	11
	Jones Falls		3.3 X 10^4	>2.4 X 10^6	17		5.0 X 10^3	>1.6 X 10^6	17		2.6 X 10^3	8.0 X 10^5	17	11
	Bush Street		7.9 X 10^3	2.4 X 10^6	17		1.7 X 10^3	2.4 X 10^6	17		2.5 X 10^3	1.9 X 10^6	17	11
	Northwood		1.3 X 10^3	1.7 X 10^5	14		80	7.9 X 10^4	14		1.7 X 10^3	3.0 X 10^5	14	11
Durham, NC	NA					2.5 X 10^5	3.0 X 10^3	1.9 X 10^6	37					5
	NA					2.3 X 10^2	1	2.0 X 10^3						7
Nashville, TN	Highway site		1.3 X 10^3	2.9 X 10^6			1.5 X 10^2	2.6 X 10^5			3.9 X 10^3	3.5 X 10^6		13
Knoxville, TN	Plantation Hills suburban					2.0 X 10^4	6.7 X 10^2	7.0 X 10^5	40					3
Atlanta, GA	3 suburban sites combined					6.3 X 10^3	10	10^5	53					4
Miami, FL	Parking lot	5.0 X 10^4				5.0 X 10^4				10^3				23
	Residential	5.0 X 10^3				4.0 X 10^3				7.0 X 10^2				23
	Residential	2.0 X 10^4				2.0 X 10^4				10^3				23
Oklahoma City, OK	15 areas combined					4.0 X 10^2	0	4.7 X 10^5	358					2
Houston, TX	Westberry Sq. residential	3.0 X 10^7		10^7		2.0 X 10^4		10^4		10^4		10^4		8
Denver, CO	Highway site		0	>10^5			0	2.7 X 10^3			0	>10^5		13
Boulder, CO	Snowmelt	5.0 X 10^6	2.3 X 10^3	1.1 X 10^7	1 storm	<5.0 X 10^6	<2 X 10^2	<1.1 X 10^7	1 storm					18
	Urban stream – base flow		4.0 X 10^3	6.0 X 10^3			6.0 X 10^2	10^3						18
	Semi-urban/rural		9.3 X 10^2	4.6 X 10^4			9.3 X 10^2	9.3 X 10^3						15
	urban		9.3 X 10^2	2.4 X 10^5			4.3 X 10^2	9.8 X 10^4						15
Seattle, WA	Street gutters			1.6 X 10^4										22
Tucson, AZ	High school	10^7				10^5				10^5				20
	Arcadia	10^6				10^5				10^5				20
	Railroad	10^6				10^4				10^5				20
San Diego, CA	Tecolote Creek					1.5 X 10^4	5.8 X 10^3	4.1 X 10^4	33					21
Sacramento, CA	NA						2.4 X 10^4	10^7						9
Stockholm, Sweden	Streets and parks	4.0 X 10^3		2.0 X 10^5										1
Nationwide	Urban streams					6.0 X 10^3	2.0 X 10^2	2.0 X 10^6						10
Overall minimum:			0				0				0			
Overall maximum:				3.0 X 10^7				10^7				3.0 X 10^6		
Average of site geo. means		3.0 X 10^6				3.0 X 10^4				6.0 X 10^4				

* References:

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16. Olivieri, Kruse, and Kawata 1977a
17. Ontario Ministry of the Environment 1982
18. Pontius 1977
19. Qureshi and Dutka 1979
20. Resnick and DeCook 1980
21. Setmire and Bradford 1980
22. Sylvester 1960
23. Lager, *et al*. 1977
24. Waller and Novak undated
25. Weibel, *et al*. 1964

As a comparison, Table 6 presents some typical combined sewer overflow bacteria concentrations, as reported in the literature. The fecal coliform concentrations in combined sewer overflows are seen to range from about 2 x 10^4 to a high of about 2 x 10^7 fecal coliform organisms/100 mL. The separate stormwater fecal coliform bacteria observations are at the low end of this reported range for CSOs. Typical combined sewers can therefore have 100 to 1,000 times the fecal coliform concentrations as separate stormwater. A study by Burm and Vaughan (1966) in Detroit and Ann Arbor, Michigan, found that the total coliform densities in the combined sewers were always about three to 15 times greater than those found in urban runoff. The fecal coliform densities in the combined sewers were about 90 times the stormwater values. They concluded that the bacteria densities for the combined sewer overflows were at least ten times greater than those reported for the stormwaters alone.

Table 6. Selected Combined Sewer Overflow Bacteria Data from the Literature (organisms/100 mL)

City (reference)	Total Coliforms	Fecal Coliforms	Fecal Strep.
Ottawa (Rideau R. Stormwater Management Study 1981)	na	$5x10^5$ to $9x10^6$	na
Toronto (Ontario Ministry of the Environment 1983)	10^7	10^6	na
Detroit (Geldreich 1976)	na	10^6 to 10^7	10^5
Selected data (Field and Struzeski 1972)	$2x10^4$ to $9x10^7$	$2x10^4$ to $2x10^7$	$2x10^4$ to $2x10^6$

Table 7 summarizes the pathogenic bacteria biotypes that have been observed in the Rideau River near Ottawa, Ontario (Pitt 1983). The occurrence of Salmonella biotypes is low and their reported density is less than one organism/100mL. *Pseudomonas aeruginosa* are frequently encountered at densities greater than ten organisms/100mL, but only after rains. As a comparison, Tables 8 and 9 show typical pathogenic bacteria biotype concentrations found in raw sanitary wastewaters are much greater than found in urban runoff. Table 10 summarizes the occurrence of various pathogens found in urban stormwaters at various sites. The observed ranges of concentrations and percent isolations of these biotypes vary significantly from site to site and at the same location for different times.

Table 7. Pathogenic Organisms Observed in the Rideau River (Environment Canada 1980)

Organism	Occurrence	Density
Salmonella biotypes	Low[1]	<0.2 to 0.8/100 mL
S. typhimurium		
S. infantis		
S. agona		
S. haardt		
S. saint paul		
S. nienstedten		
Pseudomonas aeruginosa	frequent	>10/100 mL only after rains
Edwardsiella tarda	Rare	--
Candida albicans (a yeast)	1 to 7% positive	1 to 2 cfu/100 mL

[1] very seldom found in Ottawa urban runoff

Table 8. Pathogenic Bacteria Types found in Raw Sanitary Wastewater in Baltimore, MD (Olivieri, *et al.* 1977b)

Staphyloccus aureus	42 to 4,600/100 mL, mean of 820/100 mL
Pseudomonas aeruginosa	Average of 220,000/100 mL

Table 9. Streptococci Biotypes found in Sanitary Wastewater (% Occurrence) (Geldreich and Kenner 1969)

City	Entercocci	*S. bovis* *S. equinus*	Aytpical *S. faecalis*	*S. faecalis liquifaciens*
Preston, ID	80	0	0	21
Fargo, ND	100	0	0	0
Moorehead, MN	90	10	0	0
Cincinnati, OH	72	3	2	24
Lawrence, MA	84	4	0	12
Monroe, MI	79	1	4	16
Denver, CO	86	11	3	0

Table 10. Pathogens Found in Urban Stormwater (organisms/100mL) (Pitt 1983)

City, Province/ State	catchment/ land use	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	Salmonella	Streptococci	Enterovirus	others	reference
Burlington, Ontario	Aldershot Plaza		14-3,000	*S. senftenberg* & *S. newport* isolated			total fungi: 2xl0^{4} -2xl0^{6}	Oureshi and Dutka 1979
	Malvern Road		<1-740	100% negative			total fungi: 9-400 Heterotroph count: 4xl0^{5}-2xl0^{7}	“
Milwaukee, WI	highway runoff	all <1,000	all <1,000	45% positive				Gupta, *et al.* 1981
Baltimore, MD	Stoney Run	<3-80	200-240,000	0.03->13				Field, *et al*. 1976
	Glen Ave.	<3-150	130-260,000	0.02->110				“
	Howard Park	6-920	790-54,000	0.04->13				“
	Jones Falls	4-11	940-1,600,000	0.17-3				“
	Bush Street	<3-4,600	110-75,000	<0.02-27				“
	Northwood	<3-460	17-9,200	<0.02-0.43				“
	stormwater			94% positive				“
	stormwater	38	1,100	0.13	50,000	0.3 PFU		Lager, *et al*. 1977
Cincinnati, OH	business district				79% positive(l)			Geldreich and Kenner 1969
	residential area				80% positive(2)			“
	rural area				87% positive(3)			“
overall:		<3-4,600	<1-1,600,000	<1-110 (mostly positive)	>80% positive			

(1) Strep. bacteria types found:
S. bovis/*S. equinus* (2%)
Atypical *S. faecalis* (1%)
S. faecalis liquifaciens (19%)
S. thompson: 4,500/100mL

(2) Strep. bacteria types found:
S. bovis/*S. equinus* (0.5%)
Atypical *S. faecalis* (1%)
S. faecalis liquifaciens (18%)

(3) Strep. bacteria types found:

S. bovis/*S. equinus* (0.5%)
Atypical *S. faecalis* (0.2%)
S. faecalis liquifaciens (12%)

However, many of the potentially pathogenic bacteria biotypes can be present in urban stormwater. Table 11 lists the pathogenic bacteria biotypes that affect mammals and birds and that can be transmitted by contaminated water. Many of these biotypes, of course, are rare but this table does demonstrate the wide range of possible diseases that can be transmitted by polluted waters, including urban runoff.

Table 11. Bacterial Parasites Affecting Mammals and Birds that can be Transmitted by Contaminated Water (Altman and Dittmer 1973)

Species (Synonym)	**Host** [1]	**Disease and Clinical Manifestation**
**Actinobacillus mallei*	Horse	Glanders (farcy)
	Man	Glanders
Bacillus anthracis	Cattle, sheep, horse	Anthrax - acute septicemia
Bordatella bronchiseptica	Swine	Anthrax - acute pharyngitis
Brucella abortus	Cattle	Brucellosis, contagious abortion (undulant fever, Bang‘s disease)
**B. melitensis*	Goat, sheep, man	Brucellosis (undulant fever)
B. suis	Swine	Brucellosis (undulant fever), abortion
Clostridium perfringens[2]	Sheep	Enterotoxemia, lamb dysentery
Corynebacterium pseudotuberculosis	Cattle, swine	Hemorrhagic enteritis
	Sheep	Caseous lymphadenitis
Erysipelothrix insidiosa (E. rhusiopathiae)	Swine, turkey	Erysipelas
**Francisella tularensis*	Lagomorphs, rodents, man	Tularemia
Haemophilus gallinarum	Chicken	Infectious coryza
Leptospira canicola	Dog, man	Leptospirosis
L. icterohemorrhagiae	Rat, man, dog	Leptospirosis
L. pomona	Cattle, swine, man	Leptospirosis
Mycobacterium avium	Turkey, chicken	Tuberculosis of intestine, spleen, and liver
M. bovis	Cattle	Tuberculosis
M. paratuberculosis	Cattle, sheep, goat	Johne's disease
Pasteurella haemolytica	Sheep	Septicemia in lambs; mastitis
	Cattle	Pneumonla, hemorrhagic septicemia
**Pseudomonas aeruginosa*	Dog	Otitis externa, dermatitis
	Man	Otitis, wound infections (burns), urinary tract infections, sinusitis, meningitis
	Cattle	Mastitis
	Horse	Abortion
**Salmonella choleraesuis*	Swine	Necrotic enteritis
	Man	Septicemia, abscesses, gastroenteritis
**S. enteritidis*	Cattle	Gastroenteritis in calves
	Man	Gastroenteritis
S. gallinarum	Fowl	Fowl typhoid
**S. paratyphi*	Man	Paratyphoid fever
S. pullorum	Chicken	Bacillary white diarrhea
**S. typhimurium*	Domestic animals, man	Gastroenteritis
**S. typhi (S. typhosa)*	Man	Typhoid fever
**Shigella dysenteriae*	Man	Shigellosis, bacillary dysentery
**S. flexneri*	Man	Shigellosis, bacillary dysentery
**S. sonnei*	Man	Shigellosis, bacillary dysentery
**Vibrio cholerae*	Man	Cholera
V. jejuni	Cattle	Dysentery
**Yersinia enterocolitica*	Man	Pseudotuberculosis, colitis

	Rodents	Pseudotuberculosis
Y. pseudotuberculosis	Rodents, turkey, swine, man	Pseudotuberculosis

* affect humans
[1] animals are listed in order of decreasing susceptibility
[2] now known to affect humans also

Sources of Bacteria and Pathogens found in Urban Runoff

Several investigations have studied potential sources of bacteria and selected pathogens that are found in urban runoff. Some of these studies have examined surface sheetflows during rainfall-runoff and during snowmelt-runoff that would not likely be contaminated by human fecal matter. More commonly, many studies have examined runoff sampled at outfalls where the runoff may have been contaminated by inappropriate discharges to the storm drainage. The following section summarizes some of the observations from these studies.

Table 12 lists some early bacteria quality data for street dirt and sewerage sediments, collected in residential areas in Ottawa (Pitt 1983). The sewerage sediments were composite samples so the minimum and maximum values are likely moderated would typically a much wider range if discrete samples were obtained. Even though these samples were obtained in the summer, with high temperatures on the streets, the bacteria levels on the streets were high. Similar findings were reported by Sartor and Boyd (1972) during their early street dirt sampling and analysis project: very hot streets did not preclude very high bacteria levels associated with the street dirt. The Ottawa catchbasin sump samples, collected where the stormwater inlets connect the street gutters to the storm drain sewerage, showed much smaller bacteria levels. It is interesting to note that the bacteria apparently survived better on the hot and dry streets than in the saturated sediments. The storm drain sediments were all much greater than the other sediments, indicating possible sanitary sewage contamination of the storm drains.

Table 12. Bacteria Quality of Street Dirt and Sewerage Sediment, Ottawa, Ontario (Pitt 1983)

	# organisms/ gram solids			
	Geometric mean	Minimum	Maximum	Number of observations
Fecal Coliforms				
Streets dirt	273	8	31,000	16
Catchbasin sump sediment	22	<10	8,900	15
Storm drain sediment	7,500	3,600	9,800	3 composites
Fecal Strep.				
Streets dirt	1,980	24	16,000	12
Catchbasin sump sediment	130	<10	39,000	15
Storm drain sediment	20,600	7,800	41,000	3 composites

Tests in Toronto examined sources of urban stormwater bacteria (Pitt and McLean 1986), as shown on Table 13, along with sheetflow bacteria data from other studies. High bacteria populations were found in sidewalk, road, and some bare ground sheetflow samples (collected from locations where dogs would most likely be “walked”). Some of the Toronto sheetflow contributions were not sufficient to explain the concentrations of some constituents observed in runoff at the outfall. Most of the fecal coliform populations observed in sheetflows were significantly lower than those observed at the outfall, especially during snowmelt periods. It is expected that some sanitary sewage was entering the storm drainage system, even though the test areas were thoroughly surveyed before the research project to ensure minimal contamination from other sources. Runoff from paved parking areas, streets, and landscaped areas generally had the highest observed bacteria densities, while runoff from roofs and freeways had low densities.

Table 13. Source Area Bacteria Sheetflow Quality Summary (means)

Pollutant and Land Use	Roofs	Paved Parking	Paved Storage	Unpaved Parking/ Storage	Paved Driveways	Unpaved Driveways	Dirt Walks	Paved Sidewalks	Streets	Land-scaped	Un-developed	Freeway Paved Lane and Shoulders
Fecal Coliforms (#/100 mL)												
Residential:	85 (3) <2 (4) 1400 (5)	250,000 (5)	100 (5)		600 (5)			11,000 (5)	920 (4) 6,900 (5)	3300 (5)	5400 (3) 49 (4)	1500 (9)
Commercial	9 (4)	2900 (3) 350 (4) 210 (1) 480 (7) 23,000 (8)										
Industrial:	1600 (5)	8660 (8)	9200 (5)	18,000 (5)	66,000 (5)	300,000 (5)		55,000 (5)	100,000 (5)			
Fecal Strep (#/100 mL)												
Residential:	170 (3) 920 (4) 2200 (5)	190,000 (5)	<100 (5)		1900 (5)		1800 (5)		>2400 (4) 7300 (5)	43,000 (5)	16,500 (3) 920 (4)	2200 (9)
Commercial:	17 (3)	11,900 (3) >2400 (4) 770 (1) 1120 (7) 62,000 (8)										
Industrial:	690 (5)	7300 (5)	2070 (5)	8100 (5)	36,000 (5)	21,000 (5)		3600 (5)	45,000 (5)			
Pseudo, Aerug (#/100 mL)												
Residential:	30,000 (5) 50 (5)	1900 (5)	100 (5)		600 (5)		600 (5)		570 (5)	2100 (5)		
Industrial:		5800 (5)	5850 (5)	14,000 (5)	14,300 (5)	100 (5)		3600 (5)	6200 (5)			

References:
(1) Bannerman, *et al*. 1983 (Milwaukee, WI) (NURP)
(3) Pitt 1983 (Ottawa)

(4) Pitt and Bozeman 1982 (San Jose)

(5) Pitt and McLean 1986 (Toronto)

(7) STORET Site #590866-2954309 (Shop-Save-Durham, NH) (NURP)
(8) STORET Site #596296-2954843 (Huntington-Long Island, NY) (NURP)
(9) Kobriger, *et al.* 1981 and Gupta, *et al*. 1977

Table 14 contains a summary of the measured fecal coliform and fecal strep. bacteria measurements at an industrial and a residential/commercial area outfall during the Humber River study in Toronto (Pitt and McLean 1986). From 35 to 50 events were monitored at each outfall during warm weather and cold weather, for both dry weather baseflows and snowmelt and runoff events. The cold weather values were substantially less than the warm weather values, indicating the reduced survival of bacteria in cold conditions after discharge.

Table 14. Toronto Area Storm Drain Outfall Bacteria Measurements (medians of 35 to 50 events) (Pitt and McLean 1986)

	Warm Weather Baseflows		Warm Weather Stormwater		Cold Weather Baseflows		Cold Weather Melting Periods	
	Residential	Industrial	Residential	Industrial	Residential	Industrial	Residential	Industrial
Fecal coliforms (#/100 mL)	33,000	7,000	40,000	49,000	9,800	400	2,320	300
Fecal Strep. (#/100 mL)	2,300	8,800	20,000	39,000	1,400	2,400	1,900	2,500

Water Body Sediment Bacteria

Matson, *et al.* (1978) studied the effects of river and lake sediments as a source of bacteria to the water column in Connecticut. They found that resuspended sediments in shallow waters can elevate the water column bacteria concentrations significantly. They stated that the physical resuspension of shallow water sediments is increased by elevated river discharges, wind induced turbulence, dredging, motorboats, swimming, walking, and wading and normal activities of aquatic microorganisms. The magnitude of sediment resuspension varies with the intensity of the mechanisms involved, and the water depth to the sediment. They stated that during stable river flows, the water bacteria populations are relatively constant, but during periods of high flows, sediment organisms can be scoured from the benthic surfaces and mixed into the water column. After peak discharges, water borne microorganisms resettle downstream, which increases those sediment bacteria populations. Geldreich, *et al.* (1980) also studied bacteria interactions between sediment and water. They found that the sediment-water interface of a water body is an attractive habitat for a variety of different bacteria involved in different biochemical processes. Shallow bottom sediments attract a variable bacteria population because of the physical and chemical requirements that can be satisfied there, in contrast to the more limited conditions available in the water or buried in the sediments.

Davis (1979) stated that bacteria contamination of waterways during and following storm events is a function of the stream sediment bacteria concentrations, the concentrations of bacteria in soils adjacent to the stream (and source areas in an urban watershed), and the stream velocities. Davis further stated that stream sediments can contain greater densities of coliform bacteria on a number per unit weight or volume basis than the water body itself; the concentrations of bacteria in the top two inches of mud can be 100 to 1,000 times greater than the concentrations of the bacteria in the water. He reported fecal coliform sediment concentrations up to 100 organisms per gram of sediment and that the suspended sediments can be a major source of bacteria contamination. Geldreich, *et al.* (1980) stated that sediment bacteria concentrations can be as high as 3,000 to 15,000 organisms per square meter of particulate surface. Pitt and Bozeman (1979), in a study of an urban lake (having high bird populations) in Oakland, California, found fecal coliform sediment concentrations that ranged from one to 35,000 organisms per gram and averaged about 1,000. McSwain (1977) found that in a rural study in North Carolina, total and fecal coliform concentration increases were more related to bottom sediment disturbances than to stream bank flushing. As indicated earlier, these high sediment-associated bacteria levels are not unusual for many urban sediments and soils.

Soil Bacteria Sources

Van Donsel, *et al.* (1967) stated that soil bacteria pollution may occur from direct defecation by livestock, pets, and wild animals, by malfunctioning or overflowing septic tank systems or by flooding of sewerage systems. Much of the total coliform indicator bacteria organisms in urban areas, however, are not from these sources. Geldreich, *et al.* (1968) found that in a Cincinnati urban runoff study, direct fecal contamination accounted for less than 10 percent of the total coliform bacteria present in the stormwater. The remaining coliforms (which were non-fecal in origin) were assumed to be contributed from soil erosion. Therefore, soil can contain large numbers of both non-fecal and fecal coliform bacteria. Because rain water contains very small bacteria concentrations, urban runoff becomes contaminated with bacteria when the rain water contacts contaminated surfaces. In wilderness areas, runoff has very little fecal coliform bacteria, while runoff from agricultural areas or urban areas can have varying amounts of fecal coliform bacteria. Seidler (1979) found that the movement of fecal coliform bacteria in saturated soils were extremely rapid. Soil can add appreciable fecal and non-fecal coliform bacteria to rain runoff. Casserly and Davis (1979) found that coliform types in urban soils were the same as they found in urban runoff, indicating a strong interaction between polluted soils and contaminated urban runoff. Davis (1979) found that irrigated soils, with high humic content, can yield greater amounts of bacteria. Evans and Owens (1972) found that the concentrations of *E. Coli* and Enterococci in stormwater runoff were affected by the soil bacteria concentrations.

Evans and Owens (1973) reported that bacteria were more likely to erode than the particulate matter in the soil. Davis (1979) found that the leaching action of rain on soil bacteria was quite erratic. The most important factors affecting bacteria concentrations in runoff were found to be the concentrations of the bacteria in soils. They reported total coliform concentrations in soils ranging from 200 to more than 500,000 total coliform organisms per gram. Fecal coliform soil concentrations ranged from less than 20 to about 300 organisms per gram and fecal strep. soil concentrations ranged from less than 20 to about 1,000 organisms per gram.

Wildlife Sources of Bacteria

Effects of Birds on Water Bacteria Concentrations

Several studies have been conducted which examined the effects of large migratory or permanent waterfowl populations on the bacteria quality of water bodies. A study at the Montezuma Bird Refuge in New York (Have 1973) found inconsistent relationships between the bird populations and the total coliform, fecal coliform, and fecal strep. counts. Peak populations of 70,000 geese and 100,000 ducks frequent this 1,000 acre refuge. In fact, they found that the concentrations of the non-pathogenic bacteria in the two major streams flowing into the refuge were greater than in the water flowing out of the refuge. The specific conductance of the inflowing water was also greater than the outflowing water. The effluent did have higher concentrations of phosphorous and nitrogen. They concluded that the settling effect of the quite waters in the refuge may help explain the improvement in the quality of water leaving the refuge.

Brierley, *et al.* (1975) studied the Rio Grande Refuge in New Mexico. This refuge supports bird populations of more than 10,000 Sandhill cranes, 2,000 Canada geese, more than 8,000 snow geese, and more than 25,000 ducks from October to early March along ten miles of river channel. The water flowing into this bird refuge area along the Rio Grande River has high concentrations of suspended sediments and bacteria. The bacteria concentrations seem to correlate directly with the high sediment concentrations. The presence of the large number of birds apparently does not affect the concentrations of the bacteria that were investigated (total heterotrophic bacteria, fecal and total coliforms, and Enterococci). Most of the birds use a single large pond at the end of their winter habitat. The draining of this pond at the end of their season did not seem to significantly change the bacteria population of the receiving channel water. The bird habitat pond, in fact, had decreased concentrations or bacteria during and following the period of maximum use. They concluded that the bacteria originated in upstream areas before it reached the refuge.

In a study at Lake Wingra in Wisconsin (Geldreich 1980), intermittent high fecal coliform counts during the late summer and early fall were found to be due to a combination of wastes from mallard ducks and the local weather. They reported that fecal coliforms in the sand due to duck defecation multiplied during the first week after deposition and then dieoff occurred. Bacteria in these near-lake sands were transported into the water primarily by stormwater runoff erosion and by the foot traffic of bathers when going into the water.

Oplinger (1977) studied the effects of waterfowl populations on the water quality of a small creek park in Pennsylvania. They felt that increasing waterfowl populations and the declining water quality were related and threatened the health and welfare of both the waterfowl and the human watershed users.

Figley and Vandraff (1974), in a study of suburban parks in New York state, noted that mallard ducks are especially attracted to suburban lagoon developments. They felt that urban concentrations of semi-wild ducks may be detrimental, by serving as the focal points for outbreaks of infectious avian diseases and as a reservoir of diseases that could be transmitted to migrating wildfowl.

A study by Fennell, *et al.* (1974) examined the effects of about 500 roosting gulls on a one million cubic meter storage reservoir. Salmonella were usually found in the reservoir waters but never in the incoming water. They also found close correlations between the number of gulls and the degree of bacteria contamination. The sources of Salmonella appeared to be household and other refuse from dumps where the gulls were foraging. When the gulls left, after bird scaring fireworks were used, the Salmonella and other bacteria concentrations almost immediately decreased. The bacteria concentrations remained at low levels for a period of five weeks until the fireworks were stopped; the birds were allowed to return, and the bacteria concentrations in the reservoir immediately increased.

Gore and Storrie/Proctor and Redfern, (1981a) summarized the results of studies made to determine the effects of birds roosting on bridges over the Rideau River on river bacteria concentrations. They found that the birds on the bridges could have a statistically significant impact on fecal coliform concentrations, especially during the low summer flows. Measured concentration increases of fecal coliform bacteria downstream from the Queensway Bridge was found to be about 300 fecal coliform organisma/100 mL.

It is evident that birds can have varying effects on the bacteria concentrations in waterbodies. Large refuges do not seem to be severely affected by the wildlife populations. In fact, the ponding of waters in refuges appears to improve the water quality through sedimentation. Waterfowl frequenting smaller bodies of water, especially creeks and small lagoons, appear to have the potential for substantially increasing the water bacteria concentrations.

Other Wildlife Bacteria Contributions

Table 15 lists samples (mostly from mammals and birds with some soil, sediment, and river samples) where specific bacteria types were not generally found. The presence or absence of certain bacteria types in environmental samples can be a very important factor in identifying the bacteria sources (feces from which animals). As an example, *Streptococcus bovis* and *S. equinus* have not been found in human feces by several projects. (These types, however, are the predominant fecal strep. type found in livestock feces.) Their absence in a sample indicates the probable absence of livestock feces contamination; however, their absence may only indicate dieoff and not absence of fecal contamination.

Table 15. Test Samples Where Specific Bacteria Types Were Not Generally Found (Pitt 1983)

Bacteria types	Organisms that tested negative	Reference
Aerobactor	Pine squirrel feces	Drake, *et al*. 1961
Escherichia	Pine squirrel feces	Drake, *et al*. 1961
	Pocket gopher feces	
Pseudomonas aeruginosa	not normally found in lower animals’ feces	Cabelli, *et al*. 1976
	not usually found in non-human feces	Olivieri, Kruse, and Kawata 1977b
Salmonella	rare in Texas stream sediments, soils and lake sediments	Davis 1979
	negligible contamination of Oregon surface waters by livestock grazing	Seidler 1979
	not found in Ottawa stormwater outfalls	Environment Canada 1980
S. agona	rare in Rideau River waters	Environment Canada 1978
S. haardt	rare in Rideau River waters	Environment Canada 1978
S. saint paul	rare in Rideau River waters	Environment Canada 1978
Fecal Streptococci	very little in remote streams and soils	Geldreich and Kenner 1969
	not permanent in fish intestines (only present when food or water is contaminated)	Geldreich 1965
Streptococcus faecalis	not found in most domestic animals	Bartley and Slanetz1960
S. faecalis var. *liquefaciens*	not found in duck feces	Geldreich and Kenner1969
Atypical *S. faecalis*	not found in human feces	Geldreich and Kenner 1969
	not found in cow feces	Geldreich and Kenner 1969
	not found in pig feces	Geldreich and Kenner 1969
	not found in sheep feces	Geldreich and Kenner 1969
	not found in duck feces	Geldreich and Kenner 1969
	not found in chicken feces	Geldreich and Kenner 1969
	not found in turkey feces	Geldreich and Kenner 1969
	not found in insects	Geldreich and Kenner 1969
	not found in agric. soils	Geldreich and Kenner 1969
S. bovis/ S. equinus	not found in human feces	Geldreich 1965 Geldreich and Kenner 1969 Seidler 1979
	none found in 3,100 Cincinnati stormwater bacteria strains	Geldreich, *et al*. 1968
S. zymogenes	none found in reptiles	Mundt 1963
Enterococci	very few on vegetation and litter	Seidler 1979
	generally not found in strongly herbivorous or subsurface ground dwellers	Mundt 1963

Table 16 lists the wildlife feces samples in which different bacteria types were found, along with their relative concentrations. Geldreich and Kenner (1969) stated that the absence of fecal strep. bacteria indicates the absence of warm blooded animal fecal pollution. The presence of *Streptococcus faecalis* indicates human fecal contamination. *S. faecalis* far outnumbers *S. inulinaceus* in sewage and in sewage polluted waters, even though *S. inulinaceus* is in great abundance in fresh feces (Bartley and Slanetz 1960). *S. faecalis* var. *liquefaciens* is ubiquitous as it is present in almost all samples tested (Geldreich and Kenner 1969; Bartley and Slanetz 1960). *S. mitis* and *S. salivarious* are considered sensitive indicators of human pollution when they are found (Seidler 1979). *S. bovis* and *S. equinus* are nearly ideal non-human mammal fecal indicators (Seidler 1979). They have rapid dieoff rates (much faster than fecal coliform dieoffs) and are the most sensitive bacteria in the fecal strep. category. Their presence indicates recent livestock pollution (Feacham 1975; Geldreich 1976; Bartley and Slanetz 1960; Geldreich and Kenner 1969).

Table 16. Test Samples Where Specific Bacteria Types Were Found (Pitt 1983)

Bacteria types	Samples that tested positively (relative abundance)	Reference*
Aerobactor	Chipmunk feces (33% positive)	6
	Ground squirrel feces (40% positive)	6
	Pocket gopher feces (100% positive)	6
	Cottontail rabbit feces (33% positive)	6
	Jackrabbit feces (75 to 100% positive)	6
	Deer mice feces (50% positive)	6
	Meadow mice feces (40% positive)	6
Escherichia	Chipmunk feces (67% positive)	6
	Ground squirrel feces (0 to 50% positive)	6
	Cottontail rabbit feces (67% positive)	6
	Jackrabbit feces (25 to 100% positive)	6
	Deer mice feces (64% positive)	6
	Meadow mice feces (80% positive)	6
Edwardsiella tarda	Gull feces (0.4% positive)	2
Klebsiella pneumoniae	Human feces (30 to 40% positive)	14
Vibrio cholerae	Construction sites (regular contributor)	4
Shigella dysenteriae	Construction sites (regular contributor)	4
Staphylococci	Bath and laundry waters	18
	Texas stream sediments (20 to 240/gram)	4
	Texas soils (160 to 1,000/gram)	4
	Texas lake sediments (15 to 2,600/gram)	4
Pseudomonas aeruginosa	Human feces (ubiquitous)	18
	Sewage	8
	Rideau River (fairly common)	7
	Deer feces	6
	Song sparrow feces	6
	Textile mill (non-fecal) effluent	3
	Texas stream sediments (<20 to 20/gram)	4
	Texas soils (<20 to 1,000/gram)	4
	Texas lake sediments (15 to 2,600/gram)	4
Salmonella	Household and kennel dog feces (15 to 20%)	16
	Agricultural animal feces (frequent)	12
	Sheep feces(from contaminated feed) (3 to 15%)	20
	Wild bird (grackles, cowbirds, starlings and gulls) feces	21
S. typhimurium	Domestic pets and wild animal (raccoon, skunk, and muskrat) feces	19
	Rideau River water	7
	Gull feces (5 to 8%)	2, 10
	Grackles (birds) feces (2%)	21
	Cowbird feces 4%)	21

Bacteria types	Samples that tested positively (relative abundance)	Reference*
	Starling feces (5%)	21
S. thompson	Domestic pets and wild animal (raccoon, skunk, and muskrat) feces	19
	Gull feces (13%)	10
S. typhi	Construction sites (regular contributor)	4
S. paratyphi	Construction sites (regular contributor)	4
S. blockley	Herring gull feces (2%)	21
	Starling feces (5%)	21
S. saint paul	Starling feces (9%)	21
S. braenderup	Herring gull feces (2%)	21
S. muncher	Herring gull feces (2%)	21
S. derby	Herring gull feces (2%)	21
S. enteritidas	Herring gull feces (2%)	2, 21
S. heidelberg	Herring gull feces (2%)	21
S. infantis	Herring gull feces (2%)	21
S. montevideo	Herring gull feces (2%)	21
S. panama	Herring gull feces (2%)	21
S. reading	Gull feces (10%)	2
Fecal Streprococci	Stormwater (greater abundance than fecal coliforms)	13
	Farm animals, dogs, cats and various wild animal feces (greater abundance than fecal coliforms)	13
	Dog feces (great variety of fecal strep. types)	1
	Canadian geese and whistling swan feces (more fecal coliform)	15
	Vegetation (due to insects)	5
Streptococcus faecalis	Human feces (may be predominant type)	1, 11, 20
	Mammal feces (11%)	1, 17, 20
	Dog feces (may be predominant type)	11
	Cat feces (may be predominant type)	11
	Hog feces	1
	Chicken feces	1
	Rodent feces (may be predominant type)	11
	Reptile feces (20%)	17
	Bird feces (9%)	17
	Insect feces	1

Bacteria types	Samples that tested positively (relative abundance)	Reference*
	Vegetation (due to insects?)	20
	Soil	20
S. faecalis var. *liquifaciens*	Present in most samples tested (ubiquitous)	1, 13
	Human feces (26%)	1, 13
	Mammal feces (22%)	17
	Dog feces (10%)	1
	Cat feces (6%)	1
	Cow feces (4%)	1
	Sheep feces (19%)	1
	Pig feces (2%)	1
	Bird feces (14%)	17
	Chicken feces (22%)	1, 13
	Turkey feces (22%)	1
	Reptile feces (61%)	17
	Rodent feces (35%)	1
	Insects (48%)	1
	Freshwater fish (17%)	1
	Vegetation (13%)	1
	Agricultural soils (35%)	1
Atypical *S. faecalis*	Human feces	17, 20
	Mammal feces (36%)	17
	Dog feces (14%)	13
	Cat feces (2%)	13
	Pig feces	1
S. facium	Bird feces (9%)	17
	Fowl feces	20
	Chicken feces	1
	Reptile feces (13%)	17
	Rodent feces (0.4%)	13
	Insects	1
	Freshwater fish (3%)	13
	Vegetation (35%)	13
	Soil	20
S. facium var. *casseliflauus*	Fowl feces	20
	Vegetation (significant)	20
	Soil	20
S. bovis and *S. equinus*	Dog feces (32% significant amount)	11, 13
	Cat feces (2%)	11, 13, 20
	Livestock (predominant fecal strep.)	11
	Cow feces (66% significant amounts)	13
	Sheep feces (42% significant amounts)	13
	Pig feces (19% significant amounts)	13
	Duck feces (49% significant amounts)	13
	Chicken feces (1% trace amounts)	13, 20
	Turkey feces (2% trace amounts)	13, 20
	Rodent feces (17% significant amounts)	11, 13
	Freshwater fish (7%)	13

Bacteria types	Samples that tested positively (relative abundance)	Reference*
	Vegetation (9%)	13
	Agricultural soils (2%)	13
S. bovis	Most non-human mammal feces	20
	Cow feces	1, 20
	Sheep feces	20
	Deer feces	20
S. equinus	Horse feces (predominant)	1, 20
	Pig feces	1
S. mitus and *S. salivarius*	Human feces (can be predominant fecal strep.)	20
	Suburban runoff (septic tank failures)	20
	Stream sediments (septic tank failures in area)	20
S. durans	Human feces	20
	Mammal feces	20
	Vegetation	20
	Soil	20
S. zymogenes	Mammal feces	17
	Bird feces	1
S. inulinaceus	Human feces (great abundance in fresh samples)	1
	Chicken feces (great abundance in fresh samples)	1
Enterococci	Ubiquitous (survive better than fecal coliforms)	9, 20
	Human feces (74% predominant)	13
	Large animals with varied diet (common)	17
	Dog feces (44%)	13
	Cat feces (90% predominant)	13
	Cow feces (30%)	13
	Sheep feces (39%)	13
	Pig feces (79% predominant)	13
	Fowl feces (predominant)	20
	Duck feces (51%)	13
	Chicken feces (77% predominant)	13
	Turkey feces (77% predominant)	13
	Rodent feces (47%)	13
	Insects (52%)	13
	Freshwater fish (74%)	13
	Vegetation (43%)	13
	Agricultural soils (63%)	13
Raffinose fermenters	Cow feces	1
	Chicken feces	1

* References:

1. Bartley and Slanetz 1960
2. Berg and Anderson 1972
3. Cabelli, Kennedy, and Levin 1976

4. Davis 1979
5. Van Donsel, Geldreich, and Clarke 1967
6. Drake, Woods, and Hammerstrom 1961
7. Environment Canada 1978
8. Environment Canada 1980
9. Feachem 1975
10. Fennell, James, and Morris 1974
11. Geldreich 1965
12. Geldreich, *et al.* 1968
13. Geldreich and Kenner 1969
14. Geldreich 1976
15. Geldreich 1980
16. Marron and Senn 1974
17. Mundt 1963
18. Olivieri, Kruse, and Kawata 1977b
19. Qureshi and Dutka 1979
20. Seidler 1979
21. Snoeyenbos, Morin, and Wetherbee 1967

Table 17 summarizes the bacteria concentrations observed in feces samples from different mammals and birds. Drake, *et al.* (1961) found a wide variation in the coliform content of some wild and domestic animal feces. Coliform bacteria were present in small numbers or were absent for some feces, such as from rabbits, shrews, deer, elk, some squirrels, and many birds. They also found that coliform bacteria were not found in some carnivores (shrews) but were present in large number in the carnivores (coyotes and bears). They also found no significant differences in the fecal coliform content of different animals of the same species that were collected in different areas. However, feces from different species of animals collected in the same area could have large differences in their fecal coliform concentrations. They also noted that some mammals (coyote, bear, some gophers, and some squirrels) had coliform concentrations in the feces that were similar to human coliform concentrations. Animals with soft or moist feces (man and many domestic animals such as cows, dogs, and pigs) had very high numbers of coliform bacteria (many thousands to millions of coliform bacteria per gram). The feces of other animals, especially those with hard or dry feces, may contain few or no coliform bacteria.

Table 17. Bacteria Content of Feces Samples (MPN of organisms/gram feces) (reference) (Pitt 1983)

Organism	Total Coliforms	Fecal Coliforms	Fecal Strep.
Mammals			
Humans	86,000 to 230,000,000 (1)	13,000,000 (med) (5)	1,900,000 (med) (5)
Farm animals			
pig		3,300,000 (med) (5)	84,000,000 (med) (5)
sheep		16,000,000 (med) (5)	38,000,000 (med) (5)
cow		230,000 (med) (5)	1,300,000 (med) (5)
horse		13,000 (med) (5)	6,300,000 (med) (5)
Rural wildlife			
coyote	1,200,000 (1)		
bear	200,000 (1)		
mule deer	2 to 27; 11 (med) (1)		
elk	4 to 10,000; 4 (med) (1)	5,100 (med) (5)	760,000 (med) (5)
Domestic pets			
cat	7,900,000 (med) (3)	7,900,000 (med) (3)	27,000,000 (med) (3)
dog	23,000,000 (med) (3)	23,000,000 (med) (5)	980,000,000 (med) (3)
Possible urban wildlife			
cottontail rabbit	1400 to 2200; 1800 (med) (1)		
white-tailed rabbit	132 (1)		
black-tailed jackrabbit	92 to 10,000; 920 (med) (1)		
rabbit	90 (med) (3)	20 (med) (3)	47,000 (med) (3)
rodents		160,000 (med) (4)	4,600,000 (med) (4)
rats	330,000 (med) (3)	180,000 (med) (4) 330,000 (med) (3)	79,000,000 (med) (5) 7,700,000 (med) (3)
shrews	none (1)		
deer mice	4600 to 330,000; >250,000 (med) (1)		
meadow mice	180,000 to 290,000; 220,000 (med) (1)		
field mice		330,000 (med) (5)	7,700,000 (med) (5)
pocket gopher	2,000,000 (1)		
chipmunks	7000 to 4,600,000; 30,000 (med) (1) 150,000 (med) (3)	150,000 (med) (3)	6,000,000 (med) (3)
golden-mantled ground squirrel	19,800 (1)		
Columbian ground squirrel	100,000 to >16,000,000; 8,000,000 (med) (1)		
pine squirrel	27 (1)		
Birds			

Organism	Total Coliforms	Fecal Coliforms	Fecal Strep.
Farm birds			
chicken		1,300,000 (med) (5)	3,400,000 (med) (5)
turkey		290,000 (med) (5)	2,800,000 (med) (5)
Rural birds			
quail	2 to 349; 7 (med) (1)		
pheasant	19 to 23,000; 1800 (med) (1)		
Possible urban land-birds			
robin	221 (1)	25,000 (med) (5)	12,000,000 (med) (5)
song sparrow	2 to 349; 6 (med) (1)		
English sparrow		25,000 (med) (5)	1,000,000 (med) (5)
Oregon junco starling		10,000 (med) (5)	12,000,000 (med) (5)
red-winged blackbird		9000 (med) (5)	11,000,000 (med) (5)
pigeon		10,000 (med) (5) to 100,000,000 (2)	12,000,000 (med) (5)
Possible urban waterbirds			
Lake Merritt waterbirds (composite)	200,000 (7)	200,000 (7)	920,000 (7)
Ottawa (fresh samples) waterbirds (composite)	91,000,000 to 4,400,000 130,000,000 (med) (2)	78,000,000 to 2,900,000,000 120,000,000 (med) (2)	470,000 to 280,000,000; 180,000,000 (med) (2)
Ottawa (aged samples) waterbirds (composite)	600,000,000 to 5,500,000,000; 3,100,000,000 (med) (2)	550,000,000 to 4,500,000,000; 2,500,000,000 (med) (2)	51,000,000 to 1,300,000,000; 650,000,000 (med) (2)
swan	480,000 (2)	320,000 (2)	45,000 (2)
herring gull		71,000,000 (6)	810,000 (6)
lesser black-backed gull		370,000,000 (2)	1,100,000 (2)
common gull		53,000,000 (2)	90,000 (2)
black-headed gull		27,000,000 (2)	200,000 (2)
duck		33,000,000 (med) (5)	54,000,000 (med) (5)
goose			840,000 (med) (5)

References:
(1) Drake, Woods, and Hammerstorm 1961
(2) Environment Canada 1980
(3) Geldreich, *et al*. l968
(4) Geldreich and Kenner 1969
(5) Geldreich 1976
(6) Gore and Storrie/Proctoi and Redfern 1981a
(7) Pitt and Bozeman 1979

Geldreich (1976) summarized a study that showed the variations in fecal strep. bacteria concentrations in human feces from different locations. Feces collected from humans living in Cincinnati had concentrations more than five times greater than samples collected from healthy people in Nagpur, India (13 million and 2 million fecal strep. organisms per gram, respectively). He also reported that fecal strep. densities in farm animal, cat, dog, mice, and chipmunk feces samples were in the order of millions of organisms per gram. Rabbit feces fecal strep. concentrations, however, may be several orders of magnitude lower than those found in other animals. The Ottawa waterbird feces samples were reported to have the largest total coliform, fecal coliform, and fecal strep. concentrations when compared to all other samples reported (except for the fecal strep. dog feces concentrations). Gull feces generally have the highest fecal coliform concentrations in their feces, followed by Ottawa pigeons, ducks, dogs, sheep, and humans. Other urban bird feces (pigeons, sparrows, robins, starlings, and blackbirds) were all reported to have much lower fecal coliform concentrations that were unusually high.

Feces Discharges from Wildlife

Table 18 summarizes reported discharges of feces from different mammals and birds. These discharges are expressed in grams per animal per day and vary quite widely, depending on the study. Animals can deposit substantial quantities of feces in an urban area, depending upon the animal’s population. Geldreich (1976) stated that major contributions of bacteria in urban communities are from fecal discharges from cats, dogs, and rodents. These feces are deposited on soil, asphalt, and cement. He stated that the one-half million dogs in New York City deposit about 150,000 pounds of feces on the streets, sidewalks, and park areas per day. Significant populations of rodents may also contribute large amounts of fecal material in urban areas. Fortunately, very little of this fecal bacteria enters receiving waters. Faust (1976), in an agricultural watershed in the Rhode River near Chesapeake Bay, found that only about one percent of the fecal coliform bacteria deposited by cattle in the watershed was washed into the receiving waters. Sometimes the yields (application rates) were higher, with high values around 5 percent and on one occasion reaching 25 percent. They concluded that fecal coliform discharges can be substantial from a watershed that has the equivalent of about one cow per two hectares. Evans and Owens (1973), from a study in Scotland, stated that most of the bacteria in the runoff water came from the soil. They found that the soil bacteria washoff yield was only about one-tenth of one percent of the estimated total soil bacteria population. They felt that the maximum annual discharge of bacteria from the contaminated soil would only be about 0.15 percent of the total soil bacteria population.

Table 18. Estimated Feces Discharges (Pitt 1983)

Animal	Discharge[1] (grams/animal/day)	Reference
Mammals		
Humans	150	Geldreich 1976
Farm animals		
pig	680	Howe 1969
sheep	1,100	Howe 1969
cow	7,000	Howe 1969
horse	7,000	Howe 1969
Domestic pets		
cat	70	Howe 1969
dog	140	Howe 1969
	23 to 100	Marron and Senn 1974
Possible urban wildlife		
rabbit	550	Howe 1969
rat	35	Howe 1969
mouse	10	Howe 1969
Birds		
Farm birds		
chicken	55	Howe 1969
	180	Geldreich 1976
turkey	160	Howe 1969
	450	Geldreich 1976
Possible urban birds		
pigeon	25 to 50	Gore & Storrie/Proctor & Redfern 1981a
gulls	10 to 25	Gould and Fletcher 1978
duck	70	Howe 1969
	340	Geldreich 1976
goose	160	Howe 1969

[1] estimated application factors (fraction reaching urban receiving waters): 0.01 for land animals and 0.5 for waterfowl

The Contamination of Groundwater by Stormwater-Associated Microorganisms

Pitt, *et al*. (1996) conducted an extensive review of the potential contamination of groundwater by stormwater infiltration. Viruses have been detected in groundwater where stormwater recharge basins were located short distances above the aquifer. Enteric viruses are more resistant to environmental factors than enteric bacteria and they exhibit longer survival times in natural waters. They can occur in potable and marine waters in the absence of fecal coliforms. Enteroviruses are also more resistant to commonly used disinfectants than are indicator bacteria, and can occur in groundwater in the absence of indicator bacteria.

The factors that affect the survival of enteric bacteria and viruses in the soil include pH, antagonism from soil microflora, moisture content, temperature, sunlight, and organic matter. The two most important attributes of viruses that permit their long-term survival in the environment are their structure and very small size. These characteristics permit virus occlusion and protection within colloid-size particles. Viral adsorption is promoted by increasing cation concentration, decreasing pH and decreasing soluble organics. Since the movement of viruses through soil to groundwater occurs in the liquid phase and involves water movement and associated suspended virus particles, the distribution of viruses between the adsorbed and liquid phases determines the viral mass available for movement. Once the virus reaches the groundwater, it can travel laterally through the aquifer until it is either adsorbed or inactivated.

The major bacterial removal mechanisms in soil are straining at the soil surface and at intergrain contacts, sedimentation, sorption by soil particles, and inactivation. Because of their larger size than for viruses, most bacteria are therefore retained near the soil surface due to this straining effect. In general, enteric bacteria survive in soil between two and three months, although survival times up to five years have been documented.

Enteroviruses likely have a high groundwater contamination potential for all stormwater percolation practices and subsurface infiltration/injection practices, depending on their presence in the stormwater (likely if contaminated with sanitary sewage). Other pathogens, including *Shigella*, *Pseudomonas aeruginosa*, and various protozoa, would also have high groundwater contamination potentials if subsurface infiltration/injection practices are used without disinfection. If disinfection (especially by chlorine or ozone) is used, then disinfection byproducts (such as trihalomethanes or ozonated bromides) would have high groundwater contamination potentials. Pathogens are most likely associated with sanitary sewage contamination of storm drainage systems, but bacterial pathogens may be found in surface runoff in residential areas.

Bacteria Survival in Stormwater

The survival of urban runoff bacteria in receiving waters is an important issue. Very little direct consumption or contact of urban runoff usually occurs. However, when the runoff is discharged into a larger receiving water, consumption or contact may occur shortly after the rain event has ended. The Rideau River Stormwater Management Study (Ottawa, Ontario) examined the dieoff of fecal coliform bacteria in the Rideau River (Droste and Gupgupoglu 1982; Environment Canada 1980; Gore and Storrie/Proctor and Redfern 1981b and 1981c). They found that the 90 percent dieoff for Rideau River fecal coliforms was about two days. Because of the long travel time on the Rideau River and short interevent times of rains in the area, the effects of bacteria discharges from stormwater from one storm can affect the river concentrations during the next storm. The persistence of fecal coliforms and the slow river velocities cause downstream beach bacteria concentrations to seldom, if ever, regain true low background bacteria concentration levels. Environment Canada (1980) reported significant increase in coliform concentrations in recently excreted moist feces.

Seidler (1979) stated that the sources of Salmonella bacteria can determine their survival. This is probably true for most types of bacteria because the different bacteria sources usually determine the specific bacteria biotypes found in the feces. Different bacteria types can have quite different dieoff rates.

Factors affecting urban runoff bacteria survival in stormwater have been found to be quite variable and site specific. Geldreich, *et al.* (1968) found that no significant differences in survival of urban runoff bacteria could be related to the chemical constituents present. Water temperature, however, did have a strong influence on urban runoff bacteria survival. Geldreich, *et al.* (1980) found in a Kentucky study that when copper sulfate was applied as an algicide in a reservoir, sharp declines in fecal coliform densities occurred. The standard plate count densities, however, sharply increased. They found that the survival of urban runoff bacteria was longer near the bottom of the reservoir than in shallower waters. They also found that reduced dissolved oxygen concentrations near the sediments was not detrimental to bacteria survival. Faust and Goff (1978) found that high clay concentrations in the Rhode River in the Chesapeake Bay area extended the survival of fecal coliform bacteria.

Many studies reported the effects of temperature on urban runoff bacteria dieoff. Geldreich, *et al.* (1968), in a series of lab tests, found that stormwater bacteria persisted at higher concentrations under winter water temperature conditions (10^oC) than they did for summer water temperature conditions (20^oC). There were some differences in survival for the various specific types of stormwater bacteria, but this trend seemed typical. Van Donzel, *et al.* (1967) found that fecal strep. did not survive as long as fecal coliform bacteria during the summer months, while in the autumn there was little difference in their survival times. In the winter and spring, the fecal strep. survived much longer than the fecal coliforms. Seidler (1979) found that Salmonella survived for longer periods of time in colder water temperatures. McSwain (1977) reported that coliform bacteria were able to multiply in bottom sediments at a rate regulated by stream temperature. They reported another study that found significant enteric bacteria concentration increases at temperatures above 16^oC, but that little or no growth occurred below 10^oC. The conditions affecting bacteria survival in water appear to be site and bacteria specific. Many of the differences are probably associated with the specific bacteria biotype present and with the water temperature. Chemical constituent concentrations do not appear to be a factor, except when they are present at very low concentrations.

Table 19 summarizes reported 90 day dieoff rates for different stormwater bacteria types. Fecal coliform dieoff values varied from less than one day to about 13 days, but can be considered quite fast. Fecal strep. dieoff values, however, were longer than the fecal coliform dieoff rates. Some of the Streptococcus bacteria types had long survival rates, while others had short survival rates. The forms likely to be associated with agricultural activities (*S. bovis* and *S. equinus*) all are shown to have much shorter survival times than more common urban Streptococcus types (*S. faecalis*).

Table 19. Survival of Stormwater Bacteria (Pitt 1983)

Bacteria type	Location and conditions	Days survival before 90% dieoff	Reference
Fecal Coliforms	Rideau River – summer	2	Droste and Gupgupogula 1982
	Cincinnati – stormwater at 10°C	10	Geldreich, *et al*. 1968
	Cincinnati – stormwater at 20°C	2	Geldreich, *et al*. 1968
	Oakland, CA – bird feces into urban lake	Rapid	Pitt and Bozeman 1979
	Stormwater – summer	3	Van Donsel, *et al*. 1967
	Stormwater – autumn	13	Van Donsel, *et al*. 1967
Fecal Strep.	Oakland, CA – bird feces into urban lake	>30	Pitt and Bozeman 1979
	Stormwater – summer	3	Van Donsel, *et al*. 1967
	Stormwater – autumn	20	Van Donsel, *et al*. 1967
Streptococcus faecalis	Cincinnati – stormwater	>14	Geldreich, *et al*. 1968
S. faecalis var. *liquifaciens*	Cincinnati – stormwater at 10°C	>14	Geldreich, *et al*. 1968
	Cincinnati – stormwater at 20°C	6	Geldreich, *et al*. 1968
S. bovis	Cincinnati – stormwater at 10°C	<1	Geldreich, *et al*. 1968
	Cincinnati – stormwater at 20°C	1	Geldreich, *et al*. 1968
S. equinus	Cincinnati – stormwater	<1	Geldreich and Kenner 1969
Salmonella	Rural Oregon Creek	>6	Seidler 1979
S. typhirmrium	Cincinnati – stormwater at 10°C	7	Geldreich, *et al*. 1968
	Cincinnati – stormwater at 20°C	2	Geldreich, *et al*. 1968
Shigella flexneri	Baltimore – stormwater	>8	Field, *et al.* 1976
Enterbactor aerogenes	Cincinnati – stormwater at 10°C	5	Geldreich, *et al*. 1968
	Cincinnati – stormwater at 20°C	4	Geldreich, *et al*. 1968

Survival of Bacteria in Soil

Because of the importance of soil bacteria as a source of urban runoff bacteria, their survival in the soil after deposition is important. If an area has long intervent times between rain events, soil bacteria survival would have to be quite long in order for the soil to be a significant urban runoff bacteria source. However, in areas having frequent rains, soil bacteria survival is less important (assuming that it is greater than the interevent period). Many site conditions have been reported to influence soil bacteria survival. Van Donsel, *et al*. (1967) found that sunlight, temperatures, rainfall, soil moisture, pH, organic matter, and the presence of other microorganisms all affect the survival of total coliforms, fecal coliforms, and fecal strep. soil bacteria. They also reported that feces bacteria deposited on dry soils are relatively immobilized and subject to the specific site conditions. After-growth of soil bacteria (increasing populations without new deposition) may account for some of the seasonal variations in runoff bacteria counts. If the soil has not been recently contaminated, the runoff would have an immediate supply of microorganisms from the soil. Contamination of the receiving waters would be out of proportion to the true sanitary history of the area. They also stated that non-fecal coliforms reappeared after fecal organisms declined. They were also present in much higher concentrations after fecal bacteria dieoff than before the soil was contaminated.

Both after-growth and decline of bacteria in soils have been reported. Soil coliforms exhibit after-growth following rainstorms and exhibit rapid declines during freezing weather. If very warm weather follows a rain, a very large increase in soil coliform bacteria was noted, while the increase was much less if cool weather followed a rain. They also found declining bacteria soil populations if the soil was dry. Alternate freezing and thawing at exposed winter sites caused significant morality of soil coliform bacteria. Evans and Owens (1972) reported that *E.Coli* and

Enterococci showed 90 percent reductions after about two or three months in soils. Van Donzel, *et al.* (1967) reported prolonged persistence of other bacteria types. Various strains of Salmonella were found to exist for long periods of time (nine months for *S typhimurium*). It is not uncommon for soil bacteria to survive for up to 200 days after inoculation.

Fecal Coliform to Fecal Strep. Bacteria Ratios

Geldreich (1965) found that the ratio of fecal coliform to fecal strep. bacteria concentrations may be indicative of the probable fecal source. In fresh human fecal material and domestic wastes, he found that the fecal coliform densities were more than four times the fecal strep. densities. However, this ratio for livestock, poultry, dogs, cats, and rodents was found to be less than 0.6. These ratios must be applied carefully, because of the effects of travel time and various chemical changes (especially pH) on the dieoff rates of the component bacteria. As a generality, he stated that fecal coliform to fecal strep. ratios greater than four indicate that the bacteria pollution is from domestic wastes, which are composed mostly of human fecal material, laundry wastes, and food refuse. If the ratio is less than 0.6, the bacteria is probably from livestock or poultry in agricultural areas or from stormwater runoff in urban areas. He found that agricultural and stormwater runoff can be differentiated by studying the types of fecal strep. bacteria found in the water samples. Geldreich and Kenner (1969) further stressed the importance of carefully using this ratio. They stressed that samples must be taken at the wastewater outfalls. At these locations, domestic waste, meat packing wastes, stormwater discharges, and feedlot drainage contain large numbers of fecal organisms recently discharged from warm blooded animals. Once these organisms are diffused into the receiving stream, however, water temperature, organic nutrients, toxic metals, and adverse pH values may alter the relationship between the indicator organisms. This ratio should only be applied within 24 hours following the discharge of the bacteria from the animal.

Feachem (1975) examined how these ratios could be used with bacteria observations taken over a period of time. Because the fecal coliform and fecal strep. bacteria dieoff rates are not the same, the ratio gradually changes with time. He found that bacteria is predominantly from human sources if the FC/FS ratios are initially high (greater than four) and then decrease with time. Non-human bacteria sources would result in initially low fecal coliform to fecal strep. ratios (less than 0.7) which then rise with time.

Table 20 summarizes the observed fecal coliform to fecal strep. bacteria population ratios in the Rideau River study area. These ratios are separated into source area sheetflow samples, Rideau River water samples and water samples collected at the swimming beaches. The source area sheetflow samples contain the most recent pollution, while the river segment and beach samples contain “older” bacteria. The initial source area samples all have ratios of less than 0.7. However, the river averages range from 0.5 to 1.2 and the beach samples (which may be “older” than the river samples) range from 1.7 to 2.8. These ratios are seen to start with values less than 0.7 and increase with time. Based on Feachem’s (1975) work, this would indicate that the major bacteria sources in the Rideau River are from non-human sources. This substantiates the previous conclusions as presented in the Phase 1 Rideau River Stormwater Management Report. Periodic high bacteria ratios in the river and at the beaches could be caused by the greater dieoff ratio of fecal strep. as compared to fecal coliform. The observed periodic high Rideau River FC/FS ratios (which can be greater than four) may therefore be from old, non-human fecal discharges and not from fresh human fecal discharges.

Table 20. Fecal Coliform to Fecal Strep. Bacteria Population Ratios in Study Area (Pitt 1983)

Source Areas	FC/FS ratio
Rooftop runoff	0.5
Vacant land sheetflow	0.3
Parking lot sheetflow	0.2
Gutter flows	0.2
Average of source area values	0.3
Rideau River Segment	
A	1.2
B	0.6
C	0.5
D	0.5
E	1.0
Average of river segment values	0.7
River Swimming Beaches	
Strathcona	2.8
Brantwood	2.3
Brighton	2.1
Mooney's Bay	1.7
Average of swimming beach values	2.2

Determination of Survival Rates for Selected Bacterial and Protozoan Pathogens

The following discussion was prepared by John Easton while he was a Ph.D. student at the University of Alabama at Birmingham and describes some of the experiments he has conducted concerning the survival of wet weather flow bacteria and pathogens after being discharged to urban receiving waters (Easton 2000). This section is not intended to be a comprehensive review of survival of microorganisms in the environment, but is intended to illustrate how actual site specific survival rates can be determined, especially for unusual conditions (affected by water temperature, turbidity, natural predation, local sources and receptors, etc.). This information is necessary for human health assessments when predicting resulting downstream pathogen conditions. Much of the literature information on microorganism survival is based on laboratory investigations that may not be applicable to actual field conditions. The simple tests described in this section allow more accurate in-stream predictions to be made.

Microorganisms have varying degrees of stability within the environment. Their numbers are dependent upon population dynamics, which is controlled by several criteria (McKinney 1992): 1) competition for food (limited food sources limit microbial numbers), 2) predator-prey relationships (some organisms consume others for food sources), 3) nature of organic matter (carbohydrates, organic acids, proteins all stimulate different organisms), and 4) environmental conditions (oxygen concentration, nutrient levels, temperature, pH, etc.). Since there are a multitude of factors that contribute to microorganism survivability, the use of an *in-situ* method to characterize the rates of growth and death is necessary to account for variable environmental conditions.

The experiments conducted to evaluate degradation of *G. lamblia* were conducted *in-situ*. The sewage matrix was spiked with approximately 10,000 cysts per liter to enable detection after significant dieoff. These cysts were formalinized in order not to risk releasing a potentially infectious pathogen into the environment. Since these organisms are in cyst form, i.e., relatively inert, it was hypothesized that the mechanism of dieoff would be predation by other organisms and formalinized organisms would be a suitable surrogate for "live" ones.

These *in-situ* experiments were conducted in specially designed chambers (Figure 1). These were designed to allow passage of water and nutrients between the inside of the chamber and the outside environment (Five-Mile Creek in Jefferson County, AL), while trapping the microorganisms inside to allow enumeration at various times during the experiment.

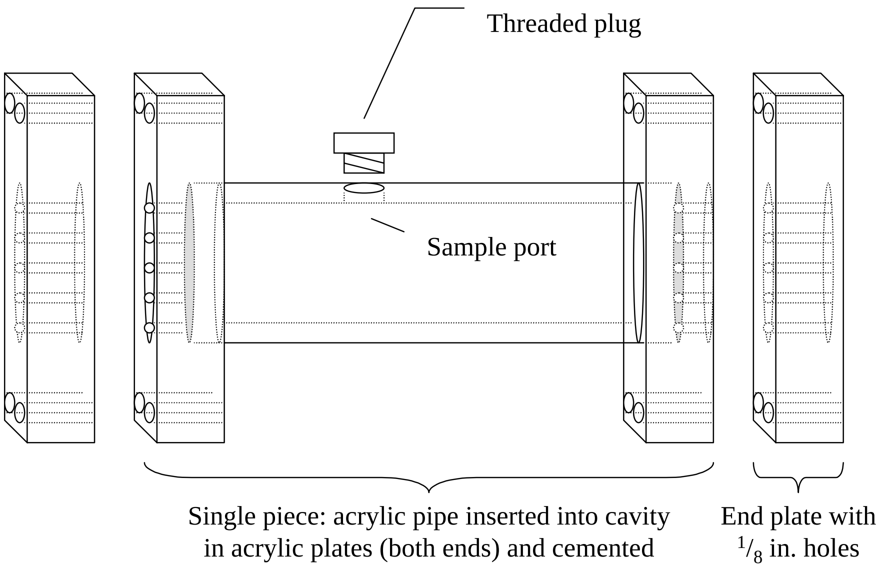

Figure 1. Acrylic components of *in-situ* chamber (Easton 2000).

These experiments included exposures over a twenty-one day period. A polyethersulfone (Supor®, Gelman Sciences) membrane filter, which is not susceptible to biological degradation, was used. This membrane material was clamped onto either end of a piece of acrylic tubing in a design devised by Easton (2000) and colleagues (Figure 2). The membrane pore size is 0.22 µm, allowing exchange of ions with the surrounding water while keeping the microorganisms inside the test chamber.

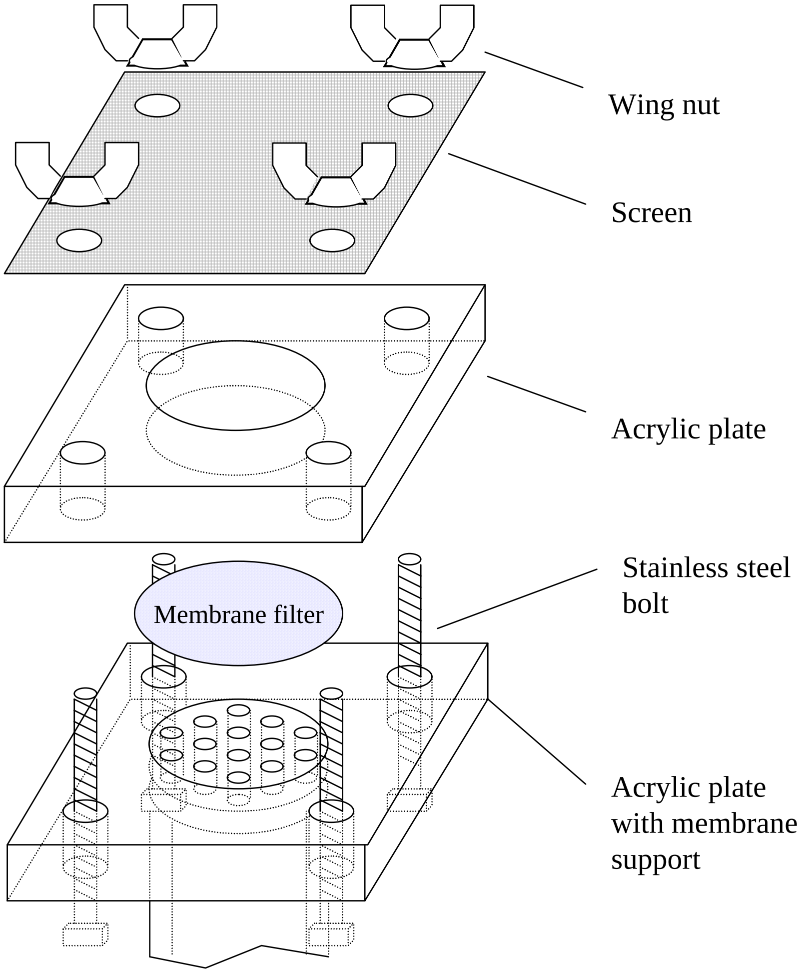

Figure 2. End-plate of *in-situ* chamber showing the location of membrane filter (Easton *2000*).

Multiple chambers containing sewage samples were placed in the creek and removed after 0, 1, 3, 7, 10, 14, and 21 days. At each time step, three separate chambers were removed and composited for analysis. Once the samples were composited, they were blended (Warring blender for two minutes) to minimize agglomeration of the microorganisms.

The results of these experiments show that the microorganisms die off at a constant, rapid rate (assumed in most receiving models) only for an initial short period. As time progressed, the dieoff rate slows. Figure 3 is a plot of the levels of *Giardia* cysts versus time. The method used to enumerate these organisms (EPA Method 1623) requires a presumptive test followed by a confirmed test. The presumptive test consists of identifying objects, of the correct size and shape, which are stained by a *Giardia*-specific antibody bound to a fluorescent probe. Next, the organisms are confirmed by identification of internal structures stained by the nuclear stain DAPI (4',6-Diamindino-2-phenylindole). Unfortunately, problems were encountered with the confirmation test in these experiments (the DAPI stain of the background was too intense to enable identification of internal structures. However, using the presumptive stain, which binds to the cyst cell wall, it was possible to detect differences in these presumptive *Giardia* cysts. Some cysts were intact (i.e., the stain covered the cell wall continuously), and some cysts were present, but degraded (i.e., the staining of the cell wall was less intense and not continuous). The levels of the former, "intact cysts," are plotted along with the levels of the latter, "degraded cysts" in Figure 3.

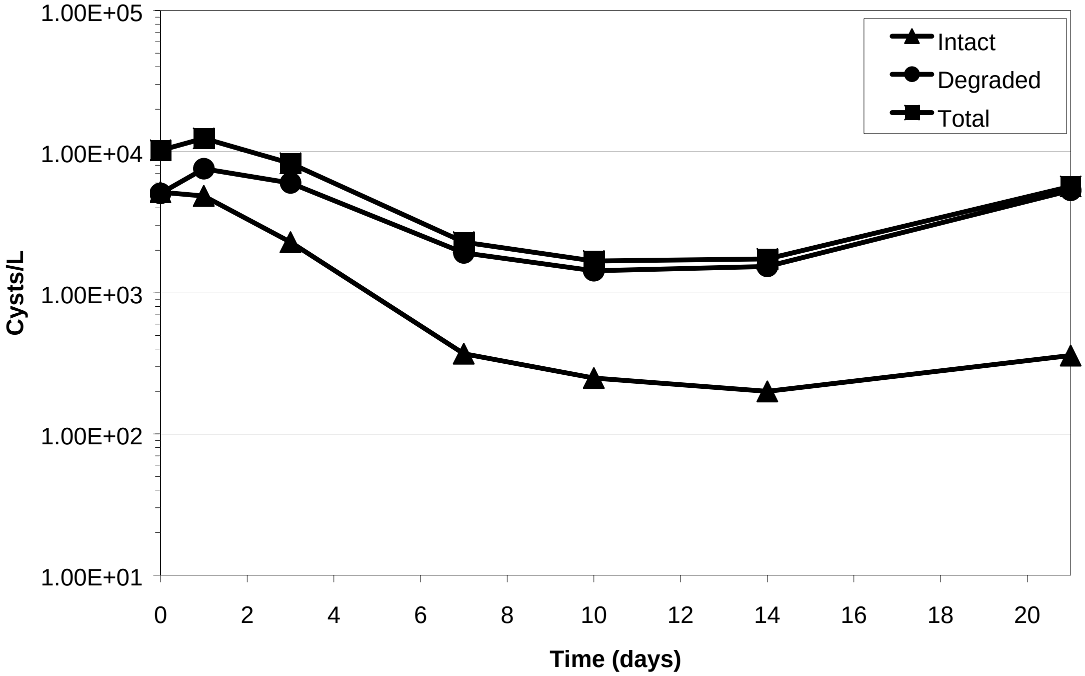

Figure 3. Degradation Plot of *Giardia* Cysts (Easton 2000).

Since the microorganisms' rate of dieoff seems to be decreasing over time, the regression model was applied in segments, starting with the first three data points, and adding one additional point until the entire twenty-one day, or 7 point, data set was used. In general, the dieoff rates decrease and T_x values correspondingly increase as data over longer time periods are included in the regression analyses. The T_{90} values (time needed for 90% dieoff) for the indicator bacteria, total coliforms and *E. coli*, are in accordance with conventional wisdom. Many studies have shown T_{90} values for these organisms to be in the range of several hours to a few days (Droste and Gupgupoglu 1982; Geldreich, *et al.* 1968; and Geldreich and Kenner 1969). The initial rapid dieoff occurred, generally, within the first seven days of the experiment. Table 21 gives a first-order dieoff constant, k (days^{-1}), and its associated ninety-five percent confidence interval, for each of the microorganisms. In addition, the results of the Mann-Kendall Test (a non-parametric test for trend) are given. All of the dieoff constants (slope of the regression line) are statistically significant except for Enterococci.

Table 21. Dieoff Rates Determined Using Day 0 to Day 7 Data (Easton 2000)

Organism	Dieoff Rate (day^{-1})	95% CI	Mann-Kendall Trend *
Total Coliforms	-0.310	± 0.152	p = 0.042
E. coli	-0.331	± 0.049	p = 0.042
Enterococci	-0.078	± 0.189	p = 0.375 †
Giardia	-0.171	± 0.074	p = 0.042

* p <0.05 indicates significant downward trend
† Not significant, no trend (dieoff)

The data generated by this study suggests that if one were using dieoff constants from indicator bacteria studies, then one may tend to under predict the length of time or distance downstream in which adverse health effects due to pathogens in sewage are present. In addition, this data indicates that assumptions regarding the constancy of dieoff rates may be invalid. There seems to be a modulation of the rate of dieoff with increased time, as all of the test organisms showed a pattern of leveling off toward some equilibrium level with increasing time.

The Enterococci results are quite different from the others, with no rapid initial dieoff, as generally reported in the literature (Facklam and Sahm 1995). This persistence is due to the enterococci being Gram+ and is therefore a better indicator of virus survival. For these reasons, the EPA has selected enterococci as an indicator organism in their new guidance documents.

The *Giardia* results were not as expected. The descriptions of this organism found in the literature seem to predict that *Giardia* will persist for much longer than observed in these tests. This study seems to show that *Giardia,* and perhaps other protozoan pathogens, exhibits dieoff characteristics similar to the bacteria included in this study. However, these cysts were treated with formalin and therefore may have been less resistant to degradation in the environment.

There are many stormwater microorganisms of interest when conducting a receiving water study. However, besides characterizing microorganism conditions, it is also necessary to understand population dynamics when predicting fate and exposures. This section briefly described some of the currently used analytical methodologies for measuring microorganism counts, along with an example *in-situ* dieoff experiment.

Inappropriate Sewage Contamination as a Source of Pathogens in Urban Wet Weather Flows

Urban stormwater runoff includes waters that find their way into storm drainage systems from many sources in addition to precipitation. In many cases, these non-stormwater sources may account for the majority of the annual discharges for some pollutants of concern from the storm drainage system. This was one of the issues which emerged from the individual projects of the U.S. EPA’s Nationwide Urban Runoff Program (NURP) (EPA 1983). Concerns regarding inappropriate connections to storm drainage systems were summarized as follows in the Final Report of the NURP executive summary:

> “A number of the NURP projects identified what appeared to be inappropriate connections of sanitary discharges to stormwater sewer systems, resulting in high bacterial counts and dangers to public health. The costs and complications of locating and eliminating such connections may pose a substantial problem in urban areas, but the opportunities for dramatic improvement in the quality of urban stormwater discharges certainly exist where this can be accomplished. Although not emphasized in the NURP effort, other than to assure that the selected monitoring sites were free from sanitary sewage contamination, this Best Management Practice (BMP) is clearly a desirable one to pursue.”

The inappropriate discharges noted during NURP were especially surprising because the monitored watersheds were carefully selected to minimize factors other than stormwater. Presumably, inappropriate discharge problems in typical watersheds would be much worse. Inappropriate entries into urban storm sewerage were identified by flow from storm sewer outfalls following substantial dry periods. Such flow could be the result of direct “inappropriate connections” as mentioned in the NURP final report, or could result from indirect connections (such as contributions from leaky sewerage infiltrating to the separate storm drainage). Many of these inappropriate dry-weather flows are continuous and would therefore also occur during rain-induced runoff periods (Pitt, *et al*. 1993).

The EPA funded an early research project to develop tools to assess and identify inappropriate discharges into storm drainage (Pitt, *et al*. 1993; Lalor 1993). This project developed simple field screening methods, heavily based on successful experience elsewhere, that were found to be highly reliable in residential and commercial test areas. In recent years, numerous screening tools have also been proposed to identify sources of contaminants found in urban drainage waters. Pitt, *et al*. (2000) reviewed many of these tools for application to inappropriate dry weather discharges into separate storm drainage.

In many cases, sanitary sewage is an important component of dry weather discharges from storm drainage systems. The effects these discharges have on the receiving waters are highly dependent on many site specific factors, including frequency and quantity of sewage discharges and the creek flows. In many urban areas, the receiving waters are small creeks in completely developed watersheds. These creeks are most at risk from inappropriate discharges, as base flows may be predominately dry weather flows from the drainage systems.

The presence of pathogens from raw, or poorly treated sewage, in urban streams, presents a potentially serious public health threat. Even if the receiving waters are not designated for water contact recreation, children are often seen playing in small city streams. From a human health perspective, it may not require much raw or poorly treated sewage to cause a receiving water problem due to pathogens.

Evidence of Sewage Contamination of Urban Streams

The following case studies present summaries of various studies conducted throughout the U.S. that investigated contamination of urban streams that were only supposed to be receiving stormwater discharges. Many of the problematic discharges were from sanitary sewage. Obviously, inappropriate discharges must be identified and corrected as part of any effort to clean up urban streams. If these sources are assumed to be non-existent in an area and are therefore not considered in the stormwater management activities, incorrect and inefficient management decisions are likely, with disappointing improvements in the receiving waters. Lalor (1993), Pitt, *et al.* (1993), and Pitt and Lalor (1997) present a strategy to support the outfall screening activities required by the NPDES Stormwater Permit Program to identify and correct these inappropriate discharges to storm drainage systems.

Washtenaw County (Ann Arbor), MI

From 1984 to 1986, Washtenaw County, Michigan, dye-tested 160 businesses in an effort to locate direct inappropriate connections to the county stormwater sewerage (Murray 1985; Schmidt and Spencer 1986; Washtenaw County 1988). Of the businesses tested, 61 (38%) were found to have improper storm drain connections. The Huron River Pollution Abatement Program was the most thorough investigation of such improper connections. Beginning in 1987, 1067 businesses, homes and other buildings located in the Huron River watershed were dye tested. The following results were reported. Inappropriate connections were detected at 60% of the automobile related businesses inspected, including service stations, automobile dealerships, car washes, and auto body and repair shops. All plating shops inspected were found to have improper storm drain connections. Additionally, 67% of the manufacturers tested, 20% of the private service agencies, and 88% of the wholesale/retail establishments tested were found to have improper storm sewer connections. Of 319 homes dye tested, 19 were found to have direct sanitary connections to storm drains. The direct discharge of rug cleaning wastes into storm drains by carpet cleaners was also noted as a common problem. Several surveys, beginning as early as 1963, identified bacterial and chemical contamination of the Allen Creek storm drainage system. Studies in 1963, 1978 and 1979 found that discharges from the Allen Creek storm drain contained significant quantities of fecal coliform and fecal streptococci. The 1979 study also documented high pollutant loads of solids, nitrates and metals. A large number of inappropriate storm drain connections originating from businesses were found, especially within automobile related facilities. Chemical pollutants, such as detergents, oil, grease, radiator wastes and solvents were causing potential problems.

The elimination of these storm drain connections prevented thousands of gallons of contaminated water from entering the Huron River from the Allen Creek storm drainage system annually. Eight sampling locations along the main stem and major lateral branches of the storm drainage system were established and monitored for 37 chemicals during rain events. From 1984 to 1986, 32 (86%) of these chemicals showed a decrease in concentrations while only 2 (5%) showed an increase. In spite of this improvement, chemical concentrations in the stormwater discharges at the Allen Creek outfall were still greater than those from the control station much of the time.

Fort Worth, TX

This program has been underway since June of 1985 (Falkenbury 1987). Investigations to date indicate few direct connections from industries to storm drains. Storm runoff, in addition to illegal dumping, accidental spills and direct discharges into the street or adjacent creeks seem to account for the majority of the contaminants entering the storm drainage system. Major problems stemmed from septic tanks, self-management of liquid wastes by industry and construction of municipal overflow bypasses from the sanitary sewer to the storm drains. The success of this program was judged by a decline in the number of undesirable features at the target outfalls. An average of 44 undesirable observations per month were made in 1986 (522 total), compared to an average of 21 undesirable observations per month in 1988.

Inner Grays Harbor, WA

In 1987, an inspection of the 90 urban stormwater outfalls draining into Inner Grays Harbor in Washington revealed 29 (32%) flowing during dry weather (Beyer, *et al*. 1979; Pelletier and Determan 1988). A total of 19 outfalls (21%) were described as suspect, based on visual observation and/or anomalous pollutant levels, as compared to those expected in typical urban stormwater runoff characterized by NURP. At least one storm drain system was later found to receive a residential sanitary sewage connection which has since been corrected. This drain exhibited no unusual visual characteristics, but was found to have atypical pH and total suspended solids levels. Notably, fecal coliform levels were within the typical range expected for stormwater.

Sacramento, CA

A Sacramento, California, investigation of urban discharges identified commercial as well as domestic discharges of oil and other automobile related fluids as a common problem based on visual observations (Montoya 1987). Montoya found that slightly less than half the water discharged from Sacramento's stormwater drainage system was not directly attributable to precipitation. Most of this water comes from unpermitted sources, including inappropriate and/or inappropriate entries to the storm drainage system.

Bellevue, WA

During the Bellevue, Washington Urban Runoff Project baseflows as well as stormwater from two residential urban basins were monitored (Pitt 1985; Pitt and Bissonnette 1983). The areas included in this study, Surrey Downs and Lake Hills, are about 5 km apart and each covered an area of about 40 ha. Both were fully developed, with predominantly single family residences. No septic tanks were present in either area and the storm drainage systems were thoroughly mapped and investigated to ensure no non-stormwater discharges to storm drainage systems or obvious illegal discharges. The Bellevue, Washington, NURP project also summarized the reported incidents of intermittent discharges and dumpings of pollutants into the local storm drainage system. During a three year period of time, about 50 citizen contacts were made to the Bellevue Storm and Surface Water Utility District concerning water quality problems. About 25 percent of the complaints concerned oil being discharged into storm drain inlets. Another important category of complaints was for aesthetic problems, such as turbid or colored water in the creeks. Various industrial and commercial discharges into the storm drainage system were detected. Concrete wastes flushed from concrete trucks at urban job sites were a frequently occurring problem. Cleaning establishment discharges into creeks were also a common problem. Vehicle accidents also resulted in discharges of gasoline, diesel fuel, hydraulic fluids, and lawn care chemicals into the storm drain inlets.

Boston, MA

A field screening program was conducted to determine the relative levels of contamination at various locations in the Stony Brook drainage system (Metcalf and Eddy 1994). During eight days of dry-weather sampling, numerous inappropriate discharges of sanitary sewage into the drainage system were identified using the investigative procedures developed by Pitt, *et al.* (1993) and a modified flow chart approach.

Minneapolis/St. Paul, MN

Water Environment & Technology (1996a) reported that the fecal coliform counts decreased from about 500 counts/100 mL to about 150 counts/100 mL in the Mississippi River after the sewer separation program in the Minneapolis and St. Paul area of Minnesota. Combined sewers in 8,500 ha were separated during this 10-year, $332 million program.

Toronto, Ontario

The Toronto Area Watershed Management Strategy (TAWMS) study monitored and characterized both stormwater and baseflows (Pitt and McLean 1986 and GLA 1983). The project involved intensive monitoring in two test areas. The Emery catchment area, located near the City of North York, covered approximately 154 ha with predominantly “medium” industrial land uses (processing goods for final consumption). The Thistledown catchment, located in the City of Etobicoke, covered approximately 39 ha with residential and commercial land uses. During cold weather, the increases in dissolved solids were quite apparent in baseflows and snowmelt for both study catchments. This increase was probably caused by high chlorides from road salt applications. In contrast, bacteria populations were noticeably lower in all outfall discharges during cold weather. Nutrient and heavy metal concentrations at the outfalls remained fairly constant during cold and warm weather. Either warm- or cold-weather baseflows were responsible for most of the yields for many constituents from the industrial catchment. These constituents included runoff volume, phosphorus, total Kjeldahl nitrogen, chemical oxygen demand and chromium. Important constituents that had high yields in the baseflow from the residential/commercial catchment included total solids, dissolved solids, chlorides, and fecal coliform and *Pseudomonas aeruginosa* bacteria.

Gartner Lee and Associates, Ltd. conducted an extensive survey of dry-weather flows in storm drainage systems in the Humber River watershed (Toronto) in an attempt to identify the most significant urban runoff pollutant sources. About 625 outfalls were sampled two times during dry-weather, with analyses conducted for many pollutants, including organics, solids, nutrients, metals, phenols, and bacteria. About 59% had dry-weather flows, and about 33% of the outfalls were discharging at rates greater than 1 L/sec. The dry-weather flows were found to contribute significant loadings of nutrients, phenols, and metals, compared to upstream conditions. About 10 to 14 percent of the outfalls were considered significant pollutant sources. Further investigations identified many industrial and sanitary sewage non-stormwater discharges into the storm drainage. An apartment building with the sanitary drains from eight units illegally connected to the storm drainage system was typical of the problems found. Other problem areas were found in industrial areas, including yard storage of animal hides and yard runoff from meat packing plants.

Ottawa, Ontario

Visual inspection of stormwater pipes discharging to the Rideau River (Ontario) found leakage from sanitary sewer joints or broken pipes to be a major source of storm drain contamination (OME 1983). A study of the lower Rideau River in the Regional Municipality of Ottawa-Carleton was conducted to establish the causes of bacteriological water quality degradation in the urbanized reach of the river and to analyze the impacts of future urbanization. Earlier programs had identified and corrected many cross-connections between sanitary sewers and stormwater sewers. Bacteriological water quality improved, but swimming standards at beaches were still not obtained.

Birmingham, AL

During the development of the methods to investigate inappropriate discharges, a three-mile section of Village Creek in Birmingham, AL, was selected for field verification of the test methods (Pitt, *et al.* 1993, Pitt and Lalor 1997). The drainage area for this section of the creek contains about 4500 acres. Residential land use comprises approximately 88% of the total area, commercial land use approximately 8%, and industrial land use less than 1%. The majority of the drainage area is serviced by sanitary sewers, but some septic tanks are also used. A total of 65 stormwater outfalls were located. Outfall diameters ranged from 2 inches to 12 feet, excluding open ditches. All sites were visited at least 8 times during the field investigation period. Of these 65 outfalls, 48 (74%) were always dry, 6 (9%) had flow intermittently, and 11 (17%) were always flowing. Eighteen direct unpermitted discharges to the creek from nearby industries and commercial areas were also located; 10 (56%) were always dry, 6 (33%) had intermittent flow, and 2 (11%) were always flowing. The dry weather flows from two of the 65 outfalls were found to be mostly sanitary sewage, while the flows from another nine were predominately washwaters. The remaining outfalls with dry weather flows were mostly affected by natural waters (most likely groundwater infiltration) or leaking domestic water.

Periodic stream surveys of tributaries of the Cahaba River in the Birmingham area (mostly the Little Cahaba River, upstream of Lake Purdy) during summer months have found that the small river contained about 1/3 treated sewage from upstream poorly operated municipal treatment facilities (since corrected), septage from failing septic tanks, and SSO discharges.

During a current EPA sponsored project investigating SSO discharges being conducted by Lalor at UAB, sewage, through SSOs and poorly operating septic tanks, were found to make up about 25% of the dry weather flows in the small, completely urbanized stream in Homewood, AL, being studied. However, sewage contributions in the much larger, and much less urbanized 5-mile Creek are very small (on a percentage standpoint), although SSOs exist in the urbanized area. These streams are still being evaluated, including future human health risk assessments associated with these discharges.

Black Warrior Watershed, AL

The Black Warrior Watershed Management Plan, through cooperation with County Public Health Departments, has identified the number of failing septic systems within the Black Warrior Watershed. The following table summarizes this information.

Estimates of Failing Septic Systems within the Black Warrior Watershed		
County	# of Septic Systems (Estimate)	Percentage of Systems Failing (Estimate)
Blount	42,000	Unknown
Cullman	10,000	20.0%
Fayette	3,100	11.5%
Greene	3,000	20 – 30%
Hale	Not Reported	Not Reported
Jefferson	100,000	1- 3%
Tuscaloosa	40,000	10%
Walker	8,000	5%
Winston	50,000	5%
Source: Prepared for the Black Warrior River Watershed Management Plan by the Tuscaloosa County Soil and Water Conservation District per conversations with County Public Health Departments.		

The 2002 303(d) listed identifies the following stream segments as being impaired for pathogens:

Stream Segments within the Black Warrior Watershed Listed as Impaired for Pathogens 2002 303(D)		
Waterbody	Waterbody ID	County
Long Branch	03160109-020_02	Cullman
Brindley Creek	03160109-030_01	Cullman
Eightmile Creek	03160109-040_01	Cullman
Broglen River	03160109-050_01	Cullman
Thacker Creek	03160109-080_01	Cullman
Rock Creek	03160110-080_01	Winston
Crooked Creek	03160110-090_01	Cullman
Dry Creek	03160111-050_02	Blount
Hurricane Creek	03160112-120_01	Tuscaloosa
Little Hurricane Creek	03160112-120_02	Tuscaloosa

Other impairments included on the 2002 303(d) list, although caused from a variety of sources, are also associated with septic and sewer failures. These impairments include: organic enrichment, low dissolved oxygen, and nutrients. The table below lists waterbodies on the 2002 303(d) list for these associated sources.

Stream Segments within the Black Warrior Watershed Listed for Associated Pathogen Sources 2002 303(D)		
Waterbody	Waterbody ID	County
Mud Creek	03160109-070_01	Cullman
Mulberry Fork	03160109-080_02	Blount/Cullman
Mulberry Fork	03160109-080_03	Blount/Cullman
Cane Creek	03160109-170_01	Walker
Locust Fork	03160111-120_01	Blount/Jefferson
Locust Fork	03160111-150_02	Jefferson
North River	03160112-100_01	Tuscaloosa

Other sources of pathogens documented in the Black Warrior Watershed Management Plan:

- The Tuscaloosa County Department of Public Health has recently documented fecal coliform bacteria in Lake Tuscaloosa in levels exceeding the standards of full body contact. (2003) Sources of fecal coliform are thought, but not documented, to be from failing septic systems around Lake Tuscaloosa.

- The Cullman County Soil & Water Conservation District currently monitors certain 303(d) listed stream segments for *E. coli*. Monitoring is performed by using Alabama Water Watch protocols. Findings are as follows:

Cullman County Soil & Water Conservation District E. Coli Sampling, 2002-2003			
Date	Source	Avg Fecal Coliform	High Fecal Coliform
July 2002	Eightmile Creek	367	1100
August 2002	Thacker Creek	8	33
September 2002	Duck Creek 1	25	67
October 2002	Duck Creek 2	277	433
February 2003	Brindley Creek	33	100
March 2003	Minnow Creek	420	1167
April 2003	Minnow Creek	275	633
May 2003	Minnow Creek	55	300
June 2003	Minnow Creek	72	200
July 2003	Crooked Creek	53	200
August 2003	Minnow Creek	56	133
Bacteria colonies per 100 mL			

- The USGS conducted a 16 month investigation (2000-2001) of water quality, aquatic-community structure, bed sediment, and fish tissue in Village (four sites) and Valley Creeks (three sites) and at two reference sites near Birmingham (Five Mile Creek/McCalla and Little Cahaba River).

- Concentration of enterococci at sites in the Birmingham area exceeded the USEPA criterion (151 col/100 mL) in 80 percent of the samples;

- *E coli* concentrations exceeded the USEPA criterion (576 col/100 mL) in 56 percent of the samples.

- Fecal coliform concentrations exceeded the ADEM criterion (4,000 col/100 mL) in 26 percent of the samples.

Summary of Inappropriate Sanitary Sewage Discharges into Urban Streams
Urban stormwater runoff includes waters from many other sources which find their way into storm drainage systems, besides from precipitation. There are cases where pollutant levels in storm drainage are much higher than they would otherwise be because of excessive amounts of contaminants that are introduced into the storm drainage system by various non-stormwater discharges. Additionally, baseflows (during dry weather) are also common in storm drainage systems. Dry-weather flows and wet-weather flows have been monitored during numerous urban runoff studies. These studies have found that discharges observed at outfalls during dry weather were significantly different from wet-weather discharges and may account for the majority of the annual discharges for some pollutants of concern from the storm drainage system.

In many cases, sanitary sewage was an important component (although not necessarily the only component) of the dry weather discharges from the storm drainage systems. From a human health perspective (associated with pathogens), it may not require much raw or poorly treated sewage to cause a receiving water problem. However, at low discharge rates, the DO receiving water levels may be minimally affected. The effects these discharges have on the receiving waters is therefore highly dependent on many site specific factors, including frequency and quantity of sewage discharges and the creek flows. In many urban areas, the receiving waters are small creeks in completely developed watersheds. These creeks are the most at risk from these discharges as dry base flows may be predominately dry weather flows from the drainage systems. In Tokyo (Fujita 1998), for example, numerous instances were found where correcting inappropriate sanitary sewage discharges resulted in the urban streams losing all of their flow. In cities that are adjacent to large receiving waters, these discharges likely have little impact (such as DO impacts from Nashville CSO discharges on the Cumberland River, Cardozo, *et al.* 1994). The presence of pathogens from raw, or poorly treated sewage, in urban streams, however, obviously presents a potentially serious public health threat. Even if the receiving waters are not designated as water contact recreation, children are often seen playing in small streams in urban areas.

Development of Bacteria Indicator Standards

Inadequacy of Indicator Bacteria

Numerous studies have been conducted that show increased health risk from exposure to recreational waters containing high levels of indicator bacteria, including an excellent recent review by Prüss (1998). The intention of this article is not to review these indicator studies, but to advocate the collection of pathogen data in future studies and the use of this data to conduct better risk assessments.

The traditional studies have used indicator bacteria such as coliforms, *E. coli*, and Enterococci. Coliforms are found in human and animal feces; however, not all of them are of human fecal origin (Bitton 1994). Animal sources can contribute to high levels of indicator bacteria in receiving waters, but these waters may or may not contain pathogens that pose a significant health risk to humans. *E. coli* has been found in pristine sites in a tropical rain forest, suggesting that they too may not be a reliable indicator of human fecal contamination (Bermudez and Hazen 1988). Members of the genus Streptococcus such as Enterococci (fecal streptococci) are present in the intestinal tract; however, one species, *Enterococcus faecalis*, has been found on some plants in addition to other habitats (Madigan, *et al*. 1997).

Additional evidence for the inadequacy of indicators comes from the published climatological or regional differences found in epidemiology studies. Different indicators correlate with disease outcome depending upon whether or not the study was conducted in fresh or marine waters. In a freshwater French study (Ferley, *et al.* 1989), fecal streptococci were better indicators for gastrointestinal disease than fecal coliforms; while in a marine Australian study (Corbett, *et al.* 1993), fecal coliforms were better predictors than fecal streptococci. Indeed, even when comparing similar environments (marine), but for studies conducted in different geographical areas, analogous inconsistencies are noted. A British study (Fleisher, *et al.* 1993) found a relationship between fecal streptococci and gastroenteritis, and no association with fecal coliforms; while a Hong Kong study (Cheung, *et al.* 1990) found *E. coli* (a fecal coliform) was the best indicator. In contrast, it is expected that numbers of a given pathogenic microorganisms will correlate quite well (and consistently) with its associated disease outcome.

As mentioned previously, recent technological advances have made laboratories increasingly more capable of enumerating pathogens. For instance, a study using these new methods was conducted to evaluate the decay rates of Cryptosporidium, Giardia, and *E. coli* O157:H7 in an urban stream in Alabama (Easton 2000). Data such as these can be used to develop more accurate risk assessments, and subsequently better standards.

Epidemiological Studies and Effects of Human Exposures to Stormwater

Epidemiology can be defined as the study of the occurrence and causes of disease in human populations and the application of this knowledge to the prevention and control of health problems. Much of the information that is used in developing environmental regulations designed to protect human health originates with epidemiological studies. Routinely used to assess risks associated with contaminants in drinking waters, epidemiology has, more recently, also been used to investigate human health risks associated with swimming in waters contaminated by stormwater.

Recently published epidemiology studies have described the increased health risks and problems associated with contact recreation in contaminated water, including water affected by stormwater, although most historical studies have focused on waters contaminated by sanitary sewage. However, as seen above, separate stormwaters are likely contaminated with sewage and therefore possibly contain similar pathogens, although the indicator conditions can vary greatly. In most cases, the levels of pathogens (see Craun, *et al*. 1997; O'Shea and Field 1992a and 1992b; Kay 1994) causing increased illness during these epidemiological studies were well within the range found in urban waters only affected by stormwater. These studies are therefore important as they indicate the risks associated with water contact recreation in receiving waters contaminated with the pathogens found in stormwater.

Before reviewing these studies, it should be noted that the results of environmental epidemiology studies have provoked controversy. An excellent review article by Craun, *et al.* (1996) on epidemiology applied to water and public health discusses many of these problems and offers suggestions to enable better interpretation of existing studies and better design of future studies.

Hong Kong Swimming Beach Study

Swimming beach studies were conducted in Hong Kong during the summers of 1986 and 1987 (Cheung, *et al.* 1990). This was one of the first major epidemiological investigations to be conducted in subtropical waters. More than 18,700 responses were obtained from beachgoers on nine beaches. Water samples were collected every two hours at the nine beaches under study. The samples were analyzed for *E. coli*, *Klebsiella* spp., fecal streptococci, fecal coliforms, staphylococci, *Pseudomonas aeruginosa*, *Candida albicans*, and total fungi. *E. coli* only represented 57% of the fecal coliforms (much lower than reported elsewhere). Beachgoers were recruited on selected weekends and given initial interviews. Follow-up telephone interviews were obtained 7 to 10 days afterwards. The beachgoers spent an average of 3.5 hours at the beach, and swimmers spent an average of 1.3 hours in the water (much longer than reported in colder climates). The individual beaches studied were affected to varying degrees by nearby submarine sewage outfalls, agricultural runoff (pig farming) or by storm drains discharging across the beaches.

The overall symptom rates for gastrointestinal, ear, eye, skin, respiratory, fever, and total illness were significantly higher for swimmers than for non-swimmers. The increased risk of swimmers developing highly credible gastrointestinal illness (HCGI) was 5 times greater than for non-swimmers. The increased risk for swimmers of developing gastrointestinal (GI), eye, skin, and total illness was 2 to 4 times greater than for non-swimmers. The incubation period for the gastrointestinal symptoms in Hong Kong were similar to those reported for the U.S., indicating a possible similar causative agent (Norwalk virus and rotavirus virus originating from human sewage being suspected). Children under 10 years of age were also found to have significantly higher symptom rates for GI, HCGI, skin, respiratory, fever, and total illness than older swimmers. *Escherichia coli* was found to be the best indicator of swimmer illness (especially gastroenteritis and skin symptoms). Staphylococci measurements were recommended as a supplement to *E. coli*, especially for ear, respiratory and total illness. Researchers contrasted this finding with typically better correlations between Enterococci and health risks at U.S. beaches, and concluded that it may not be appropriate to adopt another country’s water contact recreation water quality criteria, especially if they are vastly separated geographically. Differences may be due to differences in the immune state of the populations and the indicator-illness relationships. Geometric mean densities of 180 *E. coli* per 100 mL and 1,000 staphylococci per 100 mL were found to be the thresholds for differentiating “barely acceptable” and “relatively unpolluted” beaches. Many of the rates were also higher at “barely acceptable” beaches than at “relatively unpolluted” beaches. These observations were used to develop new swimming beach standards for Hong Kong, as shown in Table 22. This new classification scheme was in place in 1988.

Table 22. Classification of Hong Kong Beaches Based on Swimming Associated Health Risk Levels

Rank	Swimming associated gastroenteritis and skin symptom rate (per 1,000 swimmers)	Seasonal geometric mean *E. coli* density (per 100 mL)	Number of swimming beaches in category during 1988
Good	0	24	9
Acceptable	10	180	19
Barely acceptable	15	610	7
Unacceptable	>15	>610	7

Cheung, *et al.* 1990.

Sydney Beach Users Study

This study examined problems associated with sewage contaminated swimming beaches (from CSO discharges and ocean outfalls of treated sewage) (Corbett, *et al.* 1993). The research team interviewed almost 3,000 beach goers at 12 beaches during 3 months in late 1989 and early 1990. Follow-up telephone interviews were conducted about a week later concerning incidence of illness. During the 41 days of sampling, 461 samples were analyzed for fecal coliforms and fecal streptococci. Of these samples, 67% failed to meet New South Wales Department of Health water quality criteria.

Swimmers were almost twice as likely as nonswimmers to report symptoms, but the prevalence of respiratory symptoms in people aged 15 to 25 was high, irrespective of swimming status or pollution level. The incidence of respiratory, fever, eye, ear, and other problems increased with increasing bacterial counts. Fecal streptococci counts were worse predictors of the swimming risk than the fecal coliform counts. Gastrointestinal symptoms were not related to either the fecal coliforms or fecal streptococci counts monitored. Those who swam for longer than 30 minutes were more than 4 times as likely to develop gastrointestinal symptoms compared to nonswimmers or those who swam for shorter periods.

Table 23 shows the percentages of swimmers who reported various illness symptoms after swimming in waters having varying bacterial contamination levels. Increasing levels of contamination increased the health risks for all symptoms, except for gastrointestinal symptoms. Table 24 shows the odds ratios (and associated 95% confidence intervals) for illness at different levels of fecal coliform contamination. Above 1,000 cfu/100 mL fecal coliforms, the associations for these illnesses are all strong, while they are at least moderate for all levels shown, compared to the nonswimmers. However, most of the confidence intervals were quite large, indicating large variability in the observations, as expected.

Table 23. Percentages of Beachgoers Reporting Symptoms (Corbett, *et al.* 1993)

Illness	Did not swim (n=915)	Swam, low pollution (n=1770)	Swam, high pollution (n=154)	Total sample (n=2839)
Vomiting	0.9	1.0	0.6	0.9
Diarrhea	2.2	3.7	3.2	3.2
Cough, cold, flu	10.2	17.3	23.4	15.3
Ear infection	1.3	3.9	5.8	3.2
Eye infection	1.0	2.4	3.9	2.0
Fever	1.1	1.8	5.2	1.7
Other	4.7	8.0	13.0	7.2
Any condition reported	16.5	26.9	35.7	24.0
Attended a doctor	3.5	4.3	8.4	4.3
Took time off work	2.6	4.6	6.5	4.0

Table 24. Odds Ratios (OR) of Swimmers Reporting Health Problems for Different Levels of Fecal Coliform Bacteria (Corbett, *et al.* 1993)

Illness	10 – 300 cfu/100 mL		300 – 1000 cfu/100 mL		1000 – 3000 cfu/100 mL		>3000 cfu/100 mL	
	OR	CI of OR	OR	CI of OR	OR	CI of OR	OR	CI of OR
Any symptom	2.9	1.7 – 5.1	3.8	2.1 – 7.1	5.2	1.7 – 16.0	5.9	3.0 – 11.5
Cough	2.4	1.5 – 3.8	2.0	0.9 – 4.4	4.2	1.2 – 14.6	6.9	3.3 – 14.1
Ear symptoms	4.3	1.1 – 16.2	8.6	1.7 – 43.2	8.5	0.8 – 97.6	7.4	1.3 – 43.3
Eye symptoms	6.3	1.3 – 30.8	9.7	1.5 – 63.7	8.7	1.0 – 72.8	na	na
Fever	2.1	0.6 – 7.0	4.7	1.0 – 22.5	9.0	1.9 – 43.5	na	na
Any gastrointestinal symptom	4.6	1.9 – 4.9	3.1	0.7 – 13.0	3.4	0.7 – 18.0	na	na

UK Swimmer/Sewage Exposure Study

Another recent swimmer/sewage exposure study was conducted in the UK, reported by Kay, *et al.* (1994) and by Fleisher, *et al.* (1996). This study was unique in design and was able to develop dose-response relationships and critical exposure levels for a few illnesses associated with swimmer exposures to sewage contaminated waters. Adult volunteers (1528 study participants) were studied over four seasons from 1989 through 1992. After arriving at the beach, healthy volunteers were randomly separated into bather and non-bather groups with the duration and place of individual exposure being rigorously controlled. All of the study locations met European Community mandatory bacteriological marine bathing water quality criteria and were therefore not excessively contaminated.

The researchers found a clear dose-response relationship between increasing levels of fecal streptococci and increased risk of acquiring acute febrile respiratory illness. Only bathers exposed to the highest quartile of exposure (51 to 158 FS /100 mL) showed a statistically significant increase in risk compared to the non bathers. The odds ratio (OR) was 2.65 (moderate association), with a 95% confidence interval of 1.19 – 5.48 for acute fibrile respiratory illness and fecal streptococci. There was a clear dose-response relationship among the bathers. In addition, exposure to increased levels of fecal coliform organisms was found to be predictive of ear ailments among bathers. Numerous studies have repeated the strong relationship with fecal streptococci or Enterococci, such that the EU (European Union), among other regulatory agencies, intends to move to Enterococci standards from fecal coliforms.

Thresholds of exposure to indicator organisms, below which bathers were at no excess risk of illness relative to non-bathers, were estimated to be 60 fecal streptococci organisms/100 mL for febrile respiratory illness and 100 fecal coliform organisms/100 mL for ear ailments. These threshold levels are quite low and are commonly exceeded in most urban streams. No dose-response relationships or threshold levels were found for any of the indicator organisms (total coliforms, fecal coliforms, fecal streptococci, total staphylococci and *Pseudomonas aeruginosa*) and eye or skin ailments. They concluded that the use of a single illness or indicator organism for establishing swimming criteria in marine waters is incorrect.

Exposure to Pathogens in Stormwater – The Santa Monica Bay Project
This study was the first large-scale epidemiological study in the U.S. to investigate possible adverse health effects associated with swimming in ocean waters affected by discharges from separate storm drains (SMBRP 1996). This was a follow-up study after previous investigations found that human fecal waste was present in the stormwater collection systems (*Water Environment & Technology* 1996b, *Environmental Science & Technology* 1996, and Haile, *et al*. 1996).

During a four month period in the summer of 1995, about 15,000 ocean swimmers were interviewed on the beach and during telephone interviews one to two weeks later. They were queried concerning illnesses since their beach outing. The incidence of illness (such as fever, chills, ear discharge, vomiting, coughing with phlegm, and credible gastrointestinal illness) was significantly greater (from 44 to 127% increased incidence) for ocean goers who swam directly off the outfalls, compared to those who swam 400 yards away, as shown on Table 25. Disease incidence dropped significantly with distance from the storm drain. At 400 yards, and beyond, upcoast or downcoast, elevated disease risks were not found. The results did not change when adjusted for age, beach, gender, race, socioeconomic status, or worry about health risks associated with swimming at the beach.

Table 25. Comparative Health Outcomes for Swimming in Front of Storm Drain Outfalls, Compared to Swimming at least 400 Yards Away (from SMBRP 1996)

Health Outcome	Relative Risk	Rate Ratio	Estimated Association	Estimated No. of Excess Cases per 10,000 Swimmers (rate difference)
Fever	57%	1.57	Moderate	259
Chills	58%	1.58	Moderate	138
Ear discharge	127%	2.27	Moderate	88
Vomiting	61%	1.61	Moderate	115
Coughing with phlegm	59%	1.59	Moderate	175
Any of the above symptoms	44%	1.44	Weak	373
HCGI-2	111%	2.11	Moderate	95
SRD (significant respiratory disease)	66%	1.66	Moderate	303
HCGI-2 or SRD	53%	1.53	Moderate	314

These interviews were supplemented with indicator and pathogen bacteria and virus analyses in the waters. The greatest health problems were associated with times of highest concentrations (*E. coli* >320 cfu/100 mL, Enterococcus > 106 cfu/100 mL, total coliforms >10,000 cfu/100 mL, and fecal coliforms > 400 cfu/100 mL). Bacteria populations greater than these are common in urban runoff and in urban receiving waters. Symptoms were found to be associated with swimming in areas where bacterial indicator levels were greater than these critical counts.

Table 26 shows the health outcomes associated with swimming in areas having bacterial counts greater that these critical values. The association for Enterococcus with bloody diarrhea was strong, and the association of total coliforms with skin rash was moderate, but nearly strong.

Table 26. Heath Outcomes Associated with Swimming in Areas having High Bacterial Counts (from SMBRP 1996)

Indicator (and critical cutoff count)	Health Outcome	Increased Risk	Risk Ratio	Estimated Association	Excess Cases per 10,000 Swimmers
E. coli (>320 cfu/100mL)	Ear ache and nasal congestion	46% 24%	1.46 1.24	Weak Weak	149 211
Enterococcus (>106 cfu/100 mL)	Diarrhea w/blood and HCGI-1	323% 44%	4.23 1.44	Strong Weak	27 130
Total coliform bacteria (>10,000 cfu/100 mL)	Skin rash	200%	3.00	Moderate	165
Fecal coliform bacteria (>400 cfu/100 mL)	Shin rash	88%	1.88	Moderate	74

The ratio of total coliform to fecal coliform was found to be one of the better indicators for predicting health risks when swimming close to the storm drain. When the total coliforms were greater than 1,000 cfu/100 mL, the strongest effects were generally observed when the total to fecal coliform ratio was 2. The risks decreased as the ratio increased. In addition, illnesses were more common on days when enteric viruses were found in the water. The percentage of survey days exceeding the critical bacterial counts were high, especially when closest to the storm drainage, as shown on Table 27. High densities of *E. coli*, fecal coliforms and Enterococcus were observed on more than 25% of the days, however, there was a significant amount of variability in observed counts in the water samples obtained directly in front of the drains. The variability and the frequency of high counts dropped considerably with distance from the storm drains. Up-coast bacteria densities were less than down-coast densities probably because of prevailing near-shore currents.

Table 27. Percentages of Days when Samples Exceeded Critical Levels (from SMBRP 1996)

Bacterial Indicator	0 yards	1 to 100 yards up-coast	1 to 100 yards down-coast	400+ yards up-coast
E. coli (>320cfu/100 mL)	25.0%	3.5%	6.7%	0.6%
Total coliforms (>10,000 cfu/100 mL)	8.6	0.4	0.9	0.0
Fecal coliforms (>400 cfu/100 mL)	29.7	3.0	8.6	0.9
Enterococcus (>106 cfu/100 mL)	28.7	6.0	9.6	1.3
Total/Fecal coliform ratio ≤5 (and total coliforms >1,000 cfu/100 mL)	12.0	0.5	3.9	0.4

The SMBRP (1996) concluded that less than 2 miles of Santa Monica Bay’s 50 mile coastline had problematic health concerns due to the storm drains flowing into the Bay. They also concluded that the bacterial indicators currently being monitored do help predict risk. In addition, the total to fecal coliform ratio was found to be a useful additional indicator of illness. As an outcome of this study, the Los Angeles County Department of Health Services planned to post new warning signs advising against swimming near the outfalls (“Warning! Storm drain water may cause illness. No swimming”). These signs will be posted on both sides of all flowing storm drains in Los Angeles County. In addition, county lifeguards will attempt to warn and advise swimmers to stay away from areas directly in front of storm drain outlets, especially in ponded areas. The county is also accelerating their studies on sources of pathogens in stormwater.

Development of Bathing Beach Bacteriological Criteria and Associated Epidemiological Studies

Current microbiological standards seldom have clear scientific basis and regulatory authorities cannot be confident that compliance with standards currently in force will ensure appropriate levels of public health protection. Human health standards for body contact recreation (and for fish and water consumption) are based on indicator organism monitoring. Dufour (1984a) presents an excellent overview of the history of US indicator bacterial standards and water contact recreation, summarized here. Total coliforms were initially used as indicators for monitoring outdoor bathing waters, based on a classification scheme presented by W.J. Scott in 1934. Total coliform bacteria refers to a number of bacteria including *Escherichia*, *Klebsiella*, *Citrobacter*, and *Enterobacter* (DHS 1997). They are able to grow at 35°C and ferment lactose. They are all gram negative asporogenous rods and have been associated with feces of warm blooded animals. They are also present in soil. Scott had proposed four classes of water, with total coliform upper limits of 50, 500, 1,000, and >1,000 MPN/100 mL for each class. He had developed this classification based on an extensive survey of the Connecticut shoreline where he found that about 93% of the samples contained less than 1,000 total coliforms per 100 mL. A sanitary survey classification also showed that only about 7% of the shoreline was designated as poor. He therefore concluded that total coliform counts of <1,000 MPN/100 mL probably indicated acceptable waters for swimming. This standard was based on the principle of attainment, where very little control or intervention would be required to meet this standard.

In 1943, the state of California independently adopted a total coliform standard of 10 MPN/1 mL (which is the same as 1,000 MPN/100 mL) for swimming areas. This California standard was not based on any evidence, but it was assumed to relate well with the drinking water standard at the time.

H.W. Streeter used an analytical approach to develop a standard for bathing water quality in 1951. He used an equation which included both *Salmonella* and total coliforms, the number of bathers exposed, the approximate volume of water ingested by bathers daily, and the average total coliform density. Streeter concluded that water containing <1,000 MPN total coliforms/100 mL would pose no great *Salmonella typhosa* health hazard.
Dufour points out that it is interesting that all three approaches in developing a swimming water criterion resulted in the same numeric limit.

One of the earliest bathing beach studies to measure actual human health risks associated with swimming in contaminated water was directed by Stevenson (1953), of the U.S. Public Health Service's Environmental Health Center, in Cincinnati, Ohio, and was conducted in the late 1940s. They studied swimming at Lake Michigan at Chicago (91 and 190 MPN/100 mL median total coliform densities), the Ohio River at Dayton, KY (2,700 MPN/100 mL), at Long Island Sound at New Rochelle and at Mamaroneck, NY (610 and 253 MPN/100 mL). They also studied a swimming pool in Dayton, KY. Two bathing areas were studied in each area, one with historically poorer water quality than the other. Individual home visits were made to participating families in each area to explain the research program and to review the calendar record form. Follow up visits were made to each participating household to insure completion of the forms. Total coliform densities were monitored at each bathing area during the study. More than 20,000 persons participate in the study in the three areas. Almost a million person-days of useable records were obtained. The percentage of the total person-days when swimming occurred ranged from about 5 to 10 percent. The number of illnesses of all types recorded per 1,000 person-days varied from 5.3 to 8.8. They found an appreciably higher illness incidence rate for the swimming group, compared to the nonswimming group, regardless of the bathing water quality (based on total coliform densities). However, a significant increase in gastrointestinal illness was observed among the swimmers who used one of the Chicago beaches on three days when the average coliform count was 2,300 MPN/100 mL. The second instance of positive correlation was observed in the Ohio River study where swimmers exposed to the median total coliform density of 2,700 MPN/100 mL had a significant increase in gastrointestinal illness, although the illness rate was relatively low. They suggested that the strictest bacterial quality requirements that existed then (<1,000 MPN/100 mL, based on Scott's 1934 work) might be relaxed without significant detrimental effect on the health of bathers.

It is interesting to note that in 1959, the Committee on Bathing Beach Contamination of the Public Health Laboratory Service of the UK concluded that "bathing in sewage-polluted seawater carries only a negligible risk to health, even on beaches that are aesthetically very unsatisfactory" (Cheung, *et al.* 1990 and Alexander, *et al.* 1992).

Dufour (1984a) pointed out that total coliforms were an integral element in establishing fecal coliform limits as an indicator for protecting swimming uses. Fecal coliform bacteria are a subgroup of the total coliform group. They grow at 44.5°C and also ferment lactose. They are restricted to the feces of warm blooded animals and can be used to separate bacteria of soil and animal origin (DHS 1997). They do survive for variable periods of time in fecal contaminated soil and water, however. As a result of the Stevenson (1953) study, reported above, a geometric mean fecal coliform level of 200 MPN per 100 mL was recommended by the National Technical Advisory Committee (NTAC) of the Federal Water Pollution Control Administration in 1968 and was adopted by the U.S. Environmental Protection Agency in 1976 as a criterion for direct water contact recreation (Cabelli, *et al*. 1979). This criterion was adopted by almost all states in the U.S. by 1984. It was felt that fecal coliform levels were more specific to sewage contamination and had less seasonal variation than total coliform levels. Since fecal coliform exposures at swimming beaches had never been linked to disease, the NTAC reviewed the USPHS studies, as published by Stevenson (1953). The 2,300 MPN/100 mL total coliform count association with gastrointestinal disease was used in conjunction with a measured ratio of fecal coliform to total coliform counts (18%) obtained at the Ohio River site studied earlier. It was therefore assumed that a health effect could be detected when the fecal coliform count was 400 MPN/100 mL (18% of 2,300 = 414). Dufour (1984a) pointed out that a detectable health effect was undesirable and that the NTAC therefore recommended a limit of 200 MPN/100 mL for fecal coliforms. Although likely coincidental, the 1968 proposed limit for fecal coliforms (200 MPN/100 mL) was very close to being theoretically equivalent to the total coliform limit of 1,000 MPN/100 mL that was being replaced (200/0.18 = 1100).

Dufour (1984a) lists the ideal characteristics of bacterial indicators of fecal contamination, as presented by various authors. The authors were in agreement concerning many of the criteria (correlation to pathogens, unable to grow in aquatic environments, more resistant to disinfection than pathogens, and easy to isolate and enumerate), but two important aspects were seldom mentioned, namely that the indicator should have a direct relationship to fecal contamination, and that the indicator density should correlate with health hazards. Many of the follow-up studies conducted since the mid 1970s examined these additional criteria. *E. coli*, a member of the fecal coliform group, has been recently used as a better indicator of fresh fecal contamination. Table 28 indicates the species and subspecies of the Streptococcus and Enterococcus groups of bacteria that are used as indicators of fecal contamination (DHS 1997).

Table 28. Streptococcus Species used as Indicators of Fecal Contamination

Indicator organism	Enterococcus group	Streptococcus group
Group D antigen		
Streptococcus faecalis	X	X
S. faecalis subsp. *liquifaciens*	X	X
S. faecalis subsp. *zymogenes*	X	X
S. faecium	X	X
S. bovis		X
S. equines		X
Group Q antigen		
S. avium		X

Source: DHS (1997)

Fecal streptococci bacteria are indicators of fecal contamination. The Enterococcus group is a subgroup that is considered a better indication of human fecal contamination. S. bovis and S. equinus are considered related to feces from non-human warm blooded animals (such as from meat processing facilities, dairy wastes, and feedlot and other agricultural runoff), indicating that Enterococcus may be a better indication of human feces contamination. However, *S. facealis* subsp. *liquifaciens* is also associated with vegetation, insects, and some soils (DHS 1997).

The Cabelli, *et al.* (1979) study was undertaken to address many remaining questions pertaining to bathing in contaminated waters. Their study examined conditions in New York (at Coney Island beach, designated as barely acceptable, and at Rockaway beach, designated as relatively unpolluted). About 8,000 people participated in the study, approximately evenly divided between swimmers and nonswimmers at the two beaches. Total and fecal coliforms, *Escherichia*, *Klebsiella*, *Citrobacter-Enterobacter*, Enterococci, *Pseudomonas aeruginosa*, and *Clostridium perfringens* were evaluated in water samples obtained from the beaches during the epidemiological study. The most striking findings were the increases in the rates of vomiting, diarrhea, and stomach ache among swimmers relative to nonswimmers at the barely acceptable beach, but not at the relatively unpolluted beach. Ear, eye, nose, and skin symptoms, as well as fever, were higher among swimmers compared to nonswimmers at both beaches. They concluded that measurable health effects do occur at swimming beaches that meet the existing health standards. Children, Hispanic Americans, and low-middle socioeconomic groups were identified as the most susceptible portions of the population.

Cabelli, *et al*. (1982) presented data from the complete EPA sponsored swimming beach study, conducted in New York, New Orleans, and Boston. The study was conducted to address issues from prior studies conducted in the 1950s (including Stevenson's 1953 study noted above) that were apparently contradictory. They observed a direct, linear relationship between highly credible gastrointestinal illness and Enterococci. The frequency of gastrointestinal symptoms also had a high degree of association with distance from known sources of municipal wastewater. Table 29 shows correlation coefficients for total gastrointestinal (GI) and highly credible gastrointestinal (HCGI) symptoms and mean indicator densities found at the New York beaches from 1970 to 1976. The best correlation coefficients were found for Enterococci. In contrast, the correlation coefficients for fecal coliforms (the basis for most federal and state guidelines) were poor. Very low levels of Enterococcus and *E. coli* in the water (about 10 MPN/100 mL) were associated with appreciable attack rates (about 10/10,000 persons).

Table 29. Correlation Coefficients between Gastrointestinal Symptoms and Bacterial Densities at New York City Beaches (Cabelli, *et al.* 1982)

Indicator	HCGI correlation coefficient	GI correlation coefficient	Number of observations
Enterococci	0.96	0.81	9
Escherichia coli	0.58	0.51	9
Klebsiella	0.61	0.47	11
Enterobacter-Citrobacter	0.64	0.54	13
Total coliforms	0.65	0.46	11
Clostridium perfringens	0.01	-0.36	8
Pseudomonas aeruginosa	0.59	0.35	11
Fecal coliforms	0.51	0.36	12
Aeromonas hydriphila	0.60	0.27	11
Vibrio parahemoylticus	0.42	0.05	7

Regressions of swimming associated gastrointestinal symptom rates (swimmer rates minus nonswimmer rates) against the mean Enterococcus and *E. coli* densities of the water samples clearly showed that the risk of gastrointestinal symptoms associated with swimming in marine waters contaminated with municipal wastewater is related to the quality of the water, as indicated by the Enterococcus density of the water. There was a strong case for causality between Enterococci and gastrointestinal symptoms, based on the good association, the consistency at the different locations over different years, the reasonable nature of the relationship between enteric disease and fecal contamination, and the coherent association based on observations of waterborne disease transmission during prior outbreaks.

Cabelli concluded that swimming in even marginally polluted marine bathing water is a significant route of transmission for observed gastrointestinal illness. The gastrointestinal illness was likely associated with the Norwalk-like virus that had been confirmed in 2,000 cases at a shellfish associated outbreak in Australia and at several outbreaks associated with contaminated drinking water.

Fleisher (1991) reevaluated this marine swimming beach data and concluded that the limitation for Enterococci promulgated by the EPA in 1986, based on the Cabelli, *et al.* (1982) study, (35 per 100 mL, geometric mean for 5 equally spaced samples over a 30-day period, for both fresh and saline water) was too severe, due to minor adjustments of the observed data. He was also especially concerned with the use of a single criterion based on pooled data, while the data from the individual sites indicated very different probabilities of gastroenteritis among swimmers at Boston compared to New York and Lake Pontchartrain (which were similar). He also reported that previous studies found bacteria indicator, and possibly pathogen, survival to be inversely correlated with salinity. He therefore concluded that any relation between Enterococci and disease causing pathogens may be site specific, possibly related to water salinity. This EPA Enterococci criterion for swimming waters was based on an "acceptable" rate of gastroenteritis of 19 cases per 1,000 swimmers, the same rate upon which the fecal coliform criterion (200 MPN/100 mL) was based. It is interesting to note that Fleisher later participated in additional epidemiological studies in the UK and concluded that 33 fecal streptococci (essentially Enterococci)/100 mL was the threshold of increased risk for gastrointestinal illness for swimmers (Kay, *et al.* 1994).

Dufour (1984a) also reviewed a series of studies conducted at freshwater swimming beaches from 1979 to 1982, at Tulsa, OK, and at Erie, PA. Only Enterococci, *E. coli*, and fecal coliforms were monitored, based on the results of the earlier studies. Table 30 shows the correlation coefficients for these three bacterial parameters and gastrointestinal disease.

Table 30. Correlation Coefficients for Bacterial Parameters and Gastrointestinal Disease (Fresh Water Swimming Beaches)

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

These results are quite different than the results from the marine studies, in that both Enterococci and *E. coli* had high correlation coefficients between the bacterial levels and the incidence of gastrointestinal illness. However, the result was the same for fecal coliforms, in that there was no association between fecal coliform levels and gastrointestinal illness. Dufour (1984b) concluded that Enterococci would be the indicator of choice for gastrointestinal illness, based on scientific dependability. *E. coli* could also be used, if only fresh waters were being evaluated. Fecal coliforms would be a poor choice for monitoring the safety of bathing waters. However, he concluded that numeric standards should be different for fresh and saline waters because of different dieoff rates for the bacteria and viruses for differing salinity conditions.

Other studies examined symptoms other than gastrointestinal illness associated with swimming in contaminated water, and identified additional potentially useful bacterial indicators. Seyfried, *et al*. (1985), for example, examined swimming beaches in Toronto for respiratory illness, skin rashes, plus eye and ear problems, in addition to gastrointestinal illness. They found that total staphylococci correlated best with swimming associated total illness, plus ear, eye and skin illness. However, fecal streptococci and fecal coliforms also correlated (but not as well) with swimming associated total illness. Ferley, *et al*. (1989) examined illnesses among swimmers during the summer of 1986 in the French Ardèche river basin, during a time when untreated domestic sewage was entering the river. They examined total coliforms, fecal coliforms, fecal streptococci and *Pseudomonas aeruginosa* and *Aeromonas Spp*, but only two samples per week were available for each swimming area. The total morbidity rate ratio for swimmers compared to nonswimmers was 2.1 (with a 95% confidence interval of 1.8 to 2.4), with gastrointestinal illness the major illness observed. They found that fecal streptococci (FS) was the best indicator of gastrointestinal illness. A critical FS value of 20 MPN/100 mL indicated significant differences between the swimmers and nonswimmers. Skin ailments were also more common for swimmers than for nonswimmers and were well correlated with the concentrations of fecal coliforms, *Aeromonas Spp* and *Pseudomonas aeruginosa*. They noted that a large fraction (about 60%) of the fecal coliforms corresponded to *E. coli*, and that their definition of fecal streptococci essentially was what North American researchers termed Enterococci.

Koenraad, *et al*. (1997) investigated the contamination of surface waters by *Campylobacter* and its associated human health risks. They reported that campylobacteriosis is one of the most frequently occurring acute gastroenteritis diseases in humans. Typical investigations have focused on the consumption of poultry, raw milk, and untreated water as the major sources of this bacterial illness. Koenraad, *et al*. (1997) found that human exposures to *Campylobacter* contaminated surface waters is likely a more important risk factor than previously considered. In fact, they felt that *Campylobacter* infections may be more common than *Salmonella* infections. The incidence of campylobacteriosis due to exposure to contaminated recreational waters has been estimated to be between 1.2 to 170 per 100,000 individuals. The natural habitat of *Campylobacter* is the intestinal tract of warm-blooded animals (including poultry, pigs, cattle, gulls, geese, pigeons, magpies, rodents, shellfish, and even flies). It does not seem to multiply outside of its host, but it can survive fairly well in aquatic environments. It can remain culturable and infective for more than 2 months under ideal environmental conditions. Besides runoff, treated wastewater effluent is also a major likely source of *Campylobacter* in surface waters. Sanitary wastewater may contain up to 50,000 MPN of *Campylobacter* per 100 mL, with 90 to 99% reductions occurring during typical wastewater treatment.

Many of the available epidemiological studies have been confined to healthy adult swimmers, in relatively uncontaminated waters. However, it is assumed that those most at risk would be children, the elderly, and those chronically ill, especially in waters known to be degraded. Obviously, children are the most likely of this most-at-risk group to play in, or by, water. Alexander, *et al.* (1992) therefore specifically examined the risk of illness associated with swimming in contaminated sea water for children, aged 6 to 11 years old. This study was based on parental interviews for 703 child participants during the summer of 1990 at Blackpool beach, UK. Overall, 80% of the samples at the Blackpool Tower site and 93% of the samples at the South Pier site failed to meet the European Community Standards for recreational waters. All of the 11 designated beaches in Lancashire (including Blackpool beach), in the northwest region of England, continually fail the European directive imperative standards for recreational waters. During this study, statistically significant increases in disease were found for children who had water contact, compared to those who did not. Table 31 shows the prevalence and rate ratios for these symptoms. Diarrhea and loss of appetite had strong associations with the water contact group, while vomiting and itchy skin had moderate associations. No other variables examined (household income, sex of the child, sex of the respondent, general health, chronic or recurring illness in the child, age of the child, foods eaten, including ice cream, other dairy products, chicken, hamburgers, shellfish, or ice cubes, acute symptoms in other household members, presence of children under 5 in the household, and other swimming activities) could account for the significant increases in the reported symptoms for the children who had water contact.

Table 31. Illness Symptoms for Children Exposed to Sewage Contaminated Sea Water (Alexander, *et al.* 1992)

	Prevalence for water contact group, n=455 (%)	Prevalence for non-water contact group, n=248 (%)	Rate Ratio	Strength of Association
Vomiting	4.2	1.6	2.6	Moderate
Diarrhea	7.9	2.4	3.3	Strong
Itchy skin	5.1	2.8	1.8	Moderate
Loss of appetite	4.0	1.2	3.3	Strong

Other risk factors, in addition to exposure to sewage contaminated swimming waters, were investigated by Fleisher, *et al.* (1993). People visiting beaches for recreation are frequently exposed to additional risks for gastroenteritis disease, especially related to foods that are eaten. Picnic lunches and food purchased at swimming beaches may contain improperly prepared or inadequately stored foods, including food that may be especially risky such as eating sandwiches having mayonnaise, chicken, eggs, hamburgers, and hot dogs. They found that non-water related risk factors confounded the relationships between gastroenteritis and fecal streptococci densities. They also found that fecal coliform and fecal streptococci densities changed rapidly in time and location at swimming beaches, requiring many more water sample evaluations than are typically obtained during most epidemiological studies.

1986 U.S. EPA Guidance for Recreational Waters, Water Supplies, and Fish Consumption

A recreational water quality criterion can be defined as a “quantifiable relationship between the density of an indicator in the water and the potential human health risks involved in the water's recreational use.” From such a definition, a criterion can be adopted which establishes upper limits for densities of indicator bacteria in waters that are associated with acceptable health risks for swimmers.

The U.S. Environmental Protection Agency, in 1972, initiated a series of studies at marine and fresh water bathing beaches which were designed to determine if swimming in sewage-contaminated marine and fresh water carries a health risk for bathers; and, if so, to what type of illness. Additionally, the EPA wanted to determine which bacterial indicator is best correlated to swimming-associated health effects and if the relationship is strong enough to provide a criterion (EPA 1986).

Many of the above described U.S. studies were conducted as part of these EPA sponsored research activities. The quantitative relationships between the rates of swimming-associated health effects and bacterial indicator densities were determined using standard statistical procedures. The data for each summer season were analyzed by comparing the bacteria indicator density for a summer bathing season at each beach with the corresponding swimming-associated gastrointestinal illness rate for the same summer. The swimming-associated illness rate was determined by subtracting the gastrointestinal illness rate in non-swimmers from that for swimmers.

The EPA's evaluation of the bacteriological data indicated that using the fecal coliform indicator group at the maximum geometric mean of 200 organisms per 100 mL, as recommended in *Quality Criteria for Water* would cause an estimated 8 illness per 1,000 swimmers at freshwater beaches.

Additional criteria, using *E. coli* and Enterococci bacteria analyses, were developed using these currently accepted illness rates. These bacteria are assumed to be more specifically related to poorly treated human sewage than the fecal coliform bacteria indicator. The freshwater equations developed by Dufour (1984b) were used to calculate new indicator densities corresponding to the accepted gastrointestinal illness rates.

It should be noted that these indicators only relate to gastrointestinal illness, and not other problems associated with waters contaminated with other bacterial or viral pathogens. It is likely that common swimming beach problems associated with contamination by stormwater could also include skin and ear infections caused by the large concentrations of *Pseudomonas aeruginosa* and *Shigella* found in stormwater (Pitt 1983).

U.S. bacteria criteria have been established for contact with bacteria and are shown in Table 32. State standards usually also exist for fecal coliform bacteria.

Water Environment & Technology (1997) reported the new EPA BEACH (Beaches Environmental Assessment, Closure, and Health) program to help states strengthen recreational water quality monitoring programs. During the summer of 1995, state and local governments reported closing or issuing warnings for 4,000 beaches because of suspected dangerous conditions associated from wastewater and stormwater contamination of swimming areas. A new testing method for *Escherichia coli* and Enterococci bacteria was introduced that gives results in 1 day instead of the typical 2 days testing period. They also reported that these bacteria better correlate with human health risks. The EPA will survey state and local health and environmental directors about the quality of freshwater and marine recreational areas and post the results on a new Beach Watch Web site.

Table 32. U.S. EPA Water Quality Criteria for Swimming Waters

	Marine Waters	Fresh Waters
Main EPA research reference	Cabelli, et al. 1982	Dufour 1984b
Acceptable swimming associated gastroenteritis rate (per 1,000 swimmers)	Increase of 19 illnesses per 1,000 swimmers	Increase of 8 illnesses per 1,000 swimmers
Comparable fecal coliform exposure	200 fecal coliforms/100 mL	200 fecal coliforms/100 mL
Steady state geometric mean indicator density	35 Enterococci/100 mL	33 Enterococci/100 mL, or 126 *E. coli*/100 mL
Single sample limits:		
Designated bathing beach area	104 Enterococci/100 mL	61 Enterococci/100 mL, or 235 *E. coli*/100 mL
Moderate full body contact recreation	124 Enterococci/100 mL	89 Enterococci/100 mL, or 298 *E. coli*/100 mL
Lightly used full body contact recreation	276 Enterococci/100 mL	108 Enterococci/100 mL, or 406 *E. coli*/100 mL
Infrequently used full body contact recreation	500 Enterococci/100 mL	151 Enterococci/100 mL , or 576 *E. coli*/100 mL
EPA 1986		

New California Recreational Area Bacteria Standards

California Assembly Bill AB-411 was implemented in July 1999 for southern California areas. The regulations specifically only apply to beaches having over 50,000 annual visitors that receive runoff from a storm drain or natural creek and apply from April 1 to October 31 of each year. However, most of the local agencies have implemented the regulations at all beaches. These criteria are heavily based on the Santa Monica Bay study described above and recognize the danger that urban runoff presents. They recommend that recreational use of waters within stormwater drains (including manmade conveyances and also natural drains such as creeks and streams), in ponds or pools that form because of stormwater drainage, and in the immediate surf zone into which stormwater drains, should be prohibited at all times. The criteria documents state, in part, that:

> “a protocol should be developed that sets forth procedures for closing recreational waters and beach areas whenever significant amounts of rainfall results in urban runoff that enters recreational waters and beach areas. Ocean beaches that are subject to urban runoff should be closed for a minimum of 72 hours following significant rain to allow wave action to dissipate microbiological contamination, unless sampling and analysis indicates that earlier reopening is appropriate, or local health agencies have ample data and experience with the location to determine appropriate actions. Bays or other ocean water areas with poor water circulation may require a longer time to recover. (DHS 1997)”

Similar wording was also provided relating to swimming in freshwaters contaminated by urban runoff. Indicator organisms should include total and fecal coliform bacteria, at a minimum. Enterococci can also be added as an indicator. They felt that monitoring for specific pathogens (such as *Giardia* or *Cryptosporidium*) is costly and doesn’t appear to be reliable. They could be monitored if done in conjunction with the other required monitoring efforts, especially in response to specific needs.

Reopening of a closed recreational area is appropriate when two successive samples taken at least 24 hours apart are below the closure levels. If a swimming area is closed due to contamination by urban stormwater runoff, the following wording for warning signs is suggested: “Warning! Closed to swimming. Beach/swimming area is contaminated by stormwater runoff/sewage and may cause illness.” In areas that are chronically contaminated by stormwater, the following wording for permanent signs is suggested: “Warning! Storm drain water may cause illness. No swimming in storm drain water.”

WHO Guidelines for Recreational Use of Water

The World Health Organization (WHO) has been concerned with the health aspects associated with the recreational use of water for many years. In 1994, with the urging of the WHO Regional office for Europe, WHO developed *Guidelines for Safe Recreational-Water Environments*. A joint WHO and USEPA meeting was held in 1998 and the *Annapolis Protocol* (WHO 1999) was developed. The Annapolis Protocol report contains an excellent summary of health aspects of recreational waters, including standards from throughout the world and guidance for assessing the health risks for numerous discharge conditions. Separate storm drains are described as having low significance to public health, although of increasing importance if contaminated with sanitary wastewater.

Tables 33 and 34, summarized from the *Annapolis Protocol*, show example categories for recreational waters and selected bacteria standards from various counties for primary contact recreation. The *Annapolis Protocol* (WHO 1999) also specifically outlines bathing beach monitoring strategies. WHO also developed a code of good practice for monitoring recreational waters recently.

Table 33. Examples of Categories of Microbial Indicator Levels by Water Source

Water Source	Indicator(s)	Category	95th Percentile (number/100 mL)
Temperate freshwater	Fecal Streps Enterococci(1)	A	<10
		B	11-50
		C	51-200
		D	201-1000
		E	>1000
	E. coli	A	<35
		B	36-130
		C	131-500
		D	501-1000
		E	>1000
Alternative for tropical marine water and optional for tropical marine freshwater	Sulfite reducing Clostridia *Clostridium perfringens*	A	<1
		B	1-10
		C	11-50
		D	51-80
		E	>80

(1) these are the same categories and percentile values for temperate marine water

Source: from WHO 1999

Drinking Water Risks and Urban Stormwater

The National Research Council conducted an intensive review of the use of waters of impaired quality for groundwater recharge (Andelman, *et al.* 1994). Included in this book was a review of the use of stormwater to recharge groundwater for eventual use as a drinking water supply. Other potential source waters investigated for recharge included treated municipal wastewater and irrigation return flows. The following is a summary from that book, describing these potential human health risks associated with consuming stormwater.

Various chemical and bacteriological health risks associated with stormwater were examined. The major risks were identified as originating from pathogenic organisms, disinfection byproducts for water that have undergone disinfection to reduce the threat from the pathogens, synthetic organic chemicals, and inorganic chemicals. Assessments are therefore needed to identify the potential risks associated with this reuse. These assessments contain four major components: hazard identification, dose-response assessment, exposure assessment, and risk characterization. The NRC committee reviewed available epidemiological studies that had investigated the use of degraded waters for recharge and as eventual drinking water supplies.

Table 34, summarized from the NRC report, lists the health effects of known chemicals found in urban stormwater. The health effects shown are not meant to be comprehensive, but are the problems that the drinking water standards are intended to protect against. The EPA carcinogen classifications are as follows:

A = sufficient evidence for humans
B1 = limited evidence for humans and sufficient evidence in experimental animals
B2 = inadequate/limited evidence for humans, sufficient evidence in experimental animals
C = limited evidence in experimental animals with no human data
D = inadequate or no data
E = sufficient evidence for noncarcinogenicity

The concentrations presented are summarized from the EPA's Nationwide Urban Runoff Program (NURP) (EPA 1983) and show the percentage of samples where the toxicant was detected and the range of the detected values. The maximum contaminant level (MCL) is the drinking water standard established by the EPA. Also shown (in parentheses) is the concentration associated with a cancer risk of 1 in a million, the generally recognized negligible risk level. The present background cancer occurrence rate in the U.S. is 25%. This 10^{-6} risk level, associated with a lifetime exposure to a chemical, will increase the risk of getting cancer from 250,000 in 1 million to 250,001 in 1 million (Andelman, *et al.* 1994). The reference dose is the estimated daily dose that is likely to be without an appreciable risk of deleterious effects during a lifetime (expressed as mg of ingested chemical per day per kg of body weight).

Most of the listed toxicants exceed the MCL limits and the negligible risk levels (highlighted in bold). However, most of the toxicants are associated with particulates and the MCL values are not directly applicable. In addition, drinking of undiluted, untreated stormwater is not likely.

Microorganisms of concern in drinking waters may include many different types of pathogens, including bacteria, viruses, and parasites. These are excreted from infected hosts and enter sanitary sewage. Stormwater and urban receiving waters can become contaminated with these pathogens, as noted earlier. Andelman, *et al.* (1994) reviewed waterborne disease outbreaks in the U.S. from 1971 through 1990. The most common identified causative agents were *Giardia*, chemical poisoning, and *Shigella* species. During this period, the causative agents in more than 50% of the outbreaks were not able to be identified. However, reviews of past outbreaks found that the Norwalk virus (causing acute nonbacterial gastroenteritis) was the likely cause of about 40% of the outbreaks from 1976 through 1980 that had no prior identified cause. The difficulty or inability to identify many of the viruses and parasites (such as *Cryptosporidium*) is the likely reason why they are not listed as a more common cause of illness from drinking contaminated water.

Dose-response information is usually determined by exposing volunteers to different doses of the microorganisms of interest. Normally, this data does not include special problems for special at-risk individuals. Table 35 (as reported in the NRC committee report) shows infective dose information for several pathogens. Table 36 shows the probability of infection of ingestion of 100 mL of water for various levels of contamination. The levels of these microorganisms in stormwater can be much greater than the values shown on this table (enterviruses of 100 to 3000 pfu/100 L, for example was reported by Olivieri, *et al.* 1977). Of course, ingestion of untreated or undiluted stormwater is rare.

Table 34. Health Effects of Toxicants Found in Stormwater (Andelman, *et al.* 1994 and EPA 1983)

Chemical	Health Effects: Human	Health Effects: Animal/In Vitro	EPA Carcinogen classification	Reported frequency of detection (%) and observed concentrations (μg/L) (EPA 1983, NURP)		Max. contaminant level (MCL) μg/L (10^{-6} cancer risk)	Reference dose (mg/kg/day)
Pesticides:							
Lindane		Morphological changes of kidney and liver cells	C	15	0.007 – 0.1	0.2	0.0003
Chlordane		Liver hypertrophy (regional)	B2	17	0.01 – **10**	0.2 (0.03)	0.00006
Polyaromatic hydrocarbons:							
Fluoranthene		Nephrapathy; increased liver weight; hematologic alterations; clinical effects (increased SGPT levels)		16	0.3 – 21	-	0.04
Other organics:							
Pentachlorphenol		Liver and kidney pathology, feto-maternal toxicity	B2	19	**1** – **115**	1 (0.3)	0.03
Inorganics:							
Antimony	Gastrointestinal effects	Liver and kidney effects	D	13	2.6 – **23**	6	0.0004
Arsenic	Skin (hyperpigmentation, keratosis); vascular complications; neurotoxicity; liver injury	Reproductive/developmental effects; chromosomal effects	A	52	**1** – **51**	50 (0.000002)	0.0003
Beryllium	Contact dermatitis; pulmonary effects	Skeletal effects; genotoxicity	B2	12	**1 – 49**	4 (0.008)	0.005
Cadmium	Pulmonary and renal tubular effects; skeletal changes associated with effects on calcium metabolism	Reproductive/teratogenic effects; effects on myocardium	D	48	0.1 – **14**	5	0.0005
Chromium	Renal tubular necrosis	Genotoxicity	D	58	1 – **190**	100	0.005
Cyanide	Nausea, confusion, convulsion, paralysis, coma, cardiac arrhythmia, respiratory stimulation followed by respiratory failure		D	23	2 – **300**	200	0.022
Mercury	Nervous system effects; kidney effects	Genotoxicity	D	10	0.6 – 1.2	2	0.0003
Nickel	Contact dermatitis	Reproductive effects; genotoxicity	D	43	1 – **182**	100	0.005
Selenium	Nail changes; hair loss; skin lesions; nervous system effects	Reproductive effects, genotoxicity		11	2 – **77**	50	0.005
Zinc	Gastrointestinal distress; diarrhea	Poor growth	D	94	10 - 2400	-	0.3

Table 35. Values Used to Calculate Risks of Infection, Illness, and Mortality from Selected Enteric Microorganisms (Andelman, *et al.* 1994).

	Probability of infection from exposure to one organism (per one million)	Ratio of clinical illness to infection (%)	Mortality rate (%)	Secondary spread (%)
Campylobacter	7,000			
Salmonella typhi	380			
Shigella	1,000			
Vibrio cholerae	7			
Coxsackieviruses		5 – 96	0.12 – 0.94	76
Echoviruses	17,000	50	0.27 – 0.29	40
Hepatitis A virus		75	0.6	78
Norwalk virus			0.0001	30
Poliovirus 1	14,900	0.1 – 1	0.9	90
Poliovirus 3	31,000			
Rotavirus	310,000	28 – 60	0.01 – 0.12	
Giardia lamblia	19,800			

Table 36. Probability of Infection from Ingestion of 100 mL of Water Contaminated with Viruses or Protozoa

Levels in ingested water (per 100 L)	Exposure per 100 mL	Estimated risk of infection in exposed population
Rotavirus		
0.01 pfu	1.0×10^{-5}	6.2×10^{-6}
0.13 pfu	1.3×10^{-4}	6.0×10^{-5}
Echovirus		
0.01 pfu	1.0×10^{-5}	2.0×10^{-8}
0.13 pfu	1.3×10^{-4}	2.7×10^{-7}
Giardia		
0.49 cysts	4.9×10^{-4}	9.8×10^{-6}
0.89 cysts	8.9×10^{-4}	1.88×10^{-5}
1.67 cysts	1.77×10^{-3}	3.3×10^{-5}
3.3 cysts	3.3×10^{-3}	6.6×10^{-5}
Cryptosporidium		
0.75 oocysts	7.5×10^{-4}	1.5×10^{-5}
5.35 oocysts	5.35×10^{-3}	1.1×10^{-4}

Craun, *et al.* (1997) conducted evaluations of waterborne disease outbreaks from public water supplies and found that coliform bacteria monitoring is likely adequate to protect against bacterial and viral illness, but not for protozoa associated illness. Coliform bacteria monitoring has been used for many years to assess the microbiological quality of drinking waters. Except for a few strains, coliforms are not considered pathogenic. They are not very specific to fecal contamination, as most species of coliforms are free-living in the environment. Tap water having no coliforms has generally been thought to be free of agents likely to cause waterborne disease. However, Craun, *et al*. (1997) found that disease outbreaks (especially associated with *Giardia* or *Cryptosporidium*) have occurred in water systems that have not violated the maximum contaminant levels for total coliforms. The 1989 Coliform Rule for drinking waters states that systems collecting fewer than 40 samples per month may have no more than one total coliform positive sample (per 100 mL of water) per month, systems collecting more samples must have fewer than 5% of their samples positive for total coliforms. When Craun, *et al.* (1997) reviewed information from reported waterborne disease outbreaks from 1983 to 1992, they found that coliforms were detected during most of the outbreaks that were caused by bacteria, viruses, and unidentified agents, but they were found only during few of the outbreaks caused by protozoa. As an example, the 1993 Milwaukee *Cryptosporidium* outbreak (the largest documented waterborne disease outbreak in the U.S., with 400,000 cases of illness reported) occurred even though the MCL for coliforms was not violated. It is known that total coliforms are more susceptible to disinfection during water treatment than some protozoa. They concluded that "microbiological monitoring alone (for total coliforms and other indicator organisms for pathogens) cannot safeguard the public against waterborne disease. Emphasis must also be given to source water protection (watershed control programs, better control of wastewater discharges, and wellhead protection programs) and adequate water treatment and operation. The 1989 coliform rule with its more stringent requirements (periodic sanitary surveys, procedures for *E. coli* testing, and extra samples to evaluate water quality after positive total coliform results) and other USEPA regulations (e.g. the Surface Water Treatment Rule, and the pending Enhanced Surface Water Treatment Rule) are all important for reducing the risks of waterborne disease."

Summary of Wet Weather Flow Pathogen Issues

The bacterial quality of urban receiving waters is usually of great interest because of the very high levels of indicator microorganisms that occasionally are detected, and the elevated levels that are commonly detected. There is little evidence to support a workable relationship between indicator organisms and pathogens in stormwater, however, there is now limited epidemiological evidence that has associated swimming in stormwater contaminated receiving waters with increased disease. There is also increasing evidence of elevated levels of human pathogens in stormwaters, possibly from sanitary sewage contamination. Therefore, even though the traditional indicators of pathogenic contamination are not likely valid for stormwaters, pathogens may still be commonly present in stormwaters and urban receiving waters, at least associated with inadvertent contamination.

The history of the development of receiving water use guidelines and standards for contact recreation, as briefly outlined in this module, indicates how indicators and pathogens at levels commonly found in urban waters may increase disease. Again, this is becoming apparent through epidemiological studies that have examined waters contaminated with separate stormwaters and with no obvious sanitary sewage sources. It has become increasingly possible to directly monitor microorganisms that are thought to be more specifically related to fecal contamination, and to directly measure pathogens in receiving waters. It is therefore important that additional data be collected and that sources of pathogens, along with their fates, be identified in urban waters so that accurate risk assessments and control strategies can be developed. This is especially critical as children are the ones most likely to be exposed to these contaminated waters during casual play activities.

The main objective of the research to identify possible sources of *E. coli* and enterococci bacteria in dry and wet weather flows found that none of the sheetflow samples could possibly be contaminated with sanitary sewage. Even then, *E. coli* and enterococci levels higher than 2,400 and 24,000 MPN/100 mL, respectively, were observed, although the maximum values varied from site to site. The presence of high levels of bacteria in wet weather samples (both sheetflows and at outfalls) show that apart from sewage, there exists other potential sources that contribute to elevated levels. Since both the indicator organisms studied (*E. coli* and enterococci) are not of soil origin and are found in intestines of warm-blooded animals, urban birds and other animals can be considered significant sources of bacteria in stormwater.

Comparisons of samples collected from areas prone to urban animal use and those that are not, show that large overlaps exist between the bacterial concentrations found from both types of areas. Bacterial levels from roofs prone to urban animal use (squirrels and birds) were significantly higher than from roofs not exposed to such use. The other source areas did not show any significant differences between areas prone and not prone to urban animal use, except for some street areas. This could be the result of persistence of bacteria in soil, or the ubiquitous nature of these bacteria in urban areas due to movement of small animals. Dry-weather outfall samples showing *E. coli* and enterococci levels higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL respectively, are likely contaminated by sanitary sewage. Levels lower are most likely caused by other sources, such as irrigation runoff, carwash water, laundry water, etc.

Table 37. Microbiological Quality of Water, Guidelines and Standards from Several Countries for Primary Contact Recreation (number/100 mL)

	Total Coliforms	Fecal Coliforms	Other	References
Brazil	80% <500m	80% <1000m		Brazil Ministerio del Interior 1976
Colombia	1000	200		Colombia, Ministerio de Salud 1979
Cuba	1000[a]	200[a] 90% <400		Cuba, Ministerio de Salud 1986
EEC[b], Europe	80% <500[c] 95% <10,000[d]	80% <100[c] 95% <2000d	Fecal strep. 100[c] Salmonella 0/L[d] Enteroviruses 0 PFU/L[d] Enterococci 90% <100	EEC 1976 CEPPOL 1991
Ecuador	1000	200		Ecuador, Ministerio de Salud Publica 1987
France	<2000	<500	Fecal strep. <100	WHO 1977
Japan	1000			Japan, Environmental Agency 1981
Mexico	80% <1000[f] 100% < 10,000[k]			Mexico, SEDUE 1983
Peru	80% <5000f	80% <1000[f]		Peru, Ministerio de Salud 1983
Poland			*E. coli* <1000	WHO 1975
Puerto Rico		200[h] 80% <400		Puerto Rico, JCA 1983
United States, California	80% <1000[ij] 100% <10,000[k]	200[aj] 90% <400[l]		California State Water Resources Board
United States, EPA			Enterococci 35 (marine) 33 (fresh) *E. coli* 126 (fresh)	EPA 1986 Dufour and Ballentine 1986
Former USSR			E. coli <100	WHO 1977
UNEP/WHO		50% <100[n] 90% <1000[n]		WHO/UNEP 1978
Uruguay		<500[n] <1000[o]		Uruguay, DINAMA 1998
Venezuela	90% <1000 100% <5000	90% <200 100% <400		Venezuela 1978

Notes:
a. logarithmic average for a period of 30 days of at least 5 samples
b. minimum sampling frequency: every two weeks
c. guide
d. mandatory
e. monthly average
f. at least 5 sampler per month
g. minimum of 10 sampler per month
h. at least 5 samples taken sequentially from the waters in a given instance
i. period of 30 days
j. within a zone bounded by the shoreline and a distance of 1000 ft from the shoreline or the 30 ft depth contour, whichever is further from the shoreline
k. not a sample taken during the verification period of 48 hrs should exceed 10,000/100 mL
l. period of 60 days
m. “satisfactory” waters, samples obtained in each of the preceding 5 weeks
n. geometric mean of at least 5 samples
o. not to be exceeded n at least 5 samples

Source: from WHO 1999, adapted from Salas 1998

Control of Microorganisms in Urban Waters

A natural outcome of discussions after examining microorganism levels in urban waters focuses on their potential control. Unfortunately, there does not appear to be an easy (inexpensive) solution to reduce the often-times very high indicator bacteria levels found in stormwater. Table 38 is a summary of potential bacteria controls, prepared by Pitt in 1983, for the Rideau River area of Ottawa, based on extensive local testing and analyses. Although an old evaluation, it is likely still reasonably valid. It is hoped that the most basic control program would incorporate the required inappropriate discharge detection and elimination program (as described by CWP and Pitt 2004) included in the NPDES stormwater permit program, and dog feces controls. These can be highly effective and of low to moderate (or higher) cost. It is hoped that after any sewage contamination is identified and corrected, the remaining indicator bacteria, although possibly still be quite high in comparison to the current criteria, would indicate minimal risks, as they should mostly originate from urban wildlife. Dog feces control programs are a basic public health and aesthetic benefit and should also be implemented (including enforcement). In order to reduce the bacteria levels to criteria levels, much more costly control programs will be needed. These should only be implemented after a local risk-assessment is conducted and actual human health impairments are identified.

Table 38. Possible Bacteria Controls Applicable to Rideau River Urban Area, Ottawa (Pitt 1983)

	Control Effectiveness	Costs
Litter control	Low	Low/moderate
Bird control on river bridges	Moderate (to 50%)	Low/moderate
Catchbasin cleaning	Low (<10%)	Moderate/high
Street cleaning	Low/moderate (to 20%)	Very high
Dog feces control programs	Moderate (to 35%)	Very low
Inappropriate discharge detection and elimination program	High (if present)	Moderate/high
Runoff treatment and disinfection	Can be very high (>99%)	Very high

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Appendix A: Case Study: Investigation of Urban Runoff Microorganism Sources in Ottawa, Ontario

The City of Ottawa, Ontario, sponsored several studies in the early 1980s investigating the sources of the high bacteria concentrations found in the Rideau River, and possible control procedures. The following discussion (from Pitt 1983) summarizes their findings, especially relating to the relative magnitude of urban bacteria sources.

Table A1 summarizes the bacteria concentrations observed for the different samples collected in the Ottawa urban area. Except for rooftop runoff, the catchment subarea sheetflow concentrations all approach the concentrations of the urban runoff. The urban runoff bacteria concentrations are slightly greater than the river concentrations below Mooney's Bay. The catchment area sheetflow fecal strep. concentrations, again except for rooftop runoff, are all substantially greater than the river concentrations.

Table A1. Typical Bacterial Population Densities in the Ottawa Area (Pitt 1983)

	Total Coliforms	Fecal Coliforms	Fecal Strep.
Water Samples (organisms/100 mL)			
Rideau River			
Below Mooney’s Bay	7,000	1,000	500
Above Mooney’s Bay	500	50	50
Urban runoff	na	10,000	na
Snowmelt	3,000	<2	2
Catchbasin sump water	na	50	300
Gutter flows	na	4,000	20,000
Parking area sheetflow	na	3,000	10,000
Vacant land and park sheetflow	na	6,000	20,000
Rooftop runoff	na	100	200
Sediment Samples (organisms/gram solids)			
Rideau river sediments (urban area)	na	20,000	na
Sewerage sediments	na	8,000	20,000
Catchbasin sump water	400	20	100
Street dirt	na	400	2,000

Estimated Unit Area Bacteria Yields

Five to eleven storms were completely monitored for fecal coliform concentrations at four of the test catchments from 1978 to 1981. Table A2 summarizes these observations for the 34 monitored storms. The resultant calculated catchment bacteria runoff yields expressed in millions of organisms per hectare per day are shown in Table A3. Approximately 1.5 x 10^8 fecal coliforms per hectare per year and about 3.7 x 10^8 fecal strep. organisms per hectare per year are the estimated bacteria yields for the Ottawa six month runoff season.

Table A2. Catchment Runoff Fecal Coliform Bacteria Observations in Ottawa area (Pitt 1983)

	Alta Vista	Chesterton	Leonard	St. Lauraent	Overall
Geometric mean* (#/100 mL)	14,100	12,300	21,700	4,580	10,200
Min. (#/100 mL)	5,900	720	11,500	540	540
Max. (#/100 mL)	38,000	96,600	64,100	31,400	96,600
Number of storms monitored	11	7	5	11	34
Study period	1980 and 1981	1978, 1979, and 1981	1980 and 1981	1980 and 1981	1978 through 1981

* geometric mean of flow-weighted averaged concentrations for monitored storms

Table A3. Estimated Ottawa Catchment Bacteria Runoff Yields (10^6 organisms/ha/day) (Pitt 1983)

Catchment	Fecal Coliforms Mean (range)	Fecal Strep. Mean (range)
Alta Vista	0.5 (0.3 to 1.1)	1.3 (0.8 to 3)
Chestron	0.6 (0.4 to 1.5)	1.5 (1 to 4)
Leonard	1.4 (0.7 to 3)	3.5 (2 to 8)
St. Laurent	0.6 (0.3 to 1.4)	1.5 (0.8 to 4)
Average	0.8×10^6 FC org/ha/day	2×10^6 FS org/ha/day

In order to determine the importance of each of the catchment subareas in contributing urban runoff pollutants, a small sampling effort was conducted to collect sheetflow samples during two rain events. Table A4 summarizes the results of these analyses. The rooftop bacteria samples had substantially lower fecal coliform and fecal strep. bacteria concentrations than samples collected from vacant land and park sheetflows, parking lot sheetflows and street gutter flows. The rooftop samples, however, did have important bacteria concentrations, especially when compared to Rideau River bacteria concentrations above Mooney's Bay.

Table A4. Catchment Subarea Sheetflow Bacteria in Ottawa (August and September, 1981, observations) (Pitt 1983)

		Rooftop runoff	Vacant land and park sheetflow	Parking lot sheetflow	Gutter flow
Fecal coliforms	Geometric mean (#/100 mL)	85	5,600	2,900	3,500
	Min (#/100 mL)	10	360	200	500
	Max (#/100 mL)	400	79,000	19,000	10,000
	Number of observations	4	7	6	7
Fecal Strep.	Geometric mean (#/100 mL)	170	16,500	11,900	22,600
	Min (#/100 mL)	20	12,000	1,600	1,800
	Max (#/100 mL)	3,600	57,000	40,000	1,200,000
	Number of observations	4	7	6	7

The urban runoff fecal coliform unit area yield is more than a factor of ten greater than the snowmelt yield, and about a factor of ten greater than the sewerage and catchbasin sump yields. Therefore, snowmelt and sewerage accumulations probably do not appreciably affect the total annual yields, but they may significantly affect individual snowmelt and storm event concentrations and yields. The street surface particulate fecal coliform and fecal strep. accumulations are as much as one to two orders of magnitude greater than the total urban runoff bacteria discharges. Bacteria urban runoff yields do not appear to be source-limited in that substantial quantities of bacteria reside on the street surfaces that are not washed off by rain. A large quantity of bacteria is associated with particulates that are trapped in the street textures and may be subject to significant dieoff during periods of dry weather. The many other sources of bacteria in the urban area would further increase this overabundance of bacteria sources for urban runoff.

These observed subarea bacteria concentrations were much greater than those observed in a similar sampling program in San Jose, California, (Pitt and Bozeman 1982). In San Jose, the observed fecal coliform gutter and parking lot sheetflow sample concentrations were much greater than elsewhere in the San Jose study areas, and were from several hundred to about 1000 organisms/100mL. Rooftop runoff and landscaped area runoff fecal coliform concentrations were less than ten and less than 50 organisms/100 mL., respectively. The San Jose sheetflow fecal strep. concentrations were closer to the observed Ottawa concentrations. An earlier Ottawa study reported by the Regional Munciipality of Ottawa - Careleton (1972) measured rooftop runoff bacteria concentrations. The runoff from a roof at an experimental farm that was frequented by many birds had coliform concentrations greater than 10,000 organisms/100 mL. Street surface and parking lot runoff showed total coliform concentrations in the hundreds of thousand of organisms/100 mL.

Table A5 shows the resultant percentage contributions for fecal coliforms from each of the catchment subareas. These values are calculated from the observed sheetflow pollutant concentrations and from the calculated urban runoff flow contributions from each subarea. Fecal strep. and fecal coliforms have similar source area contributions for these study areas. The differences in bacteria yields from street surfaces when comparing large rains with small rains is very large. The bacteria yields from the street surfaces decrease much more as the rains increase in depth because of the high bacteria concentrations observed in non-street surface sheetflows. Even if all of the bacteria was removed from the streets, a maximum reduction of about 60 to 70 percent in outfall bacteria yields would be achieved, and only for the runoff from residential areas and for the smallest rains. For the largest rains, and if all of the fecal coliform bacteria was removed from the streets, only about 10 to 25 percent bacteria reductions would be observed at the outfall. If sidewalks and driveways were cleaned, a greater fraction of the bacteria could be controlled. If the shopping center parking lots, along with the streets, were cleaned, then much of the bacteria in these areas could also be controlled and for almost all storms.

Table A5. Percentage of Fecal Coliform Bacteria Originating from Source Areas (Pitt 1983)

	rain depth (mm)	pervious areas (yards, vacant land and parks)	roof-tops	parking lots	sidewalks and driveways	streets
Alta Vista (resid.)	0.25	*		3	26	71
	2.5			5	32	63
	5.0	30		4	26	40
	10	52		3	16	29
	20	61		2	14	23
	40	70		2	11	17
	"max"	78		1	8	13
Clegg St. (resid.)	0.25			15	25	60
	2.5			21	26	53
	5.0	6		23	28	43
	10	20		17	22	41
	20	28		16	20	36
	40	37		15	18	30
	"max"	52		11	13	24
Leonard Ave. (resid.)	0.25			4	37	59
	2.5			6	42	52
	5.0	7		7	45	41
	10	18		5	37	40
	20	21		5	39	35
	40	29	1	5	34	31
	"max"	38	1	5	28	28
Merivale (indust.)	0.25		2**	51	14	33
	2.5		2**	60	13	25
	5.0	3	2**	63	11	21
	10	42	1**	36	7	14
	20	55	1**	29	5	10
	40	65	1**	23	3	8
	"max"	78		14	3	5
Billings Bridge (shopping center)	0.25		4**	62		34
	2.5		3**	71		26
	5.0		2**	77		21
	10	10	2**	68		20
	20	18	2**	63		17
	40	24	2**	58		16
	"max"	39	1**	46		14
St. Laurent (shopping center)	0.25		3**	52	5	40
	2.5		2**	64	4	30
	5.0		2**	69	4	25
	10	3	2**	63	4	28
	20	6	2**	63	4	25
	40	6	2**	63	4	25
	"max"	11	2**	60	4	23

* values not shown are less than 1 percent
** mostly directly connected

Mammal and Bird Populations and Bacteria Discharges in the Ottawa Urban Area

Table A6 summarizes the expected populations of mammals and birds in the lower Rideau River watershed. There are other domestic and wild animals in this watershed (such as other birds and rodents) but their population estimates are not available. It is estimated that about 16,000 dogs and the same number of cats live in this watershed, corresponding to approximately one dog or cat for every other house. The waterbird estimates are based upon actual population counts made along the river.

Table A6. Estimated Bird and Pet Populations in the Lower Rideau River Watershed (below Hogs Back) (Pitt 1983)

Animal	Population Density (animals/ha)	Total estimated animal population in the Lower Rideau River Watershed (4000 ha)
Dogs[1]	4	16,000
Cats[1]	4	16,000
Robins[2]	7	28,000
Pigeons (land)[1]	1	4,000
Pigeons (on bridges)[3]		600
Ducks (on river)[3]		100
Gulls (on river)[3]		150
Swans (on river)[3]		15
Other birds on river (sparrows and blackbirds)[3]		10

[1] estimated from Colt, *et al.* 1977
[2] estimated from Howard 1974
[3] Regional Municipality of Ottawa-Carleton 1980

Table A7 is an estimate of the total annual bacteria discharges from these mammals and birds based upon these population estimates, the fecal discharges, the application factors, and the bacteria concentrations in the feces. The total estimated discharges (2×10^{11} fecal coliforms per ha per year) are two to three orders of magnitude greater than what is expected in the annual urban runoff bacteria yield. This large difference may be associated with bacteria dieoff or analytical problems.

Table A7. Annual Bacteria Discharges to Lower Rideau River from Different Urban Sources (Pitt 1983)

Animal	Animal Pop. in Lower Rideau R. Watershed	Feces Discharge (grams/ animal-day)	Annual Feces Discharge (grams/ year)	Application Factor (fraction to river)	Feces Discharged to River (grams/ year)	Total Coliforms			Fecal Coliforms			Fecal Strep.		
						MPN/gram	MPN/yr	% of total	MPN/gram	MPN/yr	% of total	MPN/gram	MPN/yr	% of total
Discharge to Land:														
Dog	16,000	100	6x10^{8}	0.01	6x10^{6}	2.3xl0^{7}	1.4x10^{14}	54	2.3xl0^{7}	1.4x10^{14}	19	9.8x10^{8}	5.9x10^{15}	95
Cat	16,000	70	4x10^{8}	0.001	4x10^{5}	7.9x10^{6}	3.2x10^{12}	1	7.9x10^{6}	3.2x10^{12}	<1	2.7x10^{7}	1.1x10^{13}	<1
Robins	28,000	10	1x10^{8}	0.01	1x10^{6}	2.2x10^{2}	2.2x10^{8}	<1	2.5x10^{4}	2.5x10^{10}	<1	1.2x10^{7}	1.2xl0^{13}	<1
Pigeons (land)	4,000	35	5x10^{7}	0.01	5x10^{5}	1.3x10^{7}	6.5xl0^{12}	3	1.0x10^{8}	5.0x10^{13}	7	1.2x10^{7}	6.0x10^{12}	<1
Direct Discharge to River:														
Pigeons (on bridge)	600	35	8x10^{6}	0.5	4x10^{6}	1.3x10^{7}	5.2x10^{13}	20	1.0x10^{8}	4.0xl0^{4}	54	1.2x10^{7}	4.8x10^{13}	1
Ducks (on river)	100	200	2x10^{7}	0.5	3x10^{6}	1.3x10^{7}	4.8x10^{13}	18	3.3x10^{7}	1.2xl0^{4}	16	5.4x10^{7}	2.0x10^{14}	3
Gulls (on river)	150	20	1xl0^{6}	0.5	5x10^{5}	1.3x10^{7}	6.5x10^{12}	3	5.3x10^{7}	2.7x10^{13}	4	9.0x10^{4}	4.5x10^{10}	<1
Swans (on river)	15	200	1x10^{6}	0.5	5x10^{5}	4.8x10^{5}	2.4xl0^{11}	<1	3.2x10^{5}	1.6xl0^{11}	<1	4.5x10^{4}	2.3xl0^{10}	<1
Other birds (on river)	10	10	4x10^{4}	0.5	2x10^{4}	1.3x10^{7}	2.6xl0^{11}	<1	2.5xl0^{4}	5.0x10^{8}	<1	5.0x10^{6}	1.0x10^{11}	<1
Total from urban runoff							1.5x10^{14}	58		1.9x10^{14}	26		5.9x10^{15}	96
Total direct discharge to river							1.1x10^{14}	42		5.5x10^{14}	74		2.5x10^{14}	4
Grand Total							2.6x10^{14}			7.4x10^{14}			6.2x10^{15}	

As a rough estimate, the values in Table A7 may all be considered to be affected by the same dieoff rates and analytical measurement errors. The percentage contributions associated with each animal may, therefore, be considered approximate. The major source of fecal coliforms in the Rideau River is expected to be pigeons (when using the high Ottawa pigeon fecal coliform values), followed by dogs and ducks. The other sources shown would all contribute less than a total of five percent. Dogs are expected to contribute almost half of the river total coliform organisms, while pigeons on the bridges and ducks on the river make up most of the remainder. Dogs are expected to contribute almost all of the river fecal strep. bacteria, with ducks on the river contributing to less than five percent. Pitt and Bozeman (1979) found that lake birds can contribute a significant amount of fecal strep. bacteria to a lake refuge in the middle of an urban area in Oakland, CA. However, urban runoff components contribute much more bacteria during wet weather conditions.

It is interesting to compare these calculated estimates of fecal coliform contributions with those reported elsewhere. Faust and Goff (1977) reported 10^9 to 10^{10} fecal coliforms discharged per hectare per year in the Chesapeake Bay area from cultivated lands, forests, and pastures. These values are about ten to 100 times the estimated urban area yields for the lower Rideau River watershed.

Summary of Ottawa Case Study

The limited assimilative capacity of the river and how the bacteria quality decreases as the river flows through Ottawa was previously described. The substantial bacteria density increases during wet weather indicate an urban runoff problem and the probable lengthy duration of adverse river conditions. The number of observations showing bacteria densities greater than the standards indicates that Strathcona, Brantwood, and Brighton Beaches exceed the fecal coliform criteria of 100 organisma/100 mL most of the time. Mooney's Bay Beach exceeds this criteria about ten percent of the time. A limited field program was conducted during this study that found the Rideau River bottom sediments to have substantial bacteria population densities.

An important phase in designing an urban runoff control program is to determine the sources of the problem pollutants in the watershed. An understanding of where they accumulate in the catchment is needed before appropriate controls may be selected. As an example, bacteria may accumulate almost everywhere in an urban area (on rooftops from birds, and on streets, parking lots, landscaped areas, and vacant land from dogs and other urban animals). Original sources therefore affect a variety of potential control areas. The Rideau River Stormwater Management Plan report identified urban runoff as the major source of the problem bacteria discharges. This special study summarized here included a limited field program which roughly identified the specific locations in the urban area where the bacteria originated. Feces from warm blooded animals are the only sources of fecal bacteria, while soils can contain some non-fecal bacteria. The ratio of fecal coliforms to fecal strep. bacteria population densities can be used to differentiate between human and non-human sources if the samples are obtained very close to the time of discharge. Otherwise, the different survival times of the fecal strep. biotypes can radically change this ratio with time. The periodic high ratios of these two bacteria indicator groups in the Rideau River may be explained by relatively old non-human discharges. If water bodies were small (creeks and small reservoirs), a relatively small number of birds (less than 100) were found to significantly increase various fecal bacteria biotypes in the water. However, if the water bodies were large (large bird refuges and large rivers), then large numbers of birds (as many as 100,000) did not significantly increase the bacteria population densities in the water. The water flowing from the bird refuges typically had better water quality than the inflowing water, possibly due to sedimentation in the refuge marshes. Dog feces are expected to contribute much of the fecal coliforms in urban runoff, while pigeons (on bridges) and ducks on the Rideau River may contribute most of the bacteria to the River. Polluted river sediments may also play an important role in contaminating river water.

Based on monitoring from the Rideau River Stormwater Management Study and other runoff bacteria studies, it is concluded that many potentially pathogenic bacteria biotypes can be present in the local urban runoff. Most of these pathogenic biotypes can cause health problems when ingested. Because of the low probability of ingestion of urban runoff, many of the potential human diseases associated with these biotypes are not likely to occur. The required infective doses of many of these biotypes and their relatively low concentrations in stormwater would require very large amounts of urban runoff to be ingested. As an example, Salmonella, when observed in Ottawa urban runoff and receiving waters, has been found in very low concentrations requiring the consumption of more than 20 liters of urban runoff for infections. Shigella, however, may be present in urban runoff and receiving waters and when ingested in low numbers can cause dysentery.

The pathogenic organisms of most importance in urban runoff are usually associated with skin infections and body contact. Body contact with urban runoff is not likely. However, the Rideau River retains many of the pathogenic biotypes originating from urban runoff for a long period of time after rains. The most important biotype causing skin infections is *Pseudomonas aeruginosa*. This biotype has been frequently detected in urban runoff at many locations in concentrations that may cause potential infections. However, there is little information relating increased infection hazards with increased *Pseudomonas* concentrations. *Staphylococci aureus* may also cause skin problems with body contact, but there is little information concerning the concentrations of this biotype in urban runoff. Various pathogenic yeasts and viruses may also be found in urban runoff, but their concentrations and infective pathways are not well enough known to establish criteria for urban runoff pollution. Therefore, the local bacteria concentration objectives based on fecal coliform concentrations may be unreasonable when actual potential health effects are considered.

Further studies also need to be made concerning populations of pathogenic bacteria (specifically *Pseudomonas aeruginosa*, *Staphylococci aureus* and Shigella) in the Rideau River. Population densities of these pathogens may be related to River location, storm type, and possibly indicator (fecal coliform) bacteria densities. If adverse levels of these pathogens can be predicted, or easily and quickly measured, then they should be used as the basis for beach closures in the River.

Appendix B: Case Study: Sources of *E. Coli* and Enterococci in Wet Weather and Dry Weather Flows

The following discussion is based on a presentation by Shergill and Pitt (2004) and Shergill's graduate research at the University of Alabama for his MSE degree (Shergill 2004).

An urban area consists of many different kinds of land uses such as residential, institutional, commercial, industrial open spaces, etc. Each type of land use consists of various types of source areas, such as roofs, parking lots, landscaped areas, playgrounds, driveways, undeveloped areas, sidewalks. Four representative source area types were sampled during this research; including rooftops, parking lots, open spaces, and streets. Two parallel sites were sampled for each source area type; one affected by birds and other animals, and another set with less influence from birds and other animals. A section of Cribbs Mill Creek in Tuscaloosa, Alabama, was also selected for dry weather sampling at outfalls. The section of the creek was selected such that the drainage areas contributing to outfalls had either commercial or residential land uses. Potential inappropriate discharge water samples were also obtained, including influent samples from the Tuscaloosa sewage treatment plant, local springs, irrigation runoff water, domestic water taps, car wash, and laundry water. Overall, total coliforms, *E. coli* and enterococci bacterial analyses were conducted on 202 wet weather and 278 dry weather flow water samples. All samples were analyzed using IDEXX Quantitray enumeration procedures.

E. coli and enterococci levels larger than 2,400 and 24,000 MPN/100 mL, respectively, were observed in wet weather samples collected from various source areas which could not possibly be contaminated with sanitary sewage. The levels of indicator bacteria present in the urban runoff source area samples exceeded the EPA 1986 single sample maximum value water quality criteria in 31% of the samples for *E. coli* and in 74% of the samples for enterococci. The geometric mean criteria were exceeded in 100% of the source area samples. Since both the indicator organisms studied (*E. coli.* and enterococci) only originate in intestines of warm-blooded animals, birds and other urban animals can be considered important sources of bacteria in stormwater.

This assumption was tested by conducted additional monitoring. Comparisons of samples collected from areas prone to urban animal use and those that are not, showed that large overlaps exist between the bacterial concentrations found from both types of areas. Bacterial levels from roofs prone to urban animal use (squirrels and birds) were significantly higher than from roofs not exposed to such use. The other source areas did not show any significant differences in bacterial levels between areas prone and not prone to urban animal use, except for some street areas. This could be the result of a combination of factors, such as the persistence of bacteria in soil, the inadvertent contamination by runoff from other areas frequented by animals, the mobility of small urban animals, or the ubiquitous presence of moderate levels of these organisms in most urban areas. Statistical analyses problems were also caused by periodic very high bacteria values that exceeded the range of the experiments.

A further objective of this study was to find how *E. coli* and enterococci could be effectively used to identifying the presence of inappropriate sanitary sewage in storm drainage systems during dry weather. Many stormwater system managers believe that the presence of indicator bacteria exceeding regulatory levels indicates the likely presence of sanitary sewage. During this study, sewage samples were compared with wet weather and dry weather source area samples (from the project reference sample library). The probability of the sewage and source area sample bacteria levels being significantly different was determined using the Mann Whitney test. When the values of the probabilities were ≤ 0.05, the diluted sewage sample bacteria levels were determined to be significantly higher as compared to bacterial levels in other source area samples (with a 1 in 20 error level). It was found that the dry-weather outfall samples showing *E. coli* and enterococci levels higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL respectively, are likely contaminated by sanitary sewage. Levels lower than this can be caused by other sources, such as irrigation runoff, carwash water, or laundry water.

Other findings of this research included:

- Bacteria levels in urban areas are not source limited, i.e. measured bacteria levels did not decrease with increasing amounts of rain, or even with increasing rain intensities. The levels may increase, or decrease, somewhat with time, but stayed generally level.
- Seasons having low temperatures are associated with decreased bacterial levels.

- The ratio of *E. coli* /enterococci was not constant and varied greatly for all conditions.
- Wet weather samples had mostly higher enterococci levels than *E. coli*, while dry weather source area samples (such as springs and irrigation runoff) had higher *E. coli* levels than enterococci levels.
- Both the indicators followed the same general trend for every site; i.e. both *E. coli* and enterococci levels increased or decreased simultaneously, although by different amounts.
- Sewage samples need vigorous agitation before analyses to break up the lumps of fecal matter in which bacteria are present.
- Samples must be kept refrigerated and analyzed shortly after sample collection. Samples a day old and unrefrigerated can be expected to have decreased bacteria levels compared to chilled and fresh samples.

This research was funded as part of a 104(b)3 grant from the U.S. Environmental Protection Agency (Bryan Rittenhouse was the project officer) to the Center for Watershed Protection (under the project management of Ted Brown and Tom Schueler) in 2001. The University of Alabama was a subcontractor to the Center. Sumandeep Shergill conducted much of the research reported in this section, with the assistance of other graduate students at UA, and his master's thesis reporting this work was accepted by the University in May of 2004 (Shergill 2004).

Methodology

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

- *Effects of Urban Wildlife on Stormwater Bacteria Levels.* Four source area categories were selected for sampling. For each category of source area, two sites were selected, prone and not prone to urban animal use. The prone locations were those where urban wildlife (birds and squirrels for roofs, and dogs for ground-level surfaces) use is common and not prone locations where urban wildlife appears to be generally absent. The number of samples collected in each category during this part of the research is listed in Table B1.

Table B1. Total Number of Sample Pairs Collected From Each Source Area

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

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

- *Seasonal Variations.* The climate of Tuscaloosa, Alabama, is subtropical with four distinct seasons including winter (December through February), spring (March and April), summer (May through September) and autumn (October and November). Anticipating that bacteria levels would vary with season, an attempt was made to take samples in every season. Wet weather sampling was conducted from August 2002 to June 2003. No samples were collected during the months of December and March. This objective was to compare cold months (December through February, generally having temperatures below 50^{o} F) with samples collected during the warmer months.

- *Variations within Storm*s. Additional tests were also conducted to determine the potential causes for the large variability found during the bacteria analyses of the sheetflow samples. During a single storm on 25 September 2002, all the sites were sampled twice, once in the morning and then again in the evening. In addition, six samples from two source areas were collected at intervals of 15 to 30 minutes during a single storm on 17 October 2003. A total of 24 samples were analyzed for these tests.

- *Effect of Sample Handling.* Three factors involving sample handling were also studied. These included holding time, refrigeration, and vigorous sample shaking. For these tests, a single 5 liter sample was taken from one source area from which 100 mL sub samples were tested after 1, 2, 5, 9, 24, and 48 hrs. The 5 liter sample was split into two components, one was refrigerated, and the other was not. The effect of refrigeration over one to two days was also measured. The effect of shaking was measured by withdrawing an initial 100 mL sample from the unshaken sample bottles, and then shaking the sample bottles and testing another 100 mL sample.

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

- *Outfall Sample Collection.* A five mile stretch of Cribbs Mill Creek in Tuscaloosa, Alabama, was selected for dry weather sampling to test methods to detect inappropriate discharges to the creek. A total of 77 total outfalls were examined and bacterial analyses were conducted during three different periods from outfalls having dry weather flows. A total of 136 samples were analyzed during this test phase.

Sampling Procedures

Wet weather sampling started in August 2002 and was completed in June 2003. The objective was to represent all the seasons so that effects of season on bacterial concentrations could be examined. Samples were taken during rains once or twice a month during this period, except for December 2002 and March 2003 when no samples were obtained. Dry weather sampling involved collection of Tuscaloosa source area samples for preparing the Tuscaloosa source area reference sample library. Most of the library samples were collected during the months of May and June 2003. All samples were analyzed using the IDEXX Quantitray enumeration procedure. All samples were analyzed for total coliforms, *E. coli* and enterococci. Although dry weather samples were analyzed for various other constituents, this paper only presents results for the microbial analyses. The quality assurance /quality control (QA/QC) procedures followed are described later.

- *Wet Weather Sampling Procedure.* Samples were collected according to procedures given in *Standard Methods for the Examination of Water and Wastewater* (Standard Methods-20th edition, 1998) for microbiological examination. Sterile techniques were used to avoid sample contamination. Sterile gloves were worn during sampling and analysis, and the samples were collected in presterilized 100 mL plastic bottles supplied by IDEXX . The bottles contain sodium thiosulphate ($Na_2S_20_3$) to prevent problems with chlorine in the samples. $Na_2S_20_3$ is a dechlorinating agent that neutralizes any residual halogen and prevents continuation of bacterial disinfection during sample transit. The use of $Na_2S_20_3$ more accurately results in the true microbial content of the water at the time of sampling (Standard Methods-20th edition, 1998).

All samples were taken manually. The sample bottles were filled up to the 100 mL mark, leaving ample air space to facilitate mixing by shaking, before testing. The pre-sterilized sample bottles were filled without rinsing and care was taken so that the inner surface of stopper or cap did not become contaminated. The bottle cap was replaced immediately.

The sample bottle labels listed the date, sample I.D, and time of sampling, using waterproof markers. The sample bottles had labels on both the cap and the bottle, preventing the caps form being interchanged. Filled sample bottles were then put in a backpack for transporting to the lab. During the initial five sampling rounds, no sample dilutions were made, so two sample bottles per site (one for *E. coli* and other for enterococci) were taken. From the sixth round on, three 100 mL samples were taken per site to allow for dilution and an expanded range of MPN values.

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

Rooftop samples were obtained by placing the sample bottle directly under the downspout. The bottle was removed soon before it filled to the 100 mL mark. The bottle cap was then used to fill the sample bottle exactly to the 100mL mark. Sheetflow samples were taken from parking lots and streets. The sampling locations on the street or parking lots were selected so that runoff was not mixed with runoff from other source areas. Similarly, sampling places inside the parking lots were selected such that there was minimal mixing from other source areas. Samples were taken by holding the sample bottle near its base, keeping it tilted at an angle with mouth facing downstream. Sheetflow samples were placed into the bottle with the cap from the bottle. Care was taken not to scratch the pavement surface with the cap during sampling. It was difficult to collect sheetflow samples from open spaces. Most open space samples were obtained from ponded water.

Samples collected from different sites were kept in different Zip Lock bags, put in the backpack and transported to the laboratory. Microbiological analysis of the water samples was started as soon as possible after collection to avoid changes in the microbial population.

• ***Dry Weather Sampling Procedure.*** Cribbs Mill Creek in Tuscaloosa, Alabama, was selected for dry weather sampling. Its' watershed contains residential, commercial, open space land use areas. Other favorable characteristics were moderate flow, accessibility by road, and it was in a completely urbanized area that has been long developed. A five-mile section of the creek was selected for sampling.

The equipment taken to the field included

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

Before sampling during any day, the field crew contacted the local Tuscaloosa Police Department to let them know the area of creek being investigated that day. The field crew consisted of three people. Upon arriving at the first site, two people waded the creek in a downstream direction carrying the field equipment in backpacks, while one person with a street map, cooler (with coolant), and a walkie-talkie drove the vehicle to a convenient downstream location where the creek intersects the street. Collected samples were placed in a portable ice cooler in the vehicle after each stretch was sampled. This collection point was usually about a half mile downstream from the last collection point. About 5 or 6 samples are usually collected from each stretch of creek and iced within a half hour of collection. Heavy-duty waders were always worn while wading which provided protected from debris (broken glass and other sharp debris, bricks etc.) and certain wildlife species (rattlesnakes, cottonmouth, etc.).

The first two creek walks involved a greater effort and time to complete because of the need to locate the outfall locations. After three complete creek walks, no new outfalls were found, and the field time was appreciably shortened. A total of 77 outfalls were eventually found in the initial study reach. Outfalls were numbered using black spray paint. The average distance between the outfalls was about 50 feet, and about six flowing outfalls were sampled during a days creek walk. About 5 to 7 days were needed for every creek walk, or about one mile per day. Out of 77 total outfalls, 20-25 were flowing during every creek walk. When a branch enters the main creek, the sampling crew went to the origin of the branch and walked downstream marking outfalls along the way. All sorts of outfalls were found, including open ditches, concrete outfalls, ductile iron pipe outfalls, and PVC outfalls. A few only drained the adjacent paved parking areas, while most were conventional outfalls draining 5 to 50 acres each. The following URL includes a large aerial photograph showing all outfalls, along with individual outfall photographs: http://www.eng.ua.edu/~rpitt/Research/ID/ID2.shtml

During the last three creek walks, bacterial analyses were also conducted, requiring two 100 mL samples collected for each flowing outfall, in addition to the 1L sample.

The following steps were followed at every outfall:

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

After the third creek walk, some branches of the creek were dropped from further evaluations because of time and a redundancy of the residential land uses in which the branches were located. The dry weather sampling was conducted at least 24 to 48 hrs after rains, depending upon the rain depths. Samples were collected in the morning and refrigerated, while the 100 mL samples that were collected for bacterial analyses were analyzed immediately after arriving at the lab after each morning sample collection. All the other constituents were usually analyzed that same afternoon. Other constituents analyzed were ammonia, boron, color, conductivity, detergents, fluorescence, fluoride, hardness, potassium, pH, optical brighteners, and turbidity.

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

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

All the industries that were analyzed send water samples to the Tuscaloosa WWTP weekly for analyses as part of the local industrial pre-treatment program. Our library samples were obtained when these industrial samples were delivered to the treatment plant lab.

Irrigation water samples were mostly sheetflow water collected from the sidewalks or roads, which flowed due to over-watering of lawns. Some samples were collected from small depressions in the lawn itself and not from runoff after flowing across concrete.

Sample Analysis Procedures

All the samples were analyzed for total coliforms, *E.* c*oli*, and enterococci using EPA-approved IDEXX Laboratories methods. EPA suggested water quality criteria based upon *E. coli* and enterococci measurements in 1986. The IDEXX methods used were developed in response to these EPA microbiological guidelines. All the equipment and supplies needed were obtained from IDEXX, including Colilert or Colilert-18 reagent, Enterolert reagent, presterlized 100 mL sample bottles, Quanti-tray-2000 sample containers, Quanti-tray sealer, rubber insert pads, two incubators, two thermometers, comparartor, and a 6 watt, 365nm wavelength UV lamp. Figure B1 shows all the equipment used. Two incubators were used, one with the temperature setting for *E. coli* sample incubation and the other set for enterococci sample incubation.

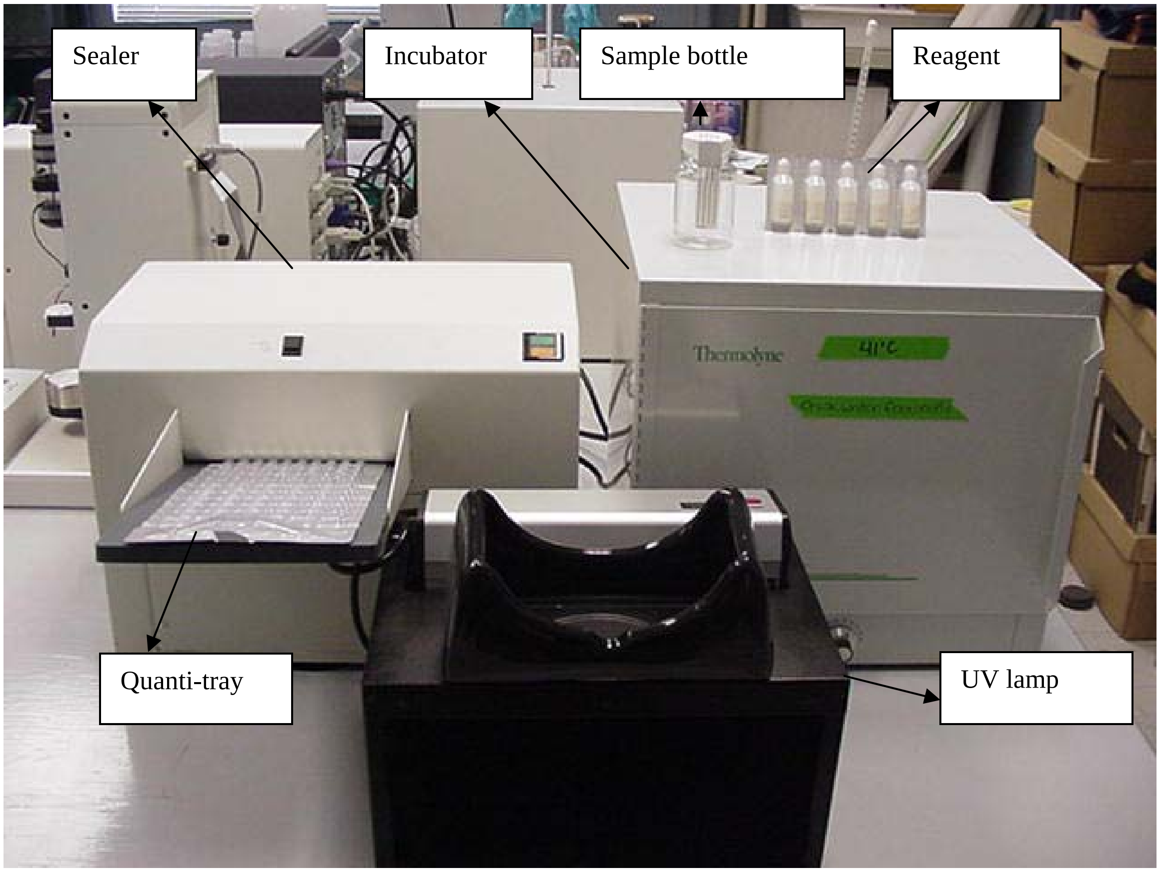

Figure B1. IDEXX Equipment Used

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

1) Personnel: Basic laboratory training was undertaken. The IDEXX training video instructions were followed.
2) Facility: Tests were done in a well-ventilated laboratory having air conditioning that reduced contamination, permitted more stable operation of incubators and decreased moisture problems with media and instruments. The work areas were kept clean and free of unnecessary chemicals. After finishing the tests, the counter and other work surfaces were wiped with an appropriate disinfecting solution (typically a bleach solution). If any sample or QA/QC solution was spilled, a sorbent material was used to soak up the material and the used sorbent was placed in the proper disposal container (Biohazard bag for on-campus disposal of biohazardous materials).
3) Laboratory equipment and instrumentation: Two separate incubators were used for testing *E. coli* and enterococci. These were maintained at temperatures of 35± 0.5° C and 41± 0.5° C, respectively. A glass thermometer with its bulb and stem submerged in water kept in a beaker inside the incubator was used to verify the incubator temperature. The water levels in the beakers were periodically checked to ensure that the bulb and stem of the thermometers were always submerged. The UV lamp and sealer were switched off after each use and were periodically cleaned.
4) Supplies: Supplies used for testing were Colilert and Colilert-18 reagent, Enterolert reagent; Quanti-cult bacterial cultures used for quality control, Quanti-trays, and 100 mL pre-sterilized sample bottles. The Quanti-cult and analytical reagents were stored in a refrigerator according to the manufacturer requirements. Quanti-trays and sample bottles supplied by IDEXX were sterile (certified by IDEXX) and disposable. This eliminates the use of glassware and any chances of contamination.

5) Analytic methods: The test used for total coliforms and *E. coli*, was the commercially available microbiological method included in *Standard Methods for the Examination of Water and Wastewater*, 20th edition (section 9223 B). Enterolert is an official ASTM method (#D6503-99). These methods are commonly used by many agencies, including the Alabama Department of Environmental Management (ADEM).
6) Analytical Quality control procedures: Every batch of Colilert and Colilert-18 reagent was checked by testing with known positive and negative control cultures (Quanti-cult®). Quanti-cult® is a set of ready to use bacterial cultures supplied by IDEXX. It consists of three sets each of three different bacterial cultures. Each set consists of 1-50 bacterial cells which were preserved in the colorless cap of a plastic vial. The contents of Quanti-cult® were kept stored in a refrigerator until time of use. Following are the contents:
   - 3 *E. coli* capped vials labeled “EC” in foil packs and 2 reusable labels
   - 3 Klebsiella pneumoniae –capped vials labeled “KP” in foil packs and 2 reusable labels. This is a total coliform bacterium.
   - 3 Pseudomonas aeruginosa – capped vials labeled “PA” in foil packs and 2 reusable labels. This is a non-coliform bacterium.
   - 12 rehydration fluid vials
   - 1 autoclavable foam vial holder

Quality control tests were run three times on different batches. All test results were acceptable and full results are reported by Shergill (2004).

Results and Discussion

This section presents the results of the wet weather and dry weather sampling and bacteria analyses. Summary tables only are included here, with detailed results provided by Shergill (2004). Statistical analyses were conducted using MINITAB, EXCEL and Pro-Stat software.

Wet Weather Sampling

Table B2 summarizes the *E. coli* and enterococci levels (MPN/100 mL) obtained from wet weather source area sampling conducted from August 2002 to June 2003.

Table B2. Wet Weather Source Area Sampling Results

Sample I.D	**Date Sample Taken**	***E. coli*** (MPN/100 mL***)	**Enterococci** (MPN/100 mL)
OPEN SPACE -Prone*	21-Sep-02	1732.9	>2419.2
	25-Sep-02	15.5	>2419.2
	25-Sep-02	41.3	>2419.2
	10-Oct-02	Not Sampled	Not Sampled
	27-Oct-02	Not Sampled	Not Sampled
	5-Nov-02	2419.2	19863
	29-Jan-03	35.4	216
	6-Feb-03	1	395
	6-Feb-03	1	Not Sampled
	24-Apr-03	82	322
	14-May-03	52	2489
	12-Jun-03	>2419.2	>24192
	27-Jun-03	3.1	4106
OPEN SPACE– Not Prone**	21-Sep-02	Not Sampled	Not Sampled
	25-Sep-02	2419.2	>2419.2
	25-Sep-02	866.4	>2419.2
	10-Oct-02	Not Sampled	Not Sampled
	27-Oct-02	Not Sampled	Not Sampled
	15-Oct-02	217.8	>2419.2
	5-Nov-02	44.8	8664
	29-Jan-03	17.7	195
	6-Feb-03	2	505
	24-Apr-03	8.6	2755
	14-May-03	307.6	9804
	12-Jun-03	63.1	>24192
	27-Jun-03	6.2	>24192
PARKING LOT- Not Prone	25-Sep-02	83.9	>2419.2
	25-Sep-02	69.7	2419.2
	10-Oct-02	14.2	>2419.2
	27-Oct-02	1553.1	48.2
	5-Nov-02	15.8	238
	29-Jan-03	4.1	238
	6-Feb-03	<1	31
	24-Apr-03	72.3	9804
	14-May-03	25.6	1130
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	5.2	613
PARKING LOT- Prone	21-Sep-02	1046.2	529.8
	25-Sep-02	137.6	>2419.2
	25-Sep-02	66.3	344.8
	10-Oct-02	980.4	>2419.2
	27-Oct-02	866.4	>2419.2
	5-Nov-02	17.3	158
	29-Jan-03	52	199
	29-Jan-03	54.6	160
	29-Jan-03	37.3	145
	6-Feb-03	6.3	150
	24-Apr-03	8.3	127
	14-May-03	290.9	805
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	29.5	416
ROOF- Prone	29-Aug-02	145.5	Not Sampled
	21-Sep-02	461.1	>2419.2
	25-Sep-02	18.7	>2419.2
	25-Sep-02	1413.6	980.4
	10-Oct-02	410.6	67.9
	27-Oct-02	>2419.2	1
	5-Nov-02	>2419.2	9.3
	29-Jan-03	2	16.4
	6-Feb-03	<1	31
	24-Apr-03	517.2	>24192
	14-May-03	Not Sampled	Not Sampled
	12-Jun-03	727	24192
	27-Jun-03	2419.2	15531

Table B2. Wet Weather Source Area Sampling Results (continued)

	29-Aug-02	<1	Not Sampled
ROOF- Not Prone	21-Sep-02	30.5	8
	25-Sep-02	2	2
	25-Sep-02	5.2	21.1
	10-Oct-02	344.8	69.1
	27-Oct-02	161.6	43.5
	5-Nov-02	29.2	1
	29-Jan-03	<1	<1
	6-Feb-03	>2419.2	3
	24-Apr-03	6.3	<1
	14-May-03	2	7
	12-Jun-03	5.2	9.5
	27-Jun-03	Not Sampled	78
	21-Sep-02	1553.1	>2419.2
STREET- Prone	25-Sep-02	920.8	>2419.2
	25-Sep-02	1119.9	>2419.2
	10-Oct-02	>2419.2	>2419.2
	27-Oct-02	>2419.2	>2419.2
	5-Nov-02	>2419.2	>24192
	29-Jan-03	Not Sampled	Not Sampled
	6-Feb-03	12.1	332
	24-Apr-03	95.9	8164
	14-May-03	>2419.2	3130
	12-Jun-03	NT	NT
	27-Jun-03	2419.2	15531
	25-Sep-02	>2419.2	>2419.2
STREET- Not Prone	25-Sep-02	980.4	>2419.2
	10-Oct-02	1046.2	>2419.2
	27-Oct-02	>2419.2	>2419.2
	5-Nov-02	1299.7	1785
	29-Jan-03	131.3	563
	6-Feb-03	52.8	749
	24-Apr-03	77.6	1401
	14-May-03	114.5	435
	12-Jun-03	Not Sampled	Not Sampled
	27-Jun-03	32.3	683

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

The upper detection limit (UDL) of this method was 2,419.2 MPN/100 mL and the lower detection limit (LDL) was 1 MPN/100 mL for all three indicator organisms. After completion of the first five rounds of sampling, it was observed that most enterococci levels exceeded the UDL. Therefore, three 100mL samples per site were collected in the subsequent rounds (two for enterococci and one for *E. coli*). One 100 mL sample was diluted 10 times to increase the range of the UDL to 24,192 MPN/100 mL. Enterococci levels were found in both diluted as well as not diluted samples. Enterococci levels found in the diluted samples were found to better represent the bacterial levels. Therefore, to maintain uniformity, the dilution results were used whenever they were available. For most of the statistical analyses, the values greater than UDL and less than LDL were replaced with the UDL and LDL values, respectively, generally resulting in conservative results. As can be seen from the table, wide ranges of bacterial levels were detected from each of the source areas. *E. coli* levels varied from <1 to >2,419.2 for most of the source areas. Since no dilutions were done for *E. coli* samples, the range was limited by the LDL and UDL values. However, the enterococci levels had a wider range due to the dilution (<1 to > 24,192). The enterococci values were much higher than the *E. coli* values. The total coliform results were mostly >UDL. Since there was little interest in these results, dilutions were not made of the total coliform and *E. coli* samples.

Dry Weather Sampling Results

Another component of this research included bacterial analyses of dry weather samples taken from outfalls flowing into Cribbs Mill Creek in Tuscaloosa, AL. Although the samples were analyzed for a number of parameters (as part of the EPA-funded Inappropriate Discharge Detection and Elimination “IDDE” project, CWP and Pitt 2004) this paper focuses on the bacterial analyses, i.e. *E. coli* and enterococci.

The “library” samples (reference samples) collected from various source areas were analyzed for various tracer materials, including *E. coli* and enterococci. This included samples from influent to sewage treatment plants, local springs, irrigation runoff, domestic water taps, car wash, and laundry water. Tables B3 and B4 show the results of the bacterial analyses of the library samples.

Statistical Analysis and Discussion

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

Table B3. *E. coli* Levels in Reference Samples (MPN/100 mL)

Sample No.	Tap Water	Spring Water	Irrigation	Laundry	Carwash	Industrial	Sewage (Dry Weather)**	Sewage (Wet Weather)
NO.1	NA	4.1	27.8	NA	1,553.1	66.3	>2,419.2	
NO.2	NA	1	8.3	NA	1,413.6	>2,419.2	NA	
NO.3	NA	NA	>2,419.2	<1	4.1	0	>2,419.2	
NO.4	NA	NA	>2,419.2	<1	14.6	3	816.4	
NO.5	NA	NA	31.8	<1	>2,419.2	NA	NA	
NO.6	<1	<1	>2,419.2	>2,419.2	1,413.6	NA	12,033,000	
NO.7	<1	290.9	>2,419.2	20.1	15.8	NA		2,851,000
NO.8	<1	172.3	>2,419.2	<1	11.9	NA		3,654,000
NO.9	<1	<1	>2,419.2	19.7	235.9	<1		2,187,000
NO.10	<1	9.7	1,299.7	<1	15.5	>2,419.2		1,785,000
NO.11	<1	1	>4,838.4	<1	1,553.1	<1		3,255,000
NO.12	<1	<1	>4,838.4	<1	<1	<1		2,282,000
Geometric mean*	1	5	771	3.9	94	19.7	15,484	2,590,319
Median	<1	1	>2,419	<1	125	2	2,419	2,566,500
COV*	0	1.96	0.76	3.09	1.21	1.81	1.99	0.26

* Values calculated by replacing <1 with 1 and >2,419.2 with 2,419.2

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

Table B4. Enterococci Levels in Reference Samples (MPN/100 mL)

Sample No.	Tap Water	Spring Water	Irrigation	Laundry	Carwash	Industrial	Sewage (Dry Weather)**	Sewage (Wet Weather)
NO.1	NA	4.1	>2,419.2	NA	>2,419.2	0	>2,419.2	
NO.2	NA	36.4	2	NA	6.20	>2,419.2	NA	
NO.3	NA	NA	>2,419.2	<1	5.2	0	>2,419.2	
NO.4	NA	NA	>2,419.2	<1	3.1	>2,419.2	43.6	
NO.5	NA	NA	>2,419.2	<1	1	NA	NA	
NO.6	<1	<1	287.7	<1	>2,419.2	NA	613,000	
NO.7	<1	412	>2,419.2	<1	<1	NA		833,000
NO.8	<1	140.8	>2,419.2	<1	11.1	NA		598,000
NO.9	<1	3.1	>2,419.2	<1	<1	<1		292,000
NO.10	<1	65.7	>2,419.2	<1	<1	866.4		328,000
NO.11	<1	<1	>4,838.4	<1	2,419.2	22.2		369,000
NO.12	<1	<1	>4,838.4	<1	<1	<1		609,000
Geometric mean*	1	10.7	1,258	1	13	69	3,536	469,578
Median	<1	4.1	>2,419	<1	4.2	12	>2,419	483,500
COV*	0	1.82	0.57	0	1.79	1.52	1.97	0.41

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

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

Due to the presence of large numbers of non-detected values, three types of paired and unpaired statistical tests were used to determine if significant differences occurred between the sites. MINITAB was used to plot box plots. For both, *E. coli* and enterococci, two separate box plots were prepared, one for warm months and the other for the whole year. Figures B2 and B3 show these box plots contrasting the observations from the sites. The box plots show the normal range box, extreme value symbols (stars) and the median symbols (circle). In order to prepare undistorted plots, values less than the lower detection limit (<1) were replaced by 0.5, and values greater than the upper detection limit values (>2,419.2) were removed. The number of observations greater than the UDL removed for each site is noted at the bottom of box plot.

As is common for most wet-weather bacteria observations, overlaps exist between different sampled categories. Larger overlaps require additional data to distinguish the data sets. The overlapping values observed for the sites prone and not prone to urban wildlife made it difficult to confirm if the sites had significantly different bacteria levels. Roof and street areas obviously had the largest differences, as shown on these figures.

The plots were supplemented with statistical tests to measure the significance of the likely differences between paired data sets. Kruskal Wallis tests were conducted, with values greater than UDL and less than LDL values were replaced by UDL and LDL values. The Kruskal-Wallis test performs a hypothesis test of the equality of the population medians for a one-way design (two or more populations). This test is a generalization of the procedure used by the Mann-Whitney test and offers a nonparametric alternative to the one-way analysis of variance (ANOVA). The Kruskal-Wallis test looks for differences among the population medians. The Kruskal-Wallis hypotheses are:

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

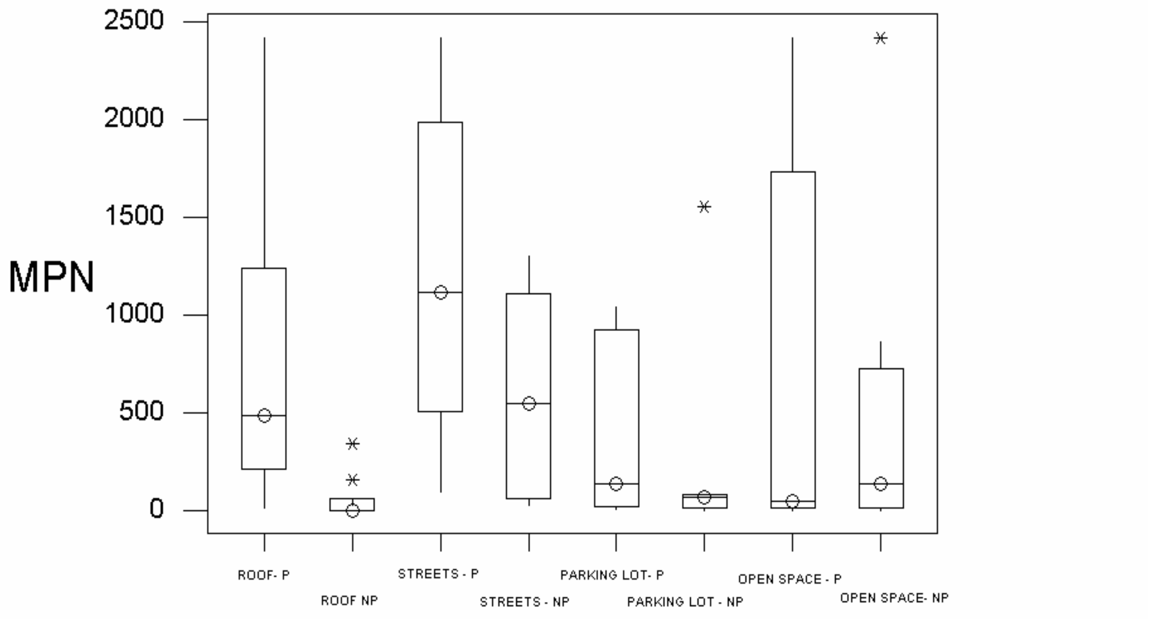

Figure B2. Group Box Plot for *E. coli* for all Warm Months*
***No. of values >2,419.2 removed: Roof- P: 2; Street-P: 4; Street- NP: 2**

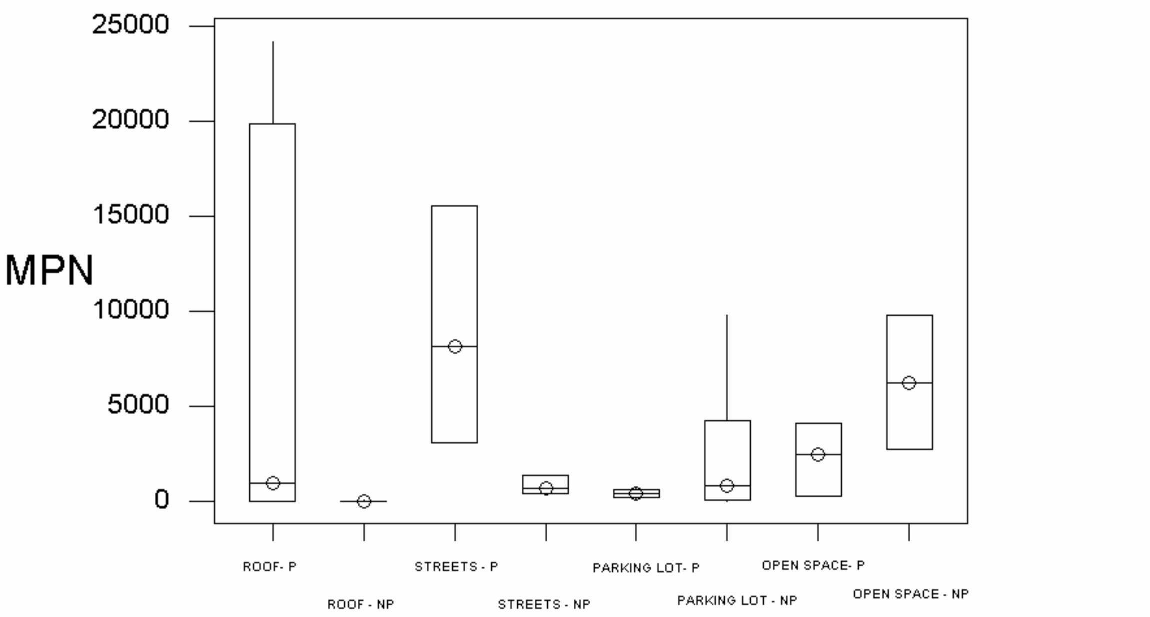

Figure B3. Group Box Plot for Enterococci for all Warm Months *
*** No. of values >2,419.2 removed: Roof- P- 3, Street-P-6 , Street- NP- 4, Parking lot -P- 3, Parking lot -NP- 2, Open space- P- 4 and Open space- NP-5**

An assumption for this test is that the samples from the different populations are independent random samples from continuous distributions, with the distributions having the same shape. The Kruskal-Wallis test is more powerful (the confidence interval is narrower, on average) than Mood's median test for analyzing data from many distributions, including data from the normal distribution, but is less robust against outliers (MINITAB help menu). Table B5 shows the results of the Kruskal Wallis tests.

Table B5. Kruskal Wallis Test Results Summary

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

* Values adjusted for ties.

In order to see if the data patterns were reasonably similar, additional tests using the paired sign test were conducted. The sign test does not require the distributions to be of same shape, or for the variance to be the same. Moreover, the values greater than and less than the quantification range can also be included. Paired tests were conducted because, except for the presence of trees, all other physical parameters that may affect the results, such as temperature, rainfall, type of land use, location etc. were very similar in both cases during each sampled event. First, the differences between the prone observations and not prone observation were found. The sign test of the median = 0 vs. >0 was performed on the difference using MINITAB. Table B6 shows the results of the paired sign tests.

Table B6. Paired Sign Test Results

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

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

The levels of indicator bacteria present in the source area stormwater exceeded the EPA 1986 water quality criteria (single sample maximum value) in 31% (*E. coli*) and 74% (enterococci) of the samples, and the geometric mean criteria was exceeded in 100% of the source area areas. Since none of these sites could be contaminated by sewage, urban birds and animals were found to be significant, but variable, contributors to elevated levels of stormwater bacteria.

Variability in Bacterial Levels

Because of the large variability found for the bacteria analyses in the sheetflow samples, additional tests were conducted to determine the potential causes for this variability.

Variability within Storms. During a single storm on 25 September 2002, all the sites were sampled twice, once in the morning and then again in the evening (Figure B4). From these figures, it is clear that bacterial levels in urban runoff from various source areas vary within storms, but there is no consistent pattern: some areas may have an increase in bacteria levels, while other areas may experience a decrease. Paired sign tests for morning vs. evening sampling gave probability (p) value of 1 for both *E. coli.* and enterococci i.e. no significant differences were observed at the 0.05 level (not enough data is available to indicate they are the same). Since no dilutions were made for enterococci samples for this storm, most of the values remained above the upper detection limit.

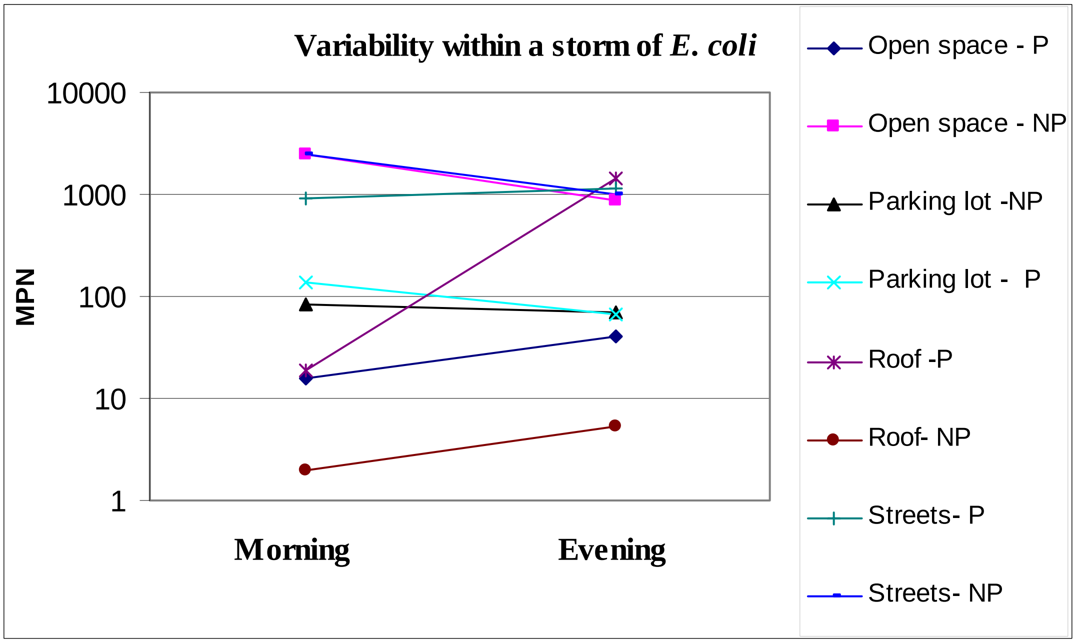

Figure B4. Variability within a Storm for *E. coli*

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

- *Climate.* The climate of Tuscaloosa, Alabama, is subtropical with four distinct seasons, and is humid with no dry season. December through February are winter months. Frosts and freezes are possible during this period. Cold periods, which are short lived, are associated with cold fronts, which may be accompanied by large amounts of rain. The average monthly temperature during these months is below 50^0 F. March and April are considered to be spring months. During this period, daily high temperatures are usually less than 80 degrees F., and freezes are rare. Spring-like temperatures are common from late February through most of April. Summer-like conditions usually begin in late April, or early May, and last until the end of September or early October. May through September are considered summer months. Summer temperatures above 90 degree F. are normal, and summer high temperatures almost never drop below 80 degrees F, and lows are usually in the 60s. October and November are considered to be the autumn months. The temperatures during these months are similar to spring, but there is less rainfall. (www.math.ua.edu/weather.htm#data, 2002).

The geometric mean values for samples collected during the cold months (December through February, with temperature below 50^0 F) were compared with samples collected during the other months (Table B7). Cold weather values were found to be much lower than the warm weather, except in the case of Roof- NP where one unusually high value was found. Thus, seasonally low temperatures may be associated with decreases in bacterial levels. Due to only two observations for winter months, statistical test could not be performed.

Table B7. Comparison of Geometric Means

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

- *Amount of Rain Occurred before Sampling.* Six samples from two different source areas were collected at an interval of 15 to 30 minutes. The total rain that occurred (in inches) before the samples were taken was noted from the weather station installed above the CEE departmental building. Table B8 shows the collected data. As can be seen from Figures B5 and B6, bacterial levels may increase or decrease with increasing amounts of rain with time, but stayed within a generally narrow band.

Table B8. Effect of Total Rain and Rain Intensity on Bacterial Levels

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

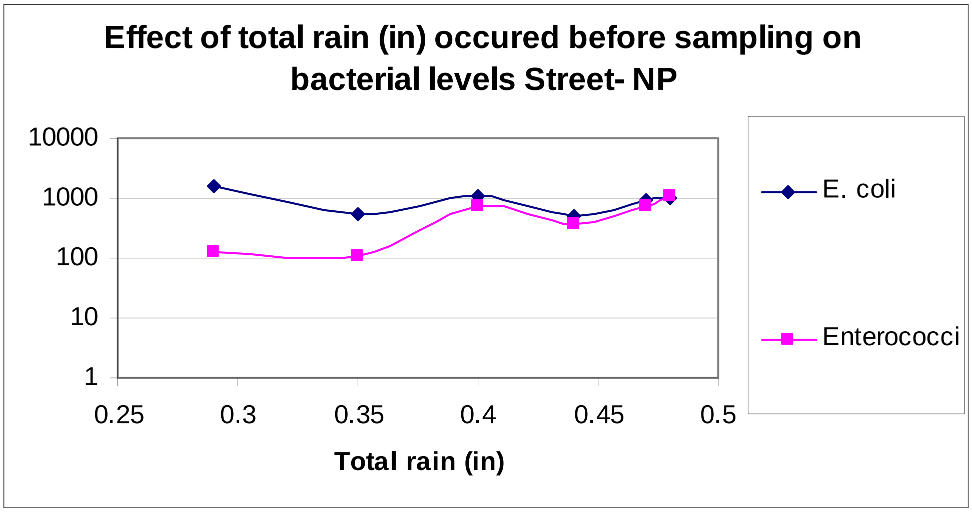

Figure B5. Effect of Total Rain on Bacterial Levels (Street- NP)

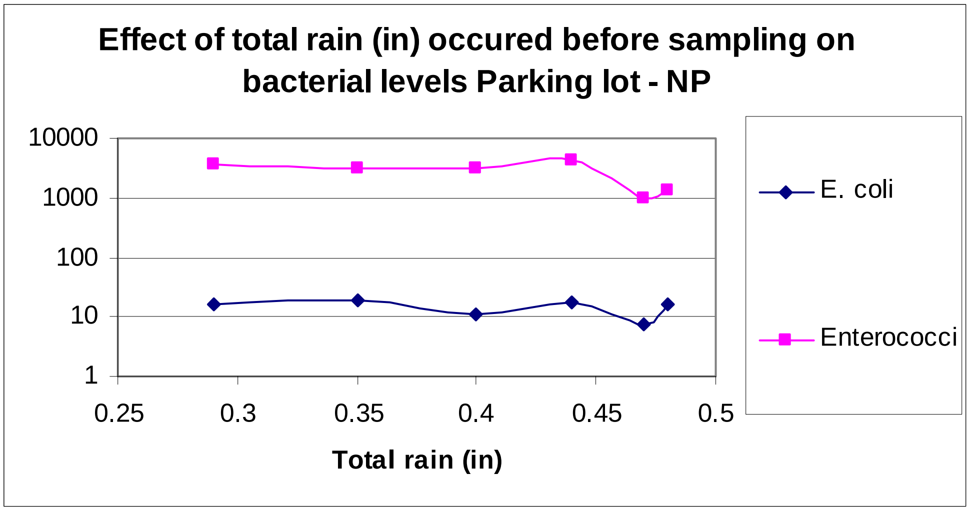

Figure B6. Effect of Total Rain on Bacterial Levels (Parking Lot- NP)

Regression analyses and associated ANOVA tests were conducted to determine the significance of the slope term in the relationship between total rain depth and bacterial levels. In all cases, no significant relationship likely exists between total rain depth and bacterial levels.

- *Rain Rate (in/hr).* Table B8 also shows the 5 minute peak rain intensity found for each of these sampling intervals and these are plotted on Figures B7 and B8.

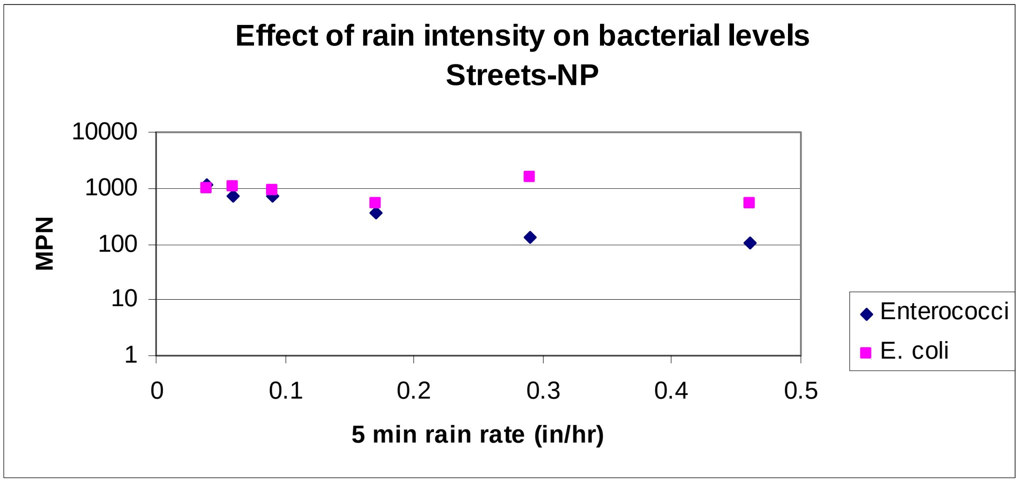

Figure B7. Effect of Rain Rate on Bacterial Levels (Streets-NP)

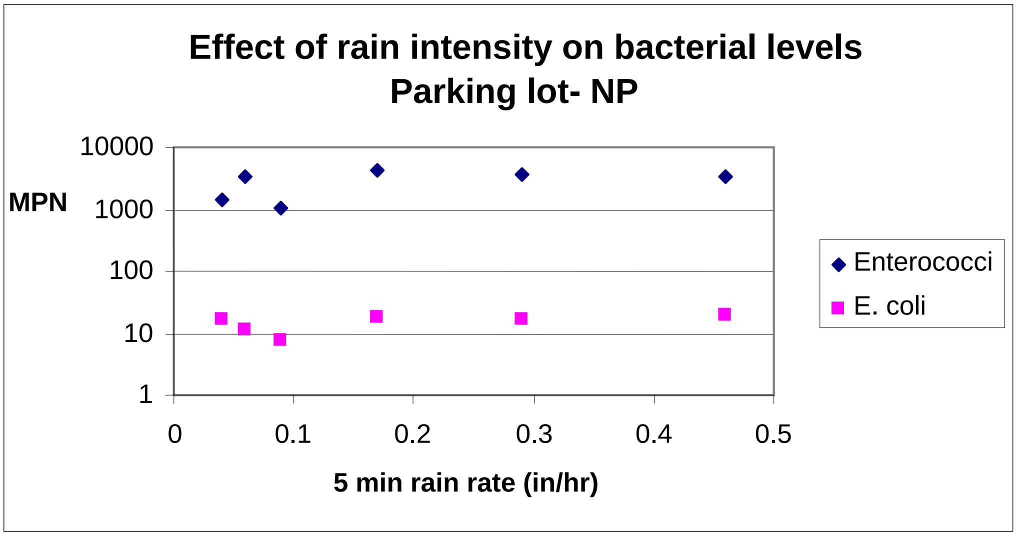

Figure B8. Effect of Rain Rate on Bacterial Levels (Parking Lot- NP)

Regression analyses and associated ANOVA tests were conducted to determine the significance of the slope term in the relationship between rain intensity and bacterial levels. Except for enterococci levels from street- NP, the slope term relating the rain rate and the bacterial levels were not significant. The enterococci levels decreased with rain rate for this site and condition.

- *Effect of sample handling.* Three factors involving sample handling were also studied which could affect the analytical results. These included holding time before analysis, refrigeration, and the effects of shaking. For these tests, a single 5 L sample was obtained from one source area. Subsamples, each as 100 mL duplicates, were tested after 1, 2, 5, 9, 24, and 48 hrs (Table B9). After the 9 hr samples were taken, the 5 liter sample was split into two components; one was kept refrigerated while the other was kept at room temperature (about 20° C). Figure B9 shows the variation of bacterial levels with sample holding time.

Table B9. Effect of Holding Time

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

Not refrigerated and not shaken

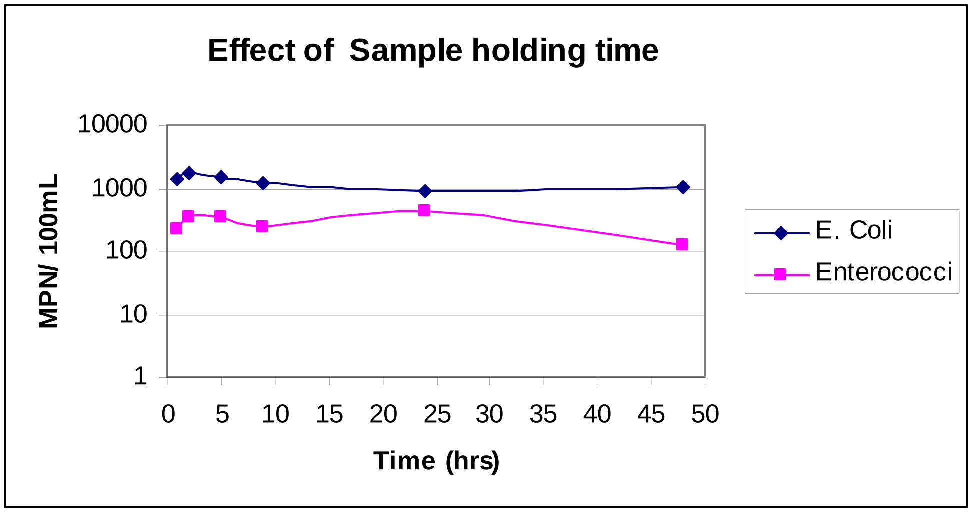

Figure B9. Variations with Sample Holding Time

The effect of refrigeration over one to two days was then measured (Table B10). All these samples were shaken before analyses.

Table B10. Effect of Refrigeration

Holding Time Hrs	Refrigeration	*E. coli* MPN/100 mL	Enterococci MPN/100 mL
24	Refrigerated	1046.2	689
24	Not Refrigerated	920.8	419
48	Refrigerated	1299.7	240
48	Not Refrigerated	1046.2	128

The effect of shaking was measured by first taking a 100 mL sample from the unshaken larger sample container, and later shaking the larger sample bottle and testing another 100 mL sample (Table B11).

Table B11. Effect of Shaking

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

A 2^3 factorial evaluation was conducted to identify the main effects and effects of interactions between these handling factors. Table B12 shows the factorial design. The calculated main effects and interaction effects are shown in Table B13 and the normal probability plot of the effects are shown on Figures B10 and B11, indicating the significant factors and interactions.

Table B12. Factorial Design

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

Table B13. Main Effects and Interaction Effects

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

Interpretations are needed for R and TS for *E. coli* and T only for enterococci, as can be seen from the probability plots of effects (Figures B10 and B11). Based on these effects, the calculated values were found using the equations:

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

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

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

Tables B14 and B15 shows the calculated and observed values for various conditions.

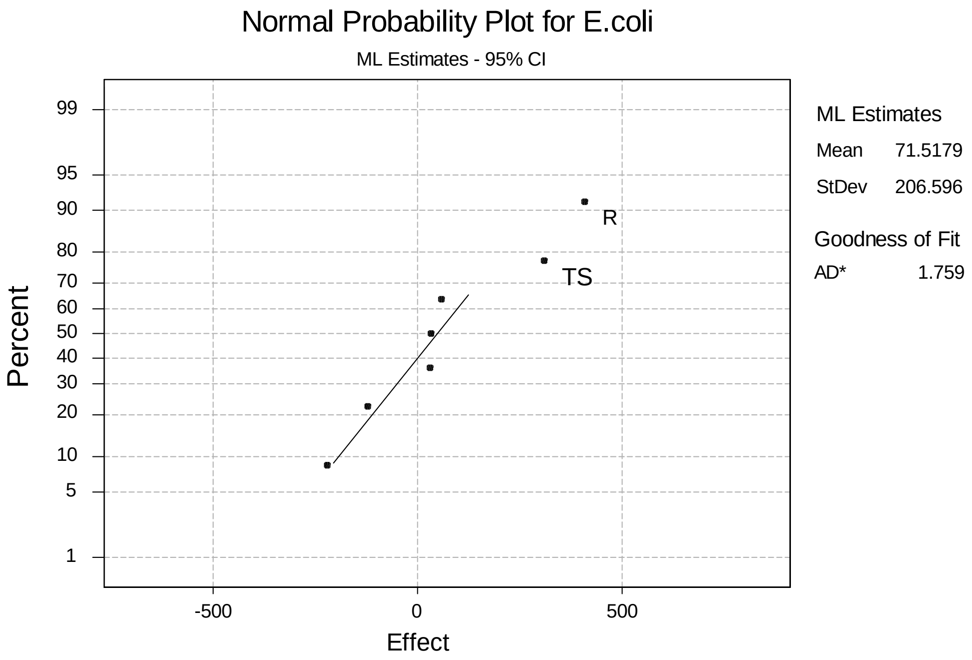

Figure B10. Normal Probability Plots for Effects (*E. coli)*

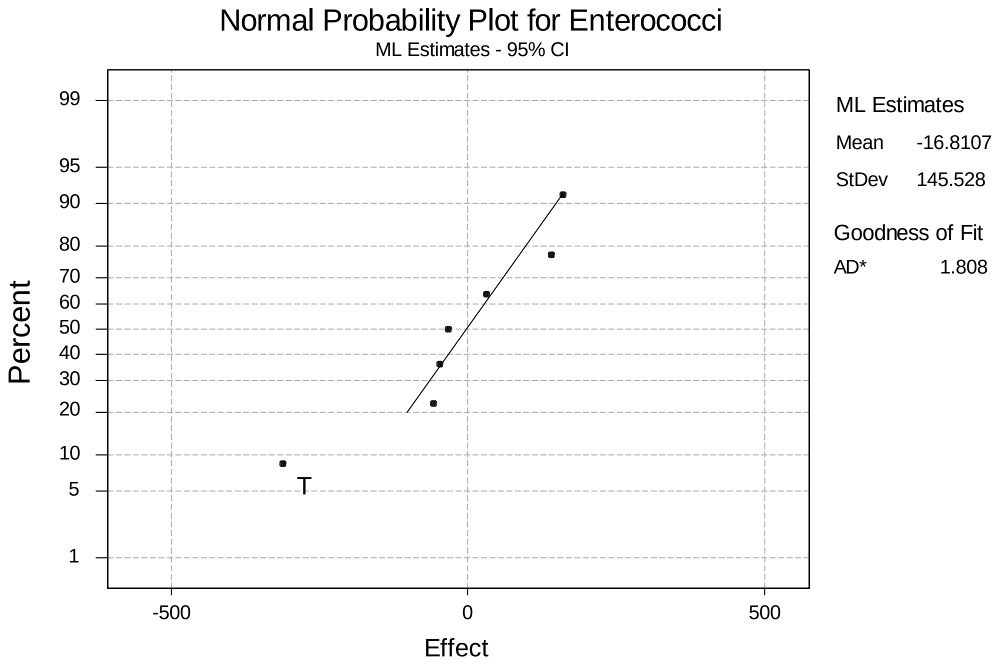

Figure B11. Normal Probability Plot for Effects (Enterococci)

Table B14. Calculated and observed values (*E. coli*)

Condition			
TS	R	**Calculated Values**	**Observed Values**
+	+	1410	1553, 1300
+	-	1098	921, 1046
-	+	1000	1120, 1046
-	-	688	488, 921

Table B15. Calculated and Observed Values (Enterococci)

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

Residuals were calculated and normal probability plots were prepared (Figures B12 and B13). From these plots and analyses, it is clear that refrigeration (R) and the time- shaking interaction (TS) affect the *E. coli* levels. Only the effect of refrigeration over a period of two days was studied, not for shorter time periods. Refrigeration of samples reduced the dieoff rates of *E. coli,* and refrigerated samples showed correspondingly higher levels of *E. coli* compared to samples that were not refrigerated, all as expected. During this research, precautions were taken to minimize the effect of these adverse factors. Samples were always transported from the field to the laboratory in an ice cooler and analyzed as soon as possible to reduce the holding time. All samples were vigorously shaken before analyses.

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

Comparison of Sewage Data with Wet Weather and Dry Weather Data

Another objective of this research was to determine if *E. coli* and enterococci could be effectively used to identify inappropriate sanitary sewage discharges in storm drainage systems. For this purpose, sewage samples were compared with wet weather and dry weather source area samples (from the project reference sample library). The most important comparison was between sewage samples collected during wet weather and wet weather urban runoff source area samples. Mann Whitney tests were conducted using MINITAB and probability (p-values) calculated to identify significant differences in the data sets.

Bacteria levels were originally measured in sewage samples collected from the Tuscaloosa wastewater treatment plant by dilution to 0.01% sewage. Calculations were then conducted to determine bacteria levels in 0.05, 1, 1.5, 2, and 5 and so on up to 100 % sewage mixtures. Runoff data from each source area were compared with the calculated values for every dilution ratio. The probability of the sewage and source area sample bacteria levels being significantly different was determined using the Mann Whitney test. Figures B14 and B15 are plots showing the resultant p-value and percentage sewage dilution. When the values of the probabilities were ≤ 0.05, the diluted sewage sample bacteria levels were determined to be significantly higher as compared to bacterial levels in the urban runoff source area samples (with a 1 in 20 error level). *E. coli* levels in diluted sewage start showing significantly higher values ($p \leq 0.05$) as compared to urban runoff (compared to streets prone which had the highest *E. coli* values) at 0.13% sewage in clear water (Figure B14). The mean value of *E. coli* corresponding to 0.13 % sewage in clear water is 3,470 MPN/100 mL. Thus, if the *E. coli* levels found from a storm drain outfall exceed 3,470 MPN/100 mL during wet weather, the most likely source (with a 1 in 20 error level) is sewage contamination (other possible contaminating sources have significantly lower bacteria levels).

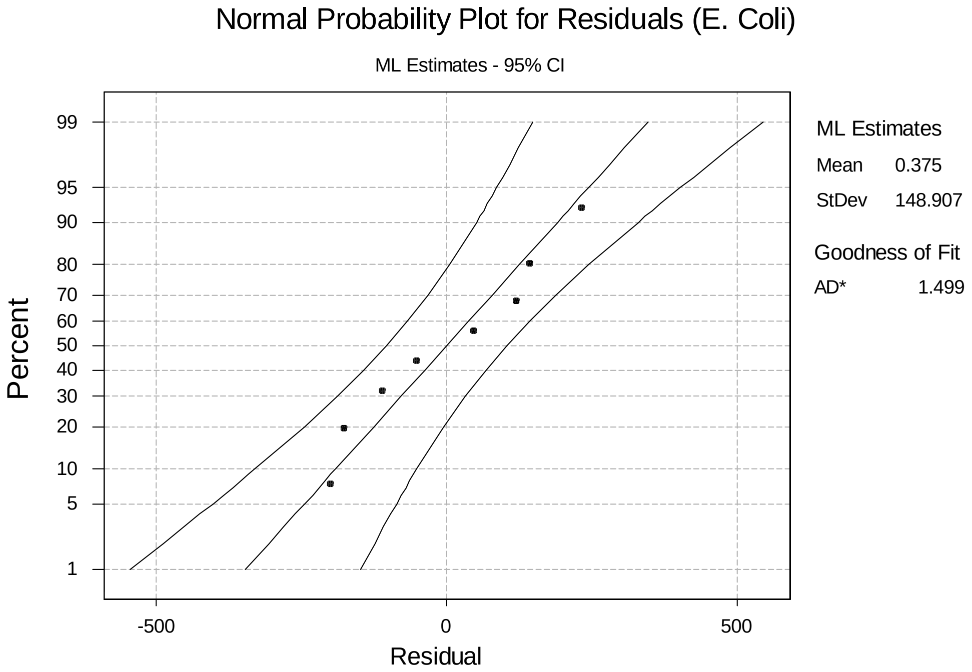

Figure B12. Normal Probability Plot for Residuals (*E. coli*)

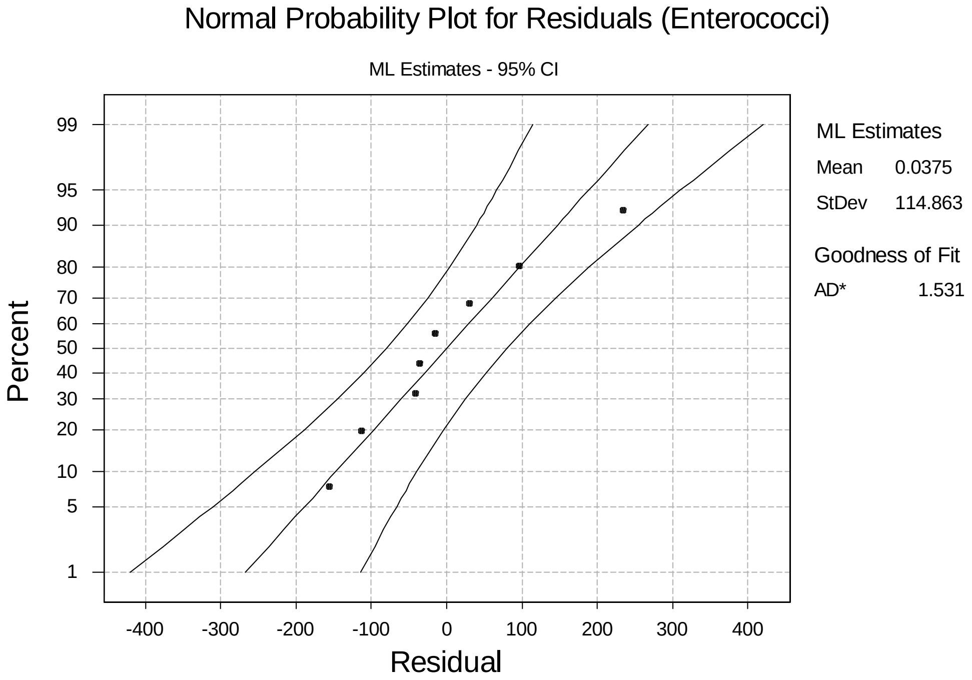

Figure B13. Normal Probability Plot for Residuals (Enterococci)

Similarly, enterococci levels in sewage start showing significantly higher values as compared to urban runoff source area samples (from Open spaces-NP which had the highest values) at 3.7% and higher sewage in clear water (Figure B15). The mean value of enterococci corresponding to 3.7% sewage in clear water is 18,530 MPN/100 mL. Thus, if the enterococci levels found at a storm drain outfall exceed 18,530 MPN/100 mL during wet weather, the high bacteria levels are most likely from sewage contamination. Lower bacteria levels at the outfalls are likely from urban animals, or sewage diluted more than these levels.

Similar plots and analyses were made between reference library samples (collected during dry weather) and percentage sewage in clear water (Figures B16 and B17). Dry weather outfall samples having *E. coli* and enterococci levels equal to or higher than 12,000 MPN/100 mL and 5,000 MPN/100 mL respectively, are most likely contaminated by sanitary sewage. Based on these observations and analyses, the earlier simple flow chart developed by Pitt, *et al.* (1993) and Lalor (1994) to identify the most significant component of flow from an outfall has been modified, as shown in Figure B18.

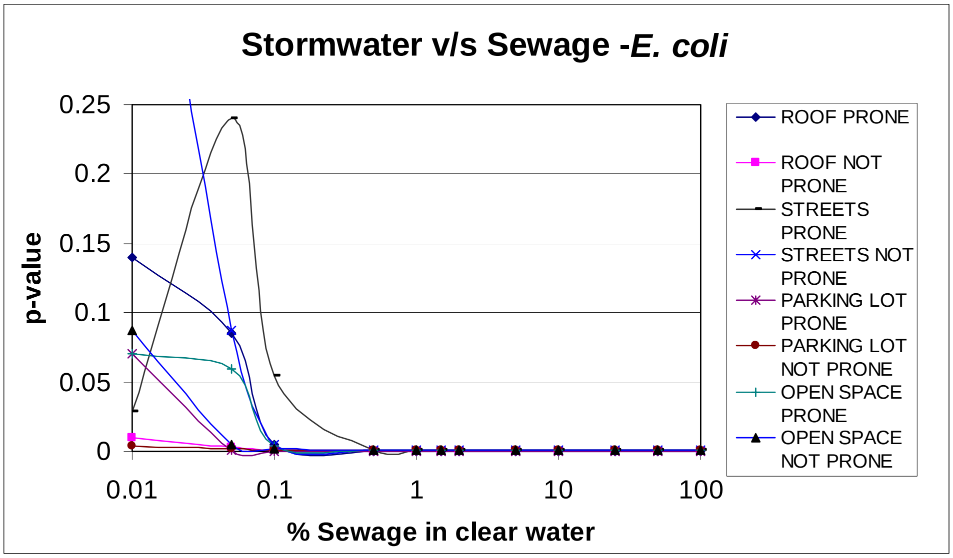

Figure B14. Comparison of Sewage with Wet Weather Data (*E. coli*)

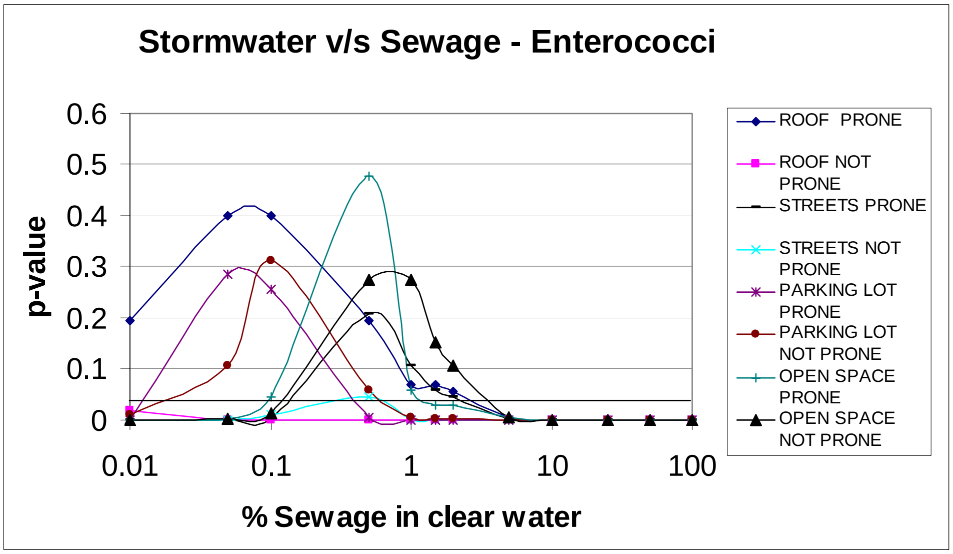

Figure B15. Comparison of Sewage with Wet Weather Data (Enterococci)

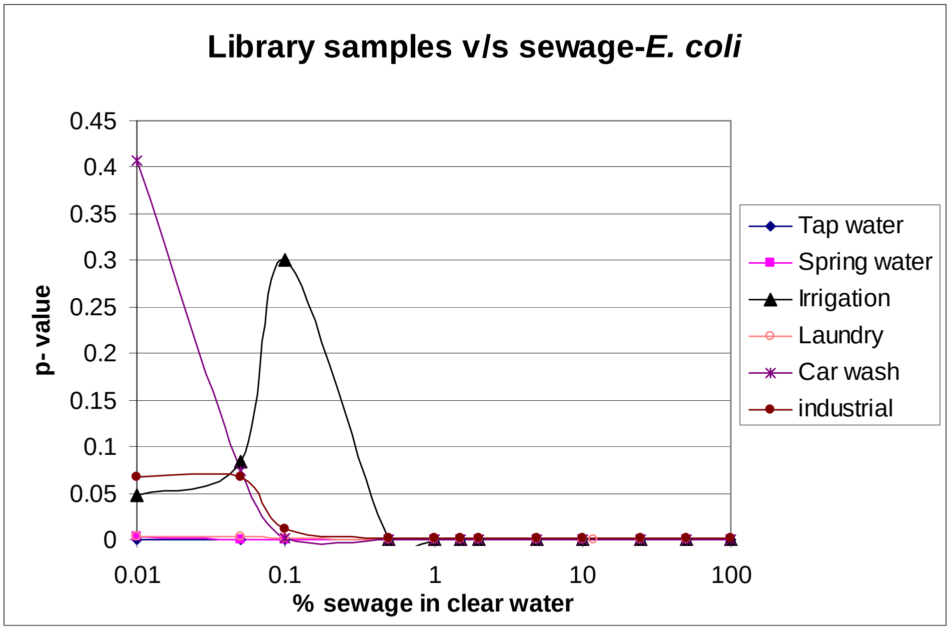

Figure B16. Comparison of Sewage with Dry Weather Data (*E. coli*)

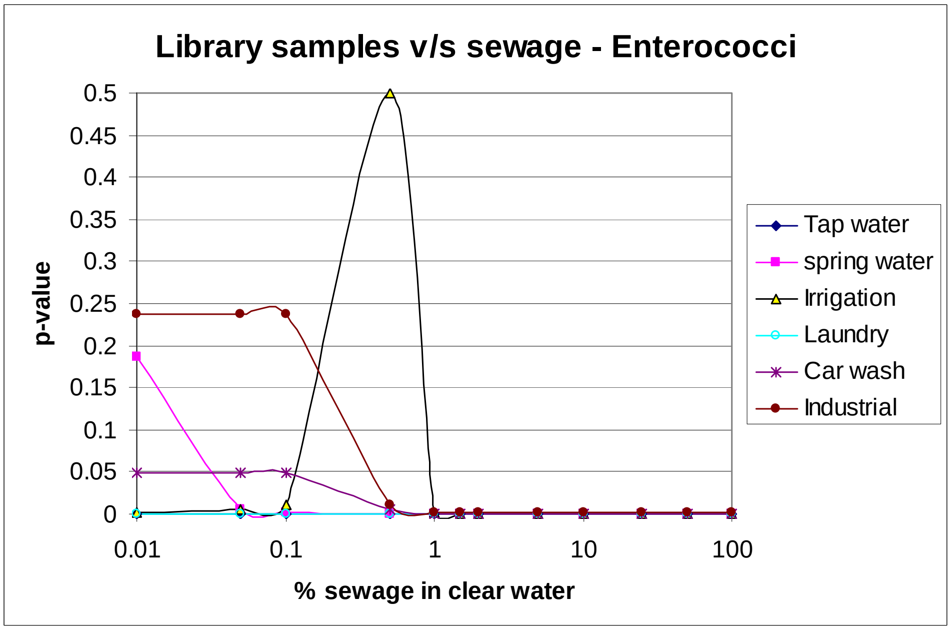

Figure B17. Comparison of Sewage with Dry Weather Data (Enterococci)

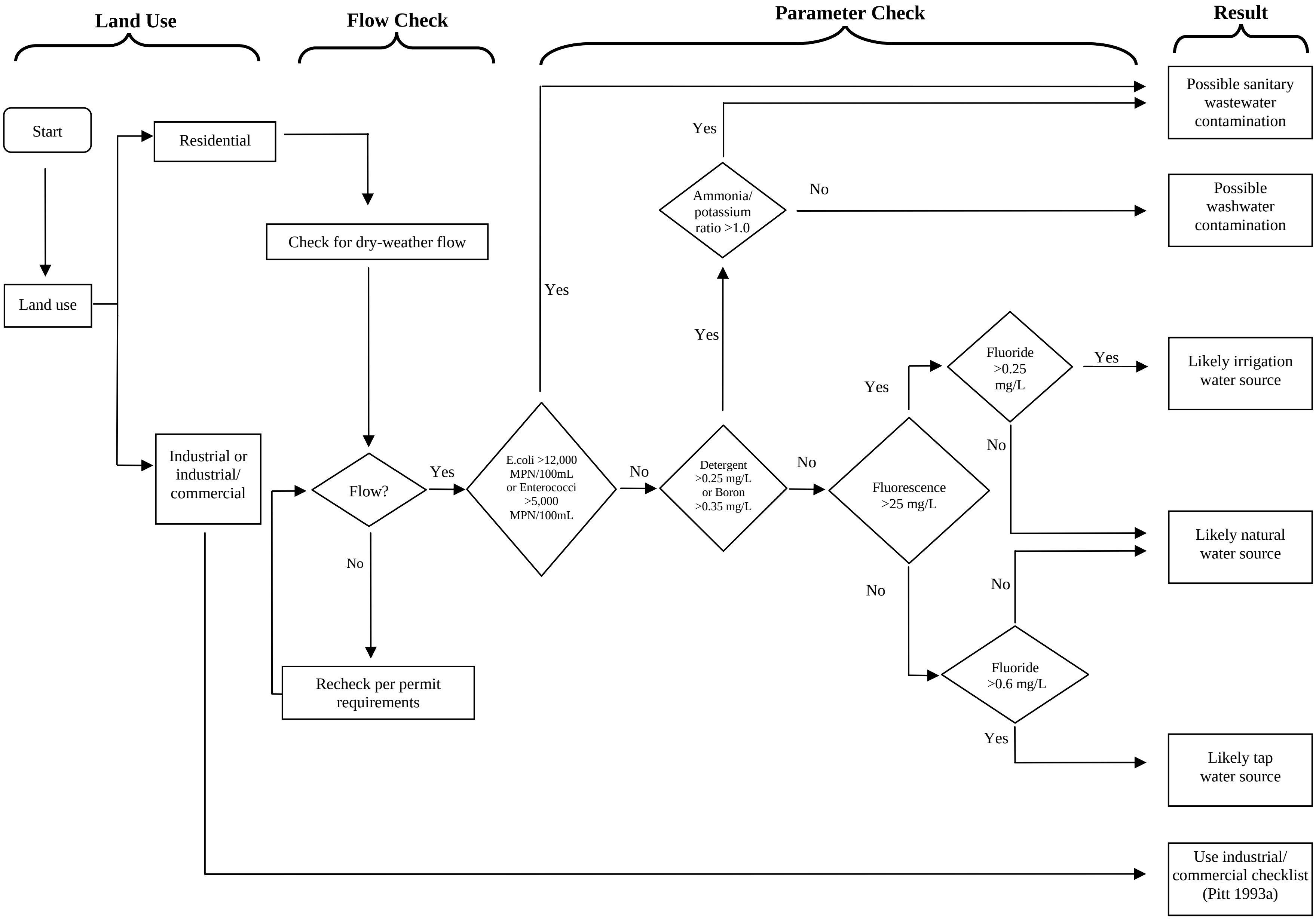

Figure B18. Modified Flow Chart to Identify Most Significant Flow Component