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Investigation of Bacterial Fecal Indicators and Coliphage Virus in Sediment and Surface Water of Parks and Beaches along the Grand River (MI) and Lake Michigan (MI)

presented by

Shikha Singh

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of the requirements for the

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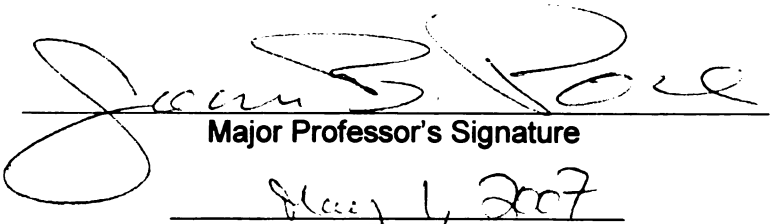

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# Investigation of Bacterial Fecal Indicators and Coliphage Virus in Sediment and Surface Water of Parks and Beaches along the Grand River (MI) and Lake Michigan (MI)

BY

Shikha Singh

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ABSTRACT

Investigation of Bacterial fecal indicators and Coliphage Virus in Sediment and Surface Water of Parks and Beaches along the Grand River (MI) and Lake Michigan (MI)

By

Shikha Singh

According to a report by the Natural Resources Defence Council (2006) the numbers of beach closing and advisory days have increased from 2003 where advisories increased in the state of Michigan by 174 % due to elevated bacterial levels and are associated with public health risks and economic vitality of the community. The *objective* of this study was to examine fecal indicator species spatially and temporally and to determine if a relationship exists between sediment and surface water quality. Eight sites in the state of Michigan along the Grand River and Lake Michigan were studied year round. Parks had significantly higher concentrations of fecal indicators compared to the beaches ($p<0.05$) indicating a large dilution effect before reaching the lake. Results were assessed for correlation between sediments and surface water for fecal coliforms, *Escherichia coli,* Enterococci, *Clostridium perfringens* and coliphage. The $R^2$ values for the aforementioned ranged between 0.444 and 0.128. While sediment quality was not directly related to the water column above it, it is a reservoir of fecal contaminants downstream and was 2-3 log10 higher. In general, most indicators had highest concentrations in surface water during the winter and spring seasons. For rainfall, fecal coliform and *E. coli* had a positive relationship with surface water and negative relationship in sediment indicating rainfall contributes to releases from sediment.

# . ACKNOWLEDGMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

**Historical notes on water contamination**

Fecal contamination of water has been a source of concern for hundreds of year and has led to spread of waterborne diseases, currently resulting in up to 5 million deaths world wide (WHO, 1992). This includes diseases such as cholera and typhoid which have long been associated with water contaminated with sewage and feces contamination. The answers to the questions of how and where to dispose of fecal matter, has evolved over time. Beginning in ancient Greek and Roman times chamber pots were used (Mattelaer, 1999) to collect bodily refuse and were subsequently tossed out windows onto busy streets (with a verbal warning...sometimes) along with development of sewers and drainage where was entered into water sources such as streams and rivers. It was not until the 1800s that the relationship between diseases (such as cholera) and contamination of water was brought to light by Dr. John Snow, known as the father of epidemiology.

Between 1849 and 1854 Dr. Snow found 286 deaths among those that used water from the river Thames with higher amount of sewage inputs in London compared to just 15 deaths from those who drew water from up river near Thames Ditton (Stewart-Tull, 2001) which at the time, was located in the suburbs. The epidemiological information gleaned from this study has influenced our understanding about the protection of water resources from fecal contamination. However, despite this information and knowledge the contamination of fresh water resources in the United States with untreated and / or improperly treated sewage (such as Combined Sewer Overflows and non-point source pollution, which will be discussed later on) remains a significant challenge for communities and public health. Better assessment of fecal contamination is needed in

order to address the overwhelming financial burden of upgrading our sewage infrastructure to improve water quality and safeguard surface waters and particularly our recreational waters.

## 1.0 Introduction

Environmental degradation and pollution of recreational and drinking water have recently moved back to the forefront of public interest. In addition to water quality concerns, the role of sediments as reservoirs of contamination in aquatic systems remains a complex and important area of study.

When dealing with water quality, there are pollution and contamination issues. *Pollution* is the introduction of substances into the environment that results in a negative effect to ecosystems. This is the accumulation and adverse affects of contaminants or pollutants and present hazards to human and animal health. Contamination is may be a result of anthropogenic waste materials produced from the activities of humans, but can also occur from natural processes such as blooms of hazardous algae, arsenic dissolution from bedrock into ground water, smoke from natural sources presence of elevated concentrations of substances in water, sediments or organisms.

Pollutants can impact the ecosystem and their inhabitants. Pollutants can bioaccumulate in organisms or become biomagnified. Bioaccumulation is the net accumulation of pollutants over time within an organism from both biotic and abiotic factors (environmental). However, when dealing with food trophic levels, the

progressive accumulation of persistent toxicants by successive trophic levels is known as biomagnification.

The higher the organism is on the food chain (top predator) the more magnified the pollutant is, as it now contains the cumulative amount of pollutant of lower prey and the prey of its prey. This is especially evident in issues of metal contamination such as mercury where whales are at risk due to the large quantities of fish (each of which has mercury) which has a magnified affect to the whale. This can be seen in the ratio of the pollutant in tissue of predator compared to its prey.

It has been recognized by the U.S Environmental Protection Agency (EPA) that nutrients (Phosphorous and Nitrogen), bulk organics (oil and grease), halogenated hydrocarbons (or persistent organics, such as DDT and PCB's), polycyclic aromatic hydrocarbons, metals (mercury, lead, manganese, cadmium, zinc) and metalloids (arsenic and selenium) are some of the major pollutants found in sediments (US EPA, 2005). For these chemical pollutants, there are guidelines, standards and risk- levels set forth by governmental agencies for water, sediment, and soils. However, there are no such guidelines for dealing with microbial contaminants and pathogens found in sediment as exist for surface water...neither in the "Clean Water Act" (US), "BEACH Act" (US) nor in the Canadian Environmental Quality Guidelines (Environment Canada, 2005).

Microbial degradation of ambient waters is a known problem. According to the 2005 report by the Natural Resources Defense Council (NRC) in 2005, the numbers of

beach closing and advisory days increased by 9% in 2004 from 2003. Beach closings and advisories increased in Michigan by 174 % in 2004 from 2003 due to elevated bacterial levels (NRDC, 2005). This increase may be a result of increased number of beaches monitored and increased amounts of rain during the sampling year. In the latest 2006 NRDC report, there was a 5% increase in ocean, bay and Great Lakes beach closings and advisories in 2005 due to bacteria and of the 14,602 advisories and closures, 63% were due to unknown sources (NRDC, 2006). While Michigan had a decrease in advisories and closures in 2005 compared to 2004, the net amount was higher than 2002. One of the Ottawa county beaches in Michigan at Lake Michigan [a site used in this study (Rosy Mound Beach)] was found to have a 13% exceedance rate (NRDC, 2006). This has potentially serious repercussions for the public who are exposed to this poor water quality. Human health risks related to these beach closings are based on high levels of fecal indicator microorganisms in the water. Indicators microorganisms are those generally found in animal and human intestines and are discharged into the environment through sewage, manure, wildlife fecal deposits and storm waters.

## 1.1 Bacterial and Coliphage Fecal Indicators

In order to assess water quality in terms of microbial pollution, fecal coliforms, *Escherichia coli*, and Enterococci are the main indicator microorganisms used and to some extent *Clostridium spp.* and bacteriophages (viruses that only infect bacteria).

Fecal Coliform bacteria belong to the family enterbacteriaceae as shown in Table 1-1. Coliforms are aerobic and facultative anaerobic, non-spore forming, gram negative

bacteria able to ferment lactose at 35°C (Total coliform) and 44.5°C (fecal coliform). Coliforms live in the digestive tract of warm-blooded animals (humans, pets, farm animals, and wildlife) (Gerba, 2000) and are excreted in the feces. In themselves, fecal coliforms generally do not pose a danger to people or animals but they indicate the possible presence of other fecal pathogens, disease-causing bacteria, such as those that cause typhoid, dysentery and cholera. *Escherichia coli* are a specific type of coliform bacteria that possess the enzyme β-glucuronidase and are capable of cleaving substrate 4-methylum-belliferyl- β-D glucuronide (MUG). Their presence indicates specifically fecal contamination, and the possibility of enteric pathogens. *E. coli* is a worldwide universal indicator of fecal contamination of water. Some strains of *E. coli* are harmful, and do cause sickness. One type of *E .coli* (O157) causes gastroenteritis (Levy *et. al.* 1998) and hemolytic uremic syndrome and has been found in water at beaches and is associated with illnesses in swimmers (Ihekweazu *et. al.*, 2006, Bruneau, A *et. al.* 2004., Harrison and Kinra, 2004, and Paunio *et. al.* 1999).

**Table 1-1.** Classification of bacteria used in this study

| **Kingdom** | Bacteria | Bacteria | Bacteria |
|---|---|---|---|
| **Phylum (P) / Division (D)** | Proteobacteria (P) | Firmicutes (D) | Firmicutes (D) |
| **Class** | Gamma | Bacilli | Clostridia |
| **Order** | Enterobacteriales | Lactobacillales | Clostridiales |
| **Family** | Enterobacteriaceae | Enterococcaceae | Clostridiaceae |
| **Genus** | *Escherichia* | *Enterococcus* | *Clostridiaceae* |
| **Species** | *coli* | *avium*<br>*durans*<br>*faecalis*<br>*faecium* | *perfringens* |
| **Shape** | Rod | Cocci (sphereical) | Rod |
| **Growth condition** | Aerobic & Facultative anaerobes | Facultative anaerobes | Anaerobic |
| **Gram Stain Reaction (+/-)** | - | + | + |
| **Sporulate** | No | No | Yes |

Enterococci are part of what is known as the fecal streptococci group; specifically *E. faecalis* and *E. faecium* are found in humans (Gerba, 2000) but are not restricted to humans. Enterococci are well suited as nosocomial pathogens because they readily colonize skin and mucous membranes (Mallon, 2002) but are excreted in the feces of animals and humans. These bacteria also tolerate temperatures from 10°C to 45°C, survive in acid and alkaline conditions and are more resistant to environmental stress and chlorination than coliform bacteria. They generally persist longer in the environment (Maier *et. al.*, 2000., Gleeson and Gray, 1997., Mellon, 2002).

One area of concern documented in literature involving water samples (such as wastewater, river and agricultural runoff) was the occurrence of *Enterococci* that showed antibiotic resistance. Rice *et. al.* (1995) found *Enterococcus faecalis*, *E. faecium*, *E. gallinarum* (collected within a 30-mile radius of Cincinnati, Ohio) demonstrating patterns

of antibiotic resistance to amino-glycosides. With agricultural drainage areas (smaller bodies of water) flowing into larger bodies of water, this could lead to higher chances of pollution containing potentially antibiotic resistant bacteria and other pathogens in rivers, lakes and recreational areas. There is a fear that some of the antibiotic resistant bacteria may be difficult to treat if a recreational user is exposed and becomes infected

*Clostridium perfringens* are spore forming bacteria which replicate under anaerobic conditions. They are excreted in the feces, and can be an indication of old pollution as the spores are able to persist in the environment. Some have suggested that this makes them more suitable as an indicator for the presence of persistent pathogens of a fecal origin (Payment and Franco, 1993) such as viruses and parasites. Due to the ability of *E. coli* and *Enterococci* to re-grow in the environment under warmer conditions, and *C. perfringens* generally unable to do so (Davies *et. al.*, 1995), it is considered a pragmatic addition to other commonly used indicator species. Due to lower decay rates, *C. perfringens* can persist in the environment significantly longer than enteric pathogens (Cabelli, 1978) making them good indicators of fecal pollution (Ashbolt, 2001), especially in warmer tropical waters.

Viruses are microscopic nano particles which infect cells of a living organism and cannot replicate on their own. Bacteriophage are virus that infect bacteria, and coliphages are bacteriophages that are specific to the host *E. coli*. Because coliphages come from fecal material, their presence in water bodies indicates fecal contamination as well as the survival and transport of potential human viral pathogens in that same source

(Noble *et. al.* 2003). Bacteriophage resemble enteric viruses in size, structure, morphology and behaviour in water (Pepper, Gerba and Brusseau, 2006), therefore their presence in the environment can indicate a possibility of human viruses if sewage is the source of fecal contamination. Bacteriophages are also advantageous to use because they are less likely to replicate in the environment. Raw sewage inputs found along the Rio Grande River basin near the United States-Mexican border region, were best represented by male specific and somatic coliphages detected in 52% (11/21) and 62% (24/39) of the samples, respectively with somatic coliphages being greater by one order of magnitude compared to male specific (Ryu *et. al.*, 2005). It was suggested that these findings were a result of surface water runoff and constant agitation of the water causing re-suspension of already present microbes, input and continuous sewage loading and combined sewer overflow events.

In order to gain a better understanding and the extent of fecal pollution, and to minimize uncertainty and extent of contamination , the use of multiple indicator species is suggested by Gerba and Rose (2003) such as *C. perfringens* and coliphage in addition to standard indicators such as *E. coli* and Enterococci.

### 1.2 Existing Water Quality Acts

In 1914, the United States Public Health Service began using the total coliform group as an indicator of contamination in drinking water (Maier *et. al.*, 2000). The public health service oversaw "safe" water until USEPA was formed in 1970. Congress rewrote and passed the "Safe Drinking Water Act" (SDWA) in 1974 to protect public health by

regulating all public drinking water supplies at a national level. The law was amended in 1986 and 1996 and requires treatment and finished water with a maximum contaminant level goal of zero for parasites and viruses (EPA, 2006).

In 1972, the "Federal Water Pollution Control Act", was amended to what is now known as the "Clean Water Act". Goals of the Clean Water Act are to have "fishable and swimmable waters" and are geared towards contaminants (both microbial and chemical) of navagatible surface waters and, maintaining the physical, chemical and biological integrity of the aquatic system. The Act gives the government power in regulating point source pollutant discharge in navigational waters (Environmental Protection Agency, 2005), wastewater management, and works to minimize non point source pollution. On October 10$^{th}$ in the year 2000, an addition to the "Clean Water Act" was made under the "Beaches Environmental Assessment and Coastal Health" Act, other wise known as the "BEACH Act".

The BEACH Act focuses on human safety and reducing human illness addressing national coastal and Great Lake recreational waters (marine and fresh) via the development of new water quality guidelines. Section 303 of the Federal Water Pollution Control Act (33U.S.C. 1313) was amended by adding "Not later than 42 months after the date of the enactment of this sub-section, each State having coastal recreation waters shall adopt and submit to the Administrator water quality criteria and standards for the coastal recreation waters of the State for those pathogens and pathogen indicators for which the Administrator has published criteria under section 304(a)". The guidelines/standards

referred to in the BEACH Act include microbial indicators Enterococci and *E. coli* measured per 100 mL of water. Table 1-2 shows the EPA water quality criteria and Michigan standards for Great Lakes recreational waters. Measures are for ambient fresh water using geometric means for multiple samples or different days and a single sample maximum for bathing beaches (Federal Register vol 69, 2004). Single sample maximum suggested by US EPA for *E. coli* and Enterococci are 235 and 61 CFU/100mL respectively. Michigan requires a maximum of 300 CFU/100 mL for *E. coli* for a single sample and a geometric mean of 130 CFU/100 mL (at present, a Michigan standard does not exist for Enterococci). Guidelines in both Clean Water and BEACH Acts suggest when and how often to monitor and these are only for the water column. Currently no standards or criteria exist for microbial contaminants in sediment or sand for recreational waters. This is important as not all recreation takes place in the water and occurs on the beach and at the sand/water interface where sediment and sand become agitated.

**Table 1-2.** Fresh water quality guidelines and standards (CFU/100 mL) for areas of recreational use.

| | **Geometric Mean[a]** | **Single Sample Maximum** |
|---|---|---|
| **US. EPA** | | |
| Enterococci | 33 | 61 |
| *E. coli* | 126 | 235 |
| **MDEQ** | | |
| *E. coli* | 130 | 300 |

[a] based on five or more samples equally spaced over a 30-day time period
MDEQ: Michigan Department of Environmental Quality

### 1.3 Fecal Contamination from Point and Non-Point Sources

There are two types of pollution: Point and Non-point pollution. Point source pollution can be traced to a specific source which directly inputs to a water body. Examples of point source pollution include waste water treatment plant, factories and combined sewer overflow pipes. Non-point source pollution comes from a variety of more diffuse sources.

Pollution caused by non-point sources is usually a result of rainfall or snowmelt runoff, which generally doesn't have an end of the pipe discharge, are very diffuse, and picks up and carry both natural and anthropogenic pollutants before depositing them into lakes, rivers, wetlands, and coastal waters. Non-point source pollution also can have an impact on underground sources of drinking water. Examples of non-point source pollution include urban and rural storm water run-off containing oil and grease, agricultural nutrients (also herbicides and pesticides), as well as fecal bacteria, viruses and parasites from wild animals, pet waste, leaky septic tanks (bacteria and nutrients) boats and manure from animal farming operations. Figure 1-1 shows how these sources of pollution creates a pathway for interaction amongst surface water, sediment and beaches and can has the potential to result in recreational human exposure to micro-organisms.

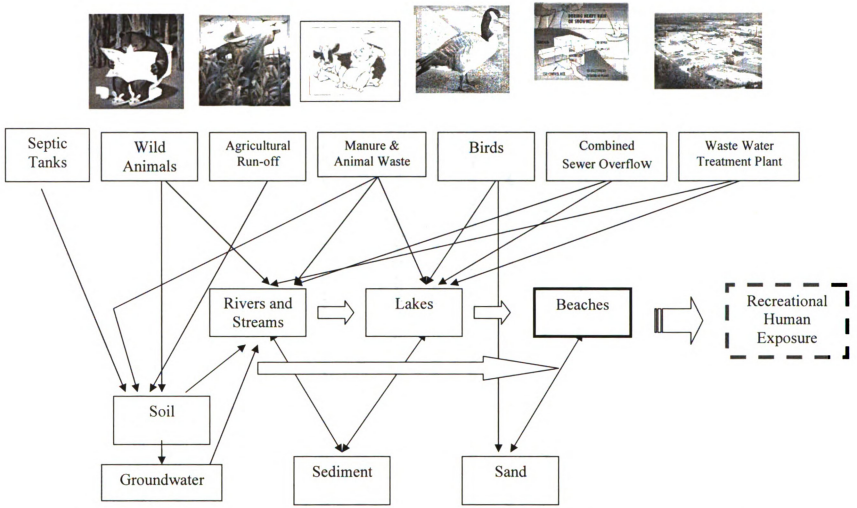


**Figure 1-1.** Sources and transport pathways for fecal micro-oganisms and how it can lead to recreational human exposure and illness.

According to the National Water Quality Inventory (EPA,2000) prepared under section 305(b) of the Clean Water Act, the causes of water body impairments were siltation (sediment), nutrients, bacteria, metals and oxygen-depleted substances. It also found that non-point source pollution in the form of urban and agricultural runoff was the leading source of impairment.

### *1.3.1 Sources of pollution*

**Septic Tank Systems**

Onsite waste disposal systems known as septic tank are individual systems for sewage storage and discharge. Septic systems are used to separate solids from liquid in waste water, after which they are partially treated or piped to a leach field for disposal to the soil. Septic systems from homes and businesses dispose of the liquid portion into the ground through soil layers. The waste water contains bacteria, parasites and viruses. It was found that $10^5$ PFU/100 mL of F-male specific phage occurred in septic tanks (Debartolomeis and Cabelli, 1991). Over 40% of homes in Michigan use septic tank systems (Lusch, 1997), this is similar to other states with large coastlines such Florida where 30% of Florida's population use septic systems (Marrella, 1990).

In the lower reaches of the Myakka and Peace Rivers (southwest Florida), fecal indicator organisms were found in areas with a high concentration of septic systems (Lipp *et. al.* 2001). During wet periods (which coincided with El Nino weather effect in end of 1997-early 1998), the fecal pollution indicators became widespread (Lipp *et. al.*

2001). Both ground waters and surface waters in Wisconsin have been shown to be impacted due to septic tanks particularly with fecal viruses (Borchardt, 2003).

**Combined Sewer Overflows (CSOs) and storm sewers**

According to the Environmental Protection Agency (2003), there are approximately 772 communities in USA which use a Combined Sewer System (CSS) which includes CSOs and accidental SSOs (Sanitary Sewer Overflows). Most CSO facilities are located in the northeast, northwest and Great Lakes regions of USA. Combined sewer systems occur when a pipe carrying storm water is combined with a pipe containing untreated sewage. This mixture is stored in a containment tank, however during periods of heavy rainfall and snow melt, and when the capacity of the tank is reached, the excess spillage is discharged without treatment into nearby streams, rivers or other water bodies. In comparison, SSO's are municipal waste, which do not contain industrial waste. Causes for SSO are generally heavy rainfall and blockage of pipes which can overflow into water bodies and/or in basements. Spillage from CSOs can contain storm water run off, raw sewage, industrial waste and toxic chemicals. Due to inputs of untreated sewage containing higher levels of total suspended solids and fecal microorganisms into bodies of water, CSOs have the capacity to impact water and sediment quality at the site of discharge as well as downstream. Greater risk may be associated with recreational areas impacted by CSO spills. These contain human fecal indicators and pathogens which can harm recreational users. Antibiotic resistance is also a concern. When examining samples from inside the CSO containment tanks, it was

found that 2 of 12 *E. coli* isolates from the sewage had multiple resistance to ampicillin, ciprofloxacin, gentamicin, and tetracycline (Edge and Hill, 2005).

Studies of intertidal mudflats in the Boston Harbour area (Savin Hill Cove) have shown CSOs to impact sediment and water (Shiaris *et. al.*, 1987). After repeated CSO events over time, bacteria were found to form a reservoir in sediment. At high tide fecal coliforms were present in sediments at two to four orders of magnitude higher than in the overlying water column. Sediment contained between 200 – 60, 000 cfu/100mL slurry of fecal coliform.

**Wastewater treatment plants**

While the United States has the Clean Water Act that has mandated secondary treatment for all sewage treatment facilities, in Canada, no such act exists. A study performed on the St. Lawrence River basin found high levels of indicator bacteria in rivers which still received raw sewage discharge (Payment *et. al.*, 2000). Geometric means for fecal coliforms in some of the polluted waters were found to be: 15, 560 CFU/L (Laval Ste-Rose), 32, 948 CFU/1L (Lavaltrie) and 26, 355 CFU/L (St. Therese). Geometric means for *C. perfringens* concentrations at the 3 sites were 957, 2676 and 1373 CFU/L respectively (Payment *et. al.*, 2000). Sediments in addition to the water column were impacted. This may be particularly important under cold climate conditions. Cold temperatures can stabilize the bacteria and allow them to remain viable for longer periods of time. At McMurdo Base (Antarctica), higher concentrations of indicator species were reported in the sediment than in the water column at the sewage

outfall (Lisle *et. al.*, 2004). The following averages were reported for the water column (CFU/100mL) /sediment (CFU/g dw): fecal coliforms ($21/1.04x10^4$), *E. coli* (15/65), Enterococci ($22/2.22x10^3$), *C. perfringens* ($4/7.78x10^2$) and coliphage (0/0).

In order to control the amount of discharge of pollutants entering into surface water, the National Pollutant Discharge Elimination System (NPDES) was created. This permitting program was born out of the Clean Water Act amendments of 1972 in the United States. Anyone or any organization proposing to discharge waste or wastewater into surface water must obtain a NPDES permit from the state. In Michigan, applications must be sent to Lansing a minimum of 180 days before the proposed use is needed. Each permit is valid for only five years. Michigan developed a five year basin plan based on each receiving water body (lake, river or stream). Every five years permits in each water basin must be renewed during the same cycle. In order to process NPDES permits efficiently, watersheds are staggered among five cycles with the Lower Grand River (part of this study site) being in cycle and its permits being renewed by April 1st in the years 2006, 2011, 2016 etc... The upper Grand River (up river of the study site) is on cycle three with next permits due to be renewed in the year 2008.

For wastewater treatment plants that discharge untreated or partially treated waste into surface water (not including CSO events), there are strict compliance laws which must be adhered to. Monthly fecal coliform bacteria must not be more than 200 CFU / 100 mL on a monthly basis and 400 CFU / 100 mL during a 7 day average. Any

chlorination or ozonation to reduce bacteria concentrations must be done before water is discharged as to minimize harm to fishes and other aquatic organisms (MDEQ, 2007).

**Animal Feeding Operations (AFO)**

Regulated large animal operations are knows as Concentrated Animal Feeding Operations (CAFOs). These are facilities where animals have been, are, or will be stabled or confined and fed or maintained for a total of at least 45 days in any 12-month period, and the animal confinement areas do not sustain crops, vegetation, forage growth, or post-harvest residues in the normal growing season (US EPA, 1993). In Michigan, a CAFO is defined as an animal feeding operation housing 1000 or more animals. Livestock excrete indicator bacteria and pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., and *Streptococcus* spp., pathogenic protozoa such as *Giardia lamblia* and *Cryptosporidium parvum*, and a number of animal specific viruses (Mawdsley *et. al.*, 1995; Rice *et. al.*, 1995).

These operations may sometimes use a lagoon system to store waste, while others concentrate the waste and dry it out to spread onto fields (Williams *et. al.*, 1999). Lagoons are generally open air pits filled with both urine and feces from animals. However lagoons are susceptible to leakages, ruptures, weather effects and mismanagement; also dried waste can enter water systems through surface runoff or ground water filtration (Mallin, 2000., Edwards and Daniel, 1992). Manure from these systems are generally applied to crops and soils both in a dry or liquid form.

These practices are another potential source of concern during rainy periods or from runoff. In instances where CAFO disposal mechanisms are aging or not up to code, microbial pollution can result in neighbouring drains, ditches, rivers, lakes and creeks. In 1995, 25 million gallons of liquid swine waste entered the New River (North Carolina) after an 8 acre hog lagoon burst. After 14 days, fecal coliforms in the water column were $10^2$-$10^3$ CFU / 100 mL water and in the sediment where the plume stayed for 5 days, fecal coliform concentrations were $\geq 10^4$ CFU / 100 mL slurry (Burkholder *et. al.*, 1997). According to NRDC (2006), during Hurricane Floyd, five manure lagoons burst while approximately 47 of them were completely inundated and flooded with water leading to contamination of well water and surface water nearby.

**Grazing Animals**

Large herds of ruminants or swine not only deposit large concentrations of fecal microbes but their grazing can further expedite the travel of microbes from soil to ground water (Celico *et. al.*, 2004b). When an area is grazed, the soil is also vulnerable to erosion during rainfall, leaving the runoff to sweep contaminated soil into nearby streams and water sources.

In a study of two springs, it was found that 29% of the samples had violated the Kentucky surface water rule (fecal coliform) before grazing occurred in the area, however after grazing in nearby pastures, 80% of samples exceeded those the standards (Howell, Coyne and Cornelius, 1995). These authors also observed that when rainfall occurred, fecal bacteria moved from soil surfaces into well water and streams. It was

found that 5% of total cattle manure deposited by cattle contributed to stream pollution (Gary, Johnson and Ponce, 1983). However, at least 150 grazing cattle were needed to significantly increase the bacterial concentration of fecal coliforms and when 40 cattle or less were grazing, concentrations went down to levels similar to no grazing as was shown in the adjacent fields.

When comparing creeks with and without grazing impacts, a significance difference was found in *E. coli* and Enterococci populations. Fisher and Endale (1999) found on average 894 MPN/100 mL at the "Grazingland Creek" compared to 88 MPN/100 mL at the Wood Creek. Enterococci were found to be 174 MPN/100 mL and 10 MPN/100 mL respectively. The wooded creek had no domestic animals within 1km of the site.

**Wild Animals**

Wildlife and animal fecal discharges cannot be discounted as a source of pollution and potential pathogens. It was found that waterfowl accounted for 67% of the fecal coliform loading to one of the coastal embayment studied (Weiskel *et. al.*, 1996). In a study using macroarray hybridization techniques for *E. coli,* 51% of urban lake water samples were identified as coming from geese and or ducks (Yan *et. al.*, 2006). When the presence of gulls on the beach was compared with concentrations of *E. coli* on foreshore sand and water the following day, R values ranged from 0.352 to 0.483 with a $p<0.05$ (Whitman and Nevers, 2003). However no correlation was found when

comparing same day samples with gull activity. Deer and other ruminants can harbour pathogens such as *E. coli* O157:H7 and shed the organism in their feces (Keene *et. al.*, 1997., Rice and Hanncock, 1995). In a study where deer were inoculated with $10^8$ CFU of *E. coli* O157:H7, on average 4.3 $\log_{10}$ *E. coli* O157:H7 CFU/ 1g feces were shed one day after inoculation, concentrations decreased substantially until day 17 (Fischer *et. al.* 2001). After day 17 *E. coli* O157:H7 was found intermittently until 25 days post inoculation however enrichment culture techniques had to be used. It was found that deer and cattle which share the same pasture can have the same strain of *E. coli* O157:H7 (Rice *et. al.* 1995).

**Seaweed and Algae**

Various studies have shown bacteria are able to accumulate and survive on seaweed in marine environments. Anderson *et. al.* (1997) found Enterococci exceeding the water quality levels in New Zealand by 2-4 magnitudes in decaying drift seaweed in recreational beaches. Shibata *et. al.* (2004) found the concentration of total coliforms and *C. perfringens* to be significantly higher in sand which was under seaweed than uncovered sand. High concentrations of Enterococci were found in marsh seaweed with a geometric mean of 2284 MPN /100 g ranging from 18-450 000 MPN /100g (Grant *et. al.*, 2001).

Studies along Lake Michigan also show that green algae (*Cladophora glomerata)* can contain high concentrations of *E. coli*. Whitman *et. al.* (2003) found mean $\log_{10}$densities to be 5.3 (*E. coli*) and 4.8 (Enterococci) g/ dw with 97% of the samples

containing these bacteria. Algal mats exposed to sunlight for 27 hours experienced lower bacteria numbers with an exponential decline in *E. coli*, but only small populational decrease occurred within the first 9 hours, even in mats only 1 mm thick. Those that were 6 mm thick maintained a 4 $\log_{10}$ g/ dw density, when exposed to sunlight for 27 hours. This was also found to be the case for Enterococci. Mats 2-4 mm thick showed Enterococci concentrations decrease by 2 $\log_{10}$ after 18 hours but remained constant afterwards. Both bacteria were dried on mats and than refrigerated at 4°C for 6 months. When the mats were re-hydrated, concentrations of bacteria increased 4 $\log_{10}$ in 24 hours.

These results have suggested that seaweed or algae can offer protection from UV rays and can sustain bacterial concentrations until they are re-suspended in surface water (during periods of agitation or wave action) and/or washed onto beach sand where these biomats can deposit bacteria or shelter bacteria already present. The algae study gives rise to the possibility that bacteria, namely *E. coli* may not necessarily be the best indicator in identifying sewage pollution in areas where seaweed and algae mats are abundant. If these fecal indicators are indicative of pathogens, then by leaving algal mats on the beach and along the swash zone (where the water meets the beach) an increased risk to recreationalists may occur if they come into contact with the mats.

### *1.3.2 Survival and Transport of Fecal Micro-organisms*

Once bacteria, parasites and viruses leave the source of fecal pollution, many factors come into play that affects survival and transport (Gerba and Bitton, 1984). Factors such as pH, salinity, nutrient abundance, solar insulation (cloud cover) and

temperature affect survival. Rainfall is a key factor in transport. Climate is the overall average weather found in a specific region and weather is the day to day events. According to Gerba and Bitton (1984) climate controls two of the most important factors associated with transport and fate, mainly rainfall and temperature. Retention of bacteria and viruses are also dependant on soil and sediment particle size and clay content (which will be discussed in the sediment section). Factors discussed here will be rainfall, temperature and salinity.

**Rainfall**

Rainfall can impact the transport of fecal micro-organisms and can impact both recreation and drinking water. A first flush effect can occur after rainfall, where sediments are mobilized in run-off carrying with them potential pathogens, nutrients and debris (Lawler *et. al.*, 2006). After heavy rainfall in Walkerton, Ontario between May 8-12 in the year 2000, the likes of which occurs in Walkerton Ontario once every 60 to 100 years, a major *E. coli* O157:H7 outbreak occurred (Auld *et. al.*, 2004). This demonstrated how rainfall can impact bacteria flow from nearby farms, eventually getting into water supply systems. Even early studies such as Goyal *et. al.* (1977) found peaks of total coliforms in both surface water and sediment samples taken from canals along coastal Texas during the months of June and November 1975 and January 1976 of over 4 $\log_{10}$ CFU/100 mL for surface water after periods of rainfall.

Many instances of non-point source pollution are facilitated through surface run-off during and after periods of heavy rainfall. Storm events increase the concentrations

of fecal bacteria downstream, and reported in some studies to be approximately two $\log_{10}$ higher than during dry weather conditions (Rechenburg, 2006). The authors also concluded that there was a significant association when looking at intensity of rainfall. High intensity rainfalls were observed to have higher levels of bacteria and parasite concentrations compared to longer lasting moderate rainfall before an overflow occurred (Rechenburg, 2006).

When examining both marine and freshwater creeks and outfall samples along the coast of Oregon for Enterococci, 99 (22 fresh water and 77 marine samples) out of 3086 samples exceeded the 158 MPN/100 mL level. The mean freshwater exceedance was 510 MPN/100 mL with a maximum concentration of 2419 MPN/100 mL. At Mill Beach (freshwater), the relationship between rainfall (3 day cumulative) and Enterococci was found be R=0.70 (Neumann *et. al.*, 2006). When analyzing the marine water samples, researchers observed that 91% of the exceedances occurred when there was some rainfall within five days preceeding the reported water exceedance. Most of those marine exceedances (55/77) of marine water exceedances occurred when the amount of rainfall was between 0.01-60.0mm (Neumann *et. al.*, 2006). The 2 day cumulative rainfall relationship for marine Enterococci levels also had an R value of 0.70.

A study performed along Lake Superior found that rainfall did not have a significant correlation with *E. coli*. The $R^2$ values ranged from 0.00005 to 0.23 in 2003 and in 2004 the range of $R^2$ was 0.0032-0.03 (Sampson *et. al.*, 2006). These measurements were taken within 24 hours of a significant rainfall of at least 6 mm.

While rainfall did not correlate well with *E. coli* in Lake Superior, there was one occurrence of high *E. coli* concentrations on one of the samples taken after heavy rainfall. After heavy rainfall (26.9 mm) on May 19$^{th,}$ 2003, the highest concentration of *E. coli* was found (>2419.2 MPN / 100 mL) between May 20 through May 21) (Sampson *et. al.*, 2006). However, this study did not take into account the possibility that over time, fecal indicator bacteria may enter into Lake Superior from rivers and through surface water runoff which may occur at time intervals greater than 24 hours after a significant rainfall.

Impact of rainfall was studied along the Milwaukee harbour along Lake Michigan. After heavy rainfall (over 10 inches or 254 mm) water samples were collected, cow specific *Bacteriodes* spp. were found in the harbour, but once the water discharged into Lake Michigan, it was not found again. Parking lot run-off near beaches were found to contain *E. coli* concentrations that ranged between 300 to 50, 000 CFU / 100 mL. After rain events, *E. coli* concentrations at two Lake Michigan beaches ranged between 110 to 5400 CFU / 100 mL with 7 out of 8 samples testing positive for Human *Bacteroides spp.* (Bower *et. al.*, 2005).

This is a world wide phenomenon and can affect large bodies of water. For example, in Tokyo Bay (Japan), it was noted that after a rainfall of 84.5mm, total coliforms increased from 13 to 240 CFU/mL and *E. coli* from 2 to 55 CFU/mL (Haramoto *et. al.* 2006). Three days later, the concentrations decreased to 21 CFU/mL (Total coliform) and 1.9 CFU/mL (fecal coliform).

Many studies have been performed examining the influence of rainfall on fecal indicator bacteria concentrations in surface water, with a larger portion of studies on marine and ocean waters. However gaps still exist in determining the extent of non-point source inputs to rivers and lakes which potentially are increased due to heavy rainfall. There is still need to examine water quality along longer reaches of rivers and creeks before they enter into larger bodies of water like lakes and oceans, as opposed to limiting sampling only near recreational beaches or harbours at the mouth of a river. As technology develops, it would be beneficial to start using species specific markers such as human sewage *esp* markers (Enterococci) and/or bird, cattle markers to determine whether sewage or agricultural run-off is the primary source of fecal indicators and pathogens during periods of intense rainfall.

**Temperature**

Temperature of water and seasonality can be related to fecal indicators in a number ways. Warm temperatures may provide conditions for optimal re-growth for some organisms, while cooler temperatures can stabilize environmental conditions for others.

In southeast Carolina, Esham and Sizemore (1998) found fecal coliforms to be in higher concentrations when temperatures were warmer (22-34°C) and when tides were low. It is has already been established that indicators such as *E. coli* and fecal coliforms may regrow in warm moist climates (Davies *et. al.*, 1995, Byappanahalli and Fujioka, 1998). Places like Hawaii use alternative indicators such as *C. perfringens* which would

not regrow along side traditional indicators to take into account bacterial regrowth in tropical and sub tropical places.

However, increasing water temperatures can also be related to higher inactivation rates. In some cases, part of this can be attributed to sunlight inactivation, as longer summer days mean more exposure to sunlight and warmer temperatures. When modeling the transport of fecal contamination in Lake Michigan, Liu *et. al.* (2006) found that sunlight played an important role in *E. coli*, fecal coliforms, Enterococci inactivation with k values of respectively. Sinton *et. al.*, (2002) found that Enterococci showed significantly faster inactivation rates in summer ($k_S$= 0.276 $m^2$ $MJ^{-1}$) than winter ($k_S$=0.110 $m^2$ $MJ^{-1}$) in rivers receiving wastewater. No significant differences were observed for inactivation of fecal coliforms, *E.coli* and Coliphage. The inactivation rates found for each indicator in summer and winter were as follows: fecal coliforms [summer ($k_S$= 0.086 $m^2$ $MJ^{-1}$) and winter ($k_S$=0.0.084 $m^2$ $MJ^{-1}$)], *E. coli* [summer ($k_S$= 0.078 $m^2$ $MJ^{-1}$) and winter ($k_S$=0.073 $m^2$ $MJ^{-1}$)] and coliphage [summer ($k_S$= 0.077 $m^2$ $MJ^{-1}$) and winter ($k_S$=0.049 $m^2$ $MJ^{-1}$)]. Enteroccocci were found to have a higher inactivation rate followed by fecal coliforms, *E. coli* and lastly coliphage (Sinton *et. al.*, 2002) .

Seasonal variation was found comparing bacterial exceedances in marine and fresh water samples in Oregon. Ninety-nine samples were over the exceedance value of 158 MPN/100mL required for Oregon with an average of 559MPN / 100mL Enterococci ranging from 160 - 4352MPN/100mL. Neumann *et. al.* (2006) reported 60% of marine

sample exceedances occurred during winter compared to only 9% freshwater exceedances during the same time period. With water temperatures in Oregon reaching 13°C in winter, full body contact and recreation occurs year round.

Bacteria have been shown to survive during colder temperatures. *Escherichia coli* O157:H7 has been shown to survive for prolonged periods in water, especially in cold water, by transforming into a viable but non-culturable state (Wang and Doyle, 1998). In this state the pathogen cannot be isolated by traditional plating methods and therefore may not be detected (Olsen *et. al.*, 2002).

In a study by Lipp *et. al.* (2001) seasonal variability was studied in southwest Florida in low salinity estuaries. It was found that bacteria and viral indicators showed significant seasonal changes over the course of one year and was related to rainfall, temperature, salinity and river discharge. No significant differences were found in sediment samples for *C. perfringens* amongst the monthly concentrations. Enterococci levels were found to be highest between December and February with a geometric range between 77-112 CFU/100 mL. Lipp *et. al.* (2001) also found coliphage to be present in higher concentrations in surface water from December to February. Coliphage peaked in December with a monthly geometric mean of 293 PFU/100 mL.

**Salinity**

Esham and Sizemore (1998) found fecal coliforms to have an inverse relationship with salinity concentrations. Coliphage was determined to be a poor survivor in warm (25°C) saline water (Chung and Sobsey, 1993). Enterococci species may be more

halotolerent (Shehane *et. al.*, 2005) however salinity was found to negatively correlate with *Enterococci, fecal coliforms* and coliphage in a Florida river system influenced by coastal tides. *Clostridium* did not seem to be affected by salinity (Shehane *et. al.*, 2005).

Concentrations of indicator species are highly variable in time and space (Shibata *et. al.*, 2004) when examining recreational marine waters. Shibata *et. al.* (2004) found the highest concentrations of indicator species to be during high tide and non-detectable levels off-shore. This suggests that the real impact of pollution is along the shoreline contributing to more recreational problems then previous recognized.

### 1.4 Public Health Concerns

Each state must have a set of guidelines and standards as mandated by the Clean Water Act and BEACH Act in order for public recreational waters to be deemed safe. Several indicators such as fecal coliforms, *E. coli* and Enterococci are monitored. However, resulting beach advisories and closures as a result of monitoring programs are often too late in capturing the pollution in real time and are generally 24 hours delayed. Another issue in beach monitoring, is that generally only water samples are taken, and not sediment and sand samples. Therefore, as of now, not all of the potential threats to recreational safety are being monitored.

In order to minimize the risk of illness in beach goers, beach managers invest millions of dollars in taking and processing water samples for fecal indicator organisms. According to Schiff *et. al.* (2002), about $3 million is spent annually to determine the

public health risk potential in southern California. Ingestion of pathogenic micro-organisms can occur through swallowing or drinking the water while recreating, through the contamination of hands or possibly washing utensils in contaminated water, contact with other materials such as algae, boats and beach toys.

Gastrointestinal illnesses and respiratory illnesses have been shown to occur in *Enterococci* polluted water (Kay *et. al.*, 1994). Eye, skin and respiratory symptoms were other symptoms found to affect swimmers of microbially polluted beach water (Kueh, C.S.W, 1995). It was found that swimmers had more episodes of diarrhoea and skin rashes compared to non-swimmers in Mission Bay (California) and the number of symptoms increased with higher exposure levels such as swallowing water (Colford *et. al.*, 2007). In the case of athletes, triathletes (those who swam, biked and ran) showed a higher attack ratio of gastroenteritis one week post triathlon competition compared to athletes who did not swim (only biked and ran) (Van Asperen *et. al.* 1998). According to the authors, triathletes were twice as likely to show symptoms of gastroenteritis compared to non swimmers in waters which had met the European and Dutch bathing water standards with waters have less than 2000 CFU / 100 mL total coliform.

After reviewing many studies, it is evident that no one indicator can predict with 100% accuracy the exact water quality of a system and therefore prevent future water born disease outbreaks from occurring. Based on a comprehensive meta analysis of recreational water quality research, Wade *et. al.* (2003) support using indicators such as *E. coli* and Enterococci over traditional indicators (fecal coliform) as the latter did not

show risk of illness if levels were increased. Their results suggested that Enterococci be used as an indicator of fecal pollution in both marine and freshwater, while *E. coli* could be used in freshwater. The authors also found that viral contamination indicators were "strong predictors" of gastrointestinal illness for both fresh and marine water environment (Wade *et. al.* 2003).

### 1.5. Water Quality in Great Lakes Basin and Michigan

The Great Lakes of North America are located along the border of Canada and the United States and compose 1/5th of the world's surface fresh water. These Lakes are an important resource as they provide drinking water, recreational opportunities and drive nearby economies. In total, the coastal line extends for 17,549 km, 5296 km of which is along the State of Michigan. Due to the importance of the Great Lakes watershed, numerous studies have been undertaken to determine the microbial water quality with a focus on fecal pollution at beaches. Important papers highlighting Great Lakes research are shown in Table 1-3.

**Table 1-3**. Summary of Great Lake published water and sediment literature.

| Reference | Microbe & water type | Year /season | Water | Sediment | Water body |
|---|---|---|---|---|---|
| Munawar *et. al.* 1994 | General Bacteria | (1991) | 2.5 x$10^6$ L. Erie<br>1.6 x$10^6$ L. Huron<br>0.9 x$10^6$ L. Superior<br>0.75 x$10^6$ L. Ontario | NA | Great Lakes |
| Sampson *et. al.*2006 | *E. coli* | 2003 & 2004 | 10.3 -192.3 MPN / 100 mL | | L. Superior |
| Francy *et. al.*, 2006 | *E. coli* | | 17 to 190 CFU/100 mL | 8 to >500 CFU/g dw | L. Erie |
| McLellan and Salmore, 2003 | *E .coli* | June – Sept. 2001 | Offshore counts (10-150m from shore)levels did not exceed 235 CFU/100 mL in more than 5%; beach samples exceeded that mark in 66% of samples | NA | L. Michigan |
| Murry *et. al.* 2001 | *E .coli* | 1997-1999 | 50% dry weather samples violated over 200 CFU /100 mL | NA | L. Michigan |
| Alm *et. al.*, 2006 | *E .coli* | 2005 | NA | Ambient sand ranged from 67 CFU/g on day 6 to 5 CFU/g on day 48. | L. Huron |
| Whitman and Nevers, 2003 | *E. coli* | | Surface water- 6.2x$10^1$CFU / 100 mL<br>Pier- 1.2.2x$10^1$CFU / 100 mL | Sediment- 7.2x$10^3$CFU / 100 $cm^3$<br>Foreshore sand- 4.0x$10^3$ CFU / 100 $cm^3$ | L. Michigan |

In 1991, a large scale study of the Great Lakes was undertaken with four great Lakes and surrounding lakes being studied. Munawar *et. al.* (1994) reported of the great lakes, highest bacterial abundance was found in Lake Erie and the lowest concentration in the oligotrophic Georgian Bay and Lake Superior. In this study, the authors were looking at total mean concentrations of bacteria and all were grouped together but were not specified. The following are the mean numbers reported / mL x$10^6$MPN: 2.5 (Lake Erie), 1.8 (Detroit River), 1.7 (Lake St. Claire), 1.6 (Lake Huron), 1.2 (St. Claire River),

0.8 (Georgian Bay), 0.9 (Lake Superior) and 0.75 (Lake Ontario). Lake Michigan was not included in the study. Because Lake Erie is the shallowest of the lakes, it tends to warm up faster and more often than the others which can influence bacterial concentrations. Historically, of all the Great Lakes, Lake Erie has generally shown higher amounts of pollution. Due to the shallowness of the lake, and chronic inputs from agriculture and urban areas conditions have been ideal for microbial pollution and the growth of algae . This study highlights that Lake Erie is still in need of consistent monitoring, and strict adherence to laws regulating discharges and inputs.

In the state of Michigan, nine rivers which drained into various Great Lakes were studied during July 2003 in the lower pennisula. Six of nine rivers had *E. coli* levels above the U.S EPA guidelines of 235 CFU / 100 mL (Jenkins *et. al.*, 2005). The range of *E. coli* for these six rivers were 235 (Raisin River) to 8500 (Rouge River, Dearborne) CFU / 100 mL. The Grand River site (upper reach of the Grand River watershed) had an *E. coli* concentration of 900 CFU / 100 mL. Seven rivers tested above the U.S. EPA guideline of 61 CFU/ 100 mL for Enterococci. Ranges for these seven rivers were 216 (Kalamazoo River) to 780 (Grand and Saginaw Rivers). Somatic coliphage (using C3000 *E.coli* as a host) ranged from 0.245 (Raison River) – 21.18 (Rouge River) PFU / 100 mL. Jenkins *et. al.* (2005) also determined that human fecal pollution was present in atleast two of the rivers studied (Grand and Rouge River) using the *esp* Enterococci human sewage marker. This study highlights the importance of inland rivers potentially contributing to the degradation of the Great Lakes water quality, and that human sewage

has the potential to make its way into Lake Michigan and Lake Erie (Rouge River empties into the Detroit River which connects Lake St. Clair to Lake Erie).

Sampson *et. al.* (2006) examined *E. coli* concentrations at 15 beaches in 2003 and 4 beaches in 2004 during beach season in Lake Superior, WI. Samples were collected on a regular basis during the beach season. In 2003, the seasonal means at the 15 beaches ranged from 10.3 MPN / 100 mL to 184 MPN / 100 mL for *E. coli* and in 2004 ranged between 118.7 to 192.3 MPN / 100 mL. In some cases, *E. coli* concentrations were found to be >2419.2 MPN / 100 mL in Ashland county, WI and 816.4 MPN / 100 mL in Bayfield county, WI.,

In a recent study, two beaches along Lake Erie in Ohio were studied for fecal contamination (Francy *et. al.*, 2006). At the Edgewater location, *E. coli* were found to be higher at sources near river mouths and outfalls away from the beach, and decreased closer to the beach. Highest counts at the beach occurred within 1-3 feet of water and decreased further out and when there were higher waves and rain (but not for all cases). Investigation in Lake Erie found that pollution was along the shoreline and came from near a pond drainage and boat launch. Researchers found physical evidence that run off from the parking lot was impacting beach quality. Sediment values at bathing beaches ranged from 8 to >500 CFU/g dw sediment at Lake shore beach. In the lake-water samples, *E. coli* ranged from 17 to 190 CFU/100 mL during both studies.

McLellan and Salmore (2003) examined potential entry points of fecal pollution into Lake Michigan. The study sites were a break wall-enclosed marina and a public

beach (South Shore Beach) on the western shore of Lake Michigan at the Milwaukee metropolitan storm-water and CSO outfalls. In offshore samples (10–150m from shore) *E. coli* did not exceed 235 CFU/100 ml in more than 5%. However, samples taken at the beach exceeded that mark in 66% of the samples (McLellan and Salmore, 2003).

During the SSO/CSO events, human-specific markers were detected at sites in nearshore Lake Michigan with >200 CFU/ 100 ml of *E. coli*. However, *E. coli* levels at distances of more than 2 km from the harbor contained < 200 CFU/100 ml *E. coli* (Bower *et. al.*, 2005). However in a study performed by Murry *et. al.* (2001), no correlation of fecal coliform abundance to CSO locations were found during the spring and summer of 1997, 1998, and 1999. Murray *et. al.* (2001) found that 50% of sites sampled during dry weather periods violated acceptable water quality standards of 200 CFU /100 mL.

The abundance of *E. coli* was measured by Marasalek *et. al.* (1996) in the St. Mary's, St. Clair and the Detroit River. Marasalek *et. al.* (1996) described water quality to be "excellent" in the St. Mary's River in Sault St. Marie which did not have any CSO's (4-162 cfu / 100 mL), but poor quality was found in the St. Clair River along a relatively short Sarnia waterfront (5 CSOs) with an average of 62-5130 cfu / 100 mL and 392-1929 cfu / 100 mL in a long stretch of the Detroit River in Windsor (25 CSOs) (Marsalek *et. al.*, 1996). In a study performed by Irvine *et. al.* (2005) in the Buffalo watershed area, on a dry day fecal coliforms were found to be between 5-450 cfu/100 mL but during rain events, fecal coliform concentrations peaked 1 to 24 hours after rain events at concentrations of approximately 1000 – 54000 cfu/100 mL. However, fecal coliform in

the Buffalo River did come from CSOs within the city boundary; higher concentrations entered the river from the upper watershed region (upstream of the city).

When examining sand, sediment and water interactions at Lake Michigan, a range of *E. coli* concentrations have been reported. Highest concentrations of *E. coli* were found in the foreshore region of the beach with a geometric mean of $4.0x10^3$ CFU / 100 $cm^3$ (volume of "whole fresh sand") followed by submerged sand at $7.2x10^3$CFU / 100 $cm^3$ (Whitman and Nevers, 2003). Water sampled 45 cm below the surface (but above the submerged sand) was $6.2x10^1$CFU / 100 mL and $1.2.2x10^1$CFU / 100 mL at the end of Casino pier. This study highlights the importance of monitoring foreshore sand, sediment and water. These results suggest that some of the foreshore sand may impact water quality and monitoring should be on a beach ecosystem level as opposed to just surface water.

A few studies (as previously mentioned) have examined fecal indicator bacteria presence in sediment and sand (Byappanhalli *et. al.*, 2003; Pettibone *et. al.*, 1996; Whitman and Nevers 2003; Whitman *et. al.* 2003). In an experiment performed on a Lake Huron beach looking at wet weight sand, *E. coli* concentrations on ambient sand went from being 67 CFU/g on day 6 to 5 CFU/g on day 48. However, initial concentrations increased from 14 CFU/g at time 0 and peaked to $7.1 x10^5$. Bacteria stayed at cultivable state for at least 48 days (Alm *et. al.*, 2006). Studies indicate that fecal indicator organisms can remain cultivatable for a prolonged period of time at ambient beach sand temperature at the Great Lakes and can make their way to the sand-

water interface during periods of rainfall, high wave action, as well as re-suspension during recreation as the previous studies have shown.

These instances of bacterial occurrences in the Great Lake basin could have consequences to human health. At a Lake Erie beach during the summer of 1991 in Ohio, 21 people acquired *E. coli* O157:H7 infections (Keene *et. al.*, 1994). All of the patients reported swimming in the lake for extended periods of time. During some of the outbreak days, Enterococci geometric means were reported to be >500 CFU / 100 mL.

Great Lake beaches remain an important source of potential recreational water borne disease. It is imperative that more comprehensive monitoring programs get established, on both American and Canadian beaches. In order to better protect the public, current and up to date research needs to be carried out using indicators which have found to be best suited to the Great Lakes environment. Also, technological advances need to be made in order to develop faster testing methods to avoid the issue of delayed beach closings and other potential sources and reservoirs of contamination must be examined to gain a better picture of the extent of pollution in the Great Lakes and where possible risks exist in a beach ecosystem.

### 1.6 Sediments as Sources of Fecal Indicators & Pathogens

Sediment is composed of loose particles such as sand, clay, silt, and other substances that settle at the bottom of a water body. Sand is usually between 0.0625 mm and 2 mm, silt is between 0.002-0.0625 mm while clay particles are generally classified

as smaller than 0.002 mm (USGS, 2005). Sand is usually shaped rather rounded or angular and coarse, thereby creating relatively large spaces between each sand particle. This promotes free drainage of water and entry of air into the substrate (Brady and Weil, 2004). Silt is characterized as having less space between particles than sand and is mainly composed of the mineral quartz. It is smooth and silky, with a tendency to retain more water (Celico *et. al.*, 2004) and less water drains through it. Clay particles are much smaller than sand and silt and therefore have smaller spaces between each particle. Clay charge (negative) can also influence permeability. Clay behaves in a colloidal manner and is difficult to separate out of water. Water moves in a very slow manner through clay. The permeability (ability of a material to allow the passage of a liquid) through sediment and soil are dependant on the ratio of clay, sand and silt with higher amounts of clay correlating with less permeability.

Sediments come from eroding soil, decomposing matter and can be carried over spatial distances by water. Soil is made up of various ratios of sand, silt and clay constituents and soil columns have multiple layers. Usually the top layer is composed of organic materials (each underlying organic layer being more compressed than that above). Following the organic layer, come minerals, clays, oxides, carbonates ending with salts. Because each layer is different, microbes travel differently through each layer. It is important to note that soil varies greatly depending on region, climate and geological aspects.

*1.6.1 Sediments as a reservoir for fecal indicator bacteria*

Sediments contain natural populations of microbes in their environment such as *Clostridium* spp (Huang *et. al.*, 2002). However, fecal indicator species originating from auchtonous sources but have been found to survive and grow in sand, sediments and soils near river banks such as *Escherichia* spp. (Burton *et. al.* 1987, Pang *et. al.*, 2003) and Enterococci (Whitman *et. al.* 2003, and Byappanahalli *et. al.*, 2003b).

Many sources of literature indicate that the majority of enteric bacteria in aquatic systems are associated with sediments (Jamieson *et. al.* 2004). As mentioned previously, these associations influenced their survival and transport characteristics. Sediments offer a protective effect on microorganisms because they protect from solar irradiation (Bitton, G., 1972) and salinity (Ghoul, M. *et. al.*, 1990). Below, several studies, generally in marine systems have reported on finding specific instances of fecal bacteria and coliphage in sediment.

Sediments near off shore breakwaters were found to contain high counts of total coliforms and Enterococci, as were instances of enteric viruses (Bitton, G., 1972). Sediments also hold organic nutrients, which can support microbial growth. Compared to water, sediments generally were found to have higher abundances of bacterial populations (Burton *et. al.* 1987, Cavallo *et. al.*, 1999) and lower die-off rates. It was found in surface flow wetlands of Arizona, die-off rates of bacteria and coliphage were greater in the water column than in the sediment (Karim, *et. al.*, 2004). Die-off rates for fecal coliforms were 0.256 $\log_{10}$ / day (water) and 0.151 $\log_{10}$ / day (sediment). For

coliphage it was determined to be $0.397\log_{10}$ / day (water) and $0.107\log_{10}$ / day (sediment) (Karim, *et. al.*, 2004). In general, the concentration of fecal coliform and coliphage were similar when compared on a volume/wet weight basis in water and sediment. When compared on a volume liquid : dry weight basis, the concentration of fecal coliforms and coliphage were one to two orders of magnitude higher in sediment (Karim, *et. al.*, 2004).

In the Ionian Sea area of Italy, it was found that the highest heterotrophic (fecal coliform, total coliform, fecal streptococci, sulphite-reducing clostridia, *Pseudomonas aeruginosa* and *Staphylococcus aureus* ) bacterial densities in water and sediment samples were found in summer ( average: $2.7\text{x}10^5$ CFU/mL for water and $5.9\ \text{x}10^5$ CFU/mL for sediment) while the lowest concentrations in fall ($3.1\text{x}10^3$ CFU/mL for water and $3.8\ \text{x}10^4$ CFU/mL for sediment) (Cavallo *et. al.*, 1999). This variance may indicate re-growth occurring in the summer coupled with larger inputs from crowds that come to beaches during summer. It was also shown that enteric bacteria were found to have extended survival ability in freshwater sediment (Burton *et. al.* 1987) compared to marine, indicating regrowth is an important issue in freshwater systems such as our study area.

In southwest Florida fecal coliform bacteria ranged (geometrically) between 7 to 2337 CFU / 100 g dw in sediment and were greater than the over laying surface water. In the water column fecal coliform ranged (geometrically) from 4 CFU/100 mL in July to 157 CFU / 100 mL in December (Lipp *et. al.*, 2001). For both sediment and surface

water samples, concentrations were observed to be highest during August and from December through February. A five order of magnitude increase was found for *C. perfringens* in sediment (184 to 36,834 CFU/100 g dw) and surface water. While the greatest concentration of *C. perfringens* in surface water was found in March (36 CFU/100 mL), concentrations varied between months.

Fecal bacteria were found to be present in sediment areas of the New York Bight apex disposal area (Babinchak *et. al.* 1977). In 1998, *C. perfringens* levels in sediment were 556 spores/g dw. Background concentrations in the same area were found to be 10-20 spores /g dw (EPA, 1998).

These studies highlight the importance of sediment as a reservoir of potential fecal indicator bacteria. Sediments were shown to protect the bacteria from UV light and offer a somewhat stable environment. In instances where high concentrations of *C. perfringens* were found, there is a potential of old pollution and harmful pathogens being re-suspended into the water column when the sediment is re-suspended through recreation or boating activity.

### *1.6.2 Re-suspension*

In areas such as lakes, beaches or canals, pathogenic microbes which are colonizing or concentrated in sediment, can be re-suspended into free flowing water. Because the sediment reservoirs allow for enteric and pathogenic bacteria survival for up

to several months (Burton *et. al.* 1987), re-suspension is a possible source of risk. Re-suspension occurs through sudden influx of water in shallow ponds, fish migration and spawning disturbances, burrowing of small animals, nesting of sediment dwelling species, earthquakes, tidal action, or seasonal flooding. Human induced re-suspension can occur through dredging, boating and recreational activities.

Pettibone *et. al.* (1996) found fecal coliforms, heterotrophic plate counts and total suspended solids increased immediately after the passage of a loaded boat along the Buffalo River. In lake studies performed near the Texas-Oklahoma border (boating marinas), *E. coli* in sediments were higher in number compared to those in lake water (An *et. al.* 2002). Authors found a direct relationship between the amount of gasoline sold which was related to recreational boating activity, and the re-suspension of *E. coli* was found. This indicated that boating activity in the marinas likely re-suspended sediments with attached *E. coli.*

In the Grand River Watershed (Ontario) a tracer study was performed to examine the effects of re-suspension of bacteria from a fine grain sediment bed (grain size 0.11mm) to the water column using *E. coli* NAR (a strain of *E. coli* resistant to nalidixic acid). Jamieson *et. al.* (2005) recorded a first order inactivation constant of K=0.005/h in the sediment. Tracer bacteria were not observed in the water column between 100 and 225 hours post seed. However, during the rising limb of a storm hydrograph at 225, 550, and 600 h, the tracer bacteria was found in the water column, along with increased total suspended solids. At 600 h *E. coli* was found to be $10^3$ CFU/g in the sediment. The

authors concluded that the *E. coli* found was due to sediment mediated re-suspension. This study is important as it highlights the risk of fecal indicator bacteria being transported from its sediment reservoir into the water column. Once in the water column, it may be transported downstream or potentially infect recreational users.

*1.6.3 Processing bacteria and coliphage from sediment & sand*

Sediment studies are limited partly due to the difficulties in collecting, sampling, and analyzing sediments for indicator organisms. Currently, no standard method exists for processing sediment samples. In order to process sediment samples, the sediment must first be eluted and mixed, it has been suggested that one part sediment be added to nine parts diluent or in some cases a 1:1 ratio of sediment to water has been used (Karim *et. al.* 2004, Davies *et. al.* 1995, Gerba and McLeod. 1976). Diluents ranged from sterilized river water, sterilized distilled water and Phosphate Buffer Water.

Two general ways to recover and quantify fecal indicators from sediment are sonication and/or some sort of elution. In order to elute the bacteria, sediment and some type of solvent (buffer, water, broth....) must be combined and shaken in such a way that the fecal indicators detach from the sediment particles and get released into the solvent. The solvent is than pipetted out. Literature surveys revealed that sonication (Craig, DL. *et a.l*, 2002, Boenigk J, 2004, Furtado and Casper, 2000., Gough, H.L and D.A. Stahl, 2003 Haglund *et. al.*, 2003), hand mixing (Karim, MR. *et. al.*, 2004), mechanical mixing (Desmarais, TR., *et. al.* 2002), rolling (An *et. al.*, 2002), density centrifugation (Furtado and Casper, 2000), and vortexing (Byappanahalli *et. al.* 2003, Burton, G. *et. al.* 1987,

Cavallo, RA *et. al.* 1996, Lisle, JT *et. al.* 2004) were commonly used to elute the sediment. Byappanahalli *et. al.* (2003) ran a comparison study using sonication, shaking, and vortexing approaches. They found that the method which revealed optimum results for eluting bacteria from sediment and soil samples involved vortexing the sample for 2 minutes. When comparing sediment to water in literature, sediment was usually displayed as CFU or PFU / 100 g dry weight ((Karim *et. al.*, 2004) or wet weight (Lee *et. al.*, 2006) to the CFU or PFU / 100 mL water;

## 1.7 Objectives of this Study

The Great Lake beaches remain at risk from CSOs and other non-point source pollution entering through river systems. However, only seasonal beach monitoring takes place in Michigan and little emphasis is placed on the potential contribution of sand and sediment on water quality. The role of sediment in fecal associated water pollution is an on going problem which needs to be further investigated in Michigan watersheds. An improved understanding of the relationship between fecal indicator species in sediments and the potential impact to water quality will allow for improved decisions made on performing beach closures and issuing beach advisories in order to protect public health. The overall aim of this project is to examine the relationship between sediments and water quality in an important river in Michigan. The specific goals of this study include:

1) Examine the spatial differences in fecal indicator concentrations along the Grand River and Lake Michigan in both parks and beaches using traditional and alternative indicators (fecal coliforms, *E. coli*, Enterococci, *C. perfringens* and coliphage) in the water column and sediment.

2) Examine the impact of seasonal changes on the concentration of fecal indicators.

3) Determine if a relationship exists between fecal indicators in surface water and sediment.

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## 2.0 Materials & Methods
### 2.1 Site description

The study sites (8 in total) were selected along the Grand River which flows from east to west across mid Michigan, USA and ranged from Grand Rapids (Kent County) to Grand Haven and Ferrysburg (Ottawa County). Six sites (three beach and three parks) were studied in Ottawa County, Michigan and two sites (both parks) in Kent County, Michigan (Table 2-1 and Figure 2-1). The three beach sites [North Shore Pier (NS), Rosy Mound (RM), and North Beach Park (NBP)] were located along Lake Michigan – north, south and at the entry point where the Grand River empties into Lake Michigan. Parks studied were Riverside Park (RSP), Deer Creek Park (DC), Grand River Park (GRP), Johnson Park (JP) and Sixth Street Park (SSP) - with Sixth Street Park located in Grand Rapids and being the most easterly.

**Table 2-1.** Location of study sites with corresponding GIS information.

| City/Township | Name (Abbrev.) | MSU ID site+sample date | Latitude/Longitude | Description |
|---|---|---|---|---|
| Grand Haven | North Shore Pier (NS) | NSmm/dd/yy | N43 03.469 W86 15.324 | B |
| Ferrysburg | North Beach Park (NBP) | NBPmm/dd/yy | N43 04.946 W86 15.265 | B, Pi |
| Grand Haven | Rosy Mound Natural Area (RM) | RMmm/dd/yy | N43 01.164 W86 14.000 | B |
| Robinson Township | Riverside Park (RSP) | RSPmm/dd/yy | N43 01.728 W86 02.352 | P, Pi, BL |
| Polkton Township | Deer Creek Park (DC) | DCmm/dd/yy | N43 00.634 W85 56.199 | P, Pi, BL |
| Georgetown Township | Grand River Park (GRP) | GRPmm/dd/yy | N42 56.671 W85 51.262 | P, Pi, BL |
| Grand Rapids | Johnson Park (JP) | 1JPmm/dd/yy | N42 58.330 W85 53.237 | P, BL |
| Grand Rapids | Sixth Street Park (SSP) | SSPmm/dd/yy | N42 58.603 W85 40.444 | P, BL, F, D |

Note: (B)Beach, (P) Park, (Pi) Picnic area, (BL) Boat launch, (F) Fishing area, (D) Dam few feet upstream, (Abbrev) Abbreviations. Note MSU ID for sediment includes "sed" prior to date

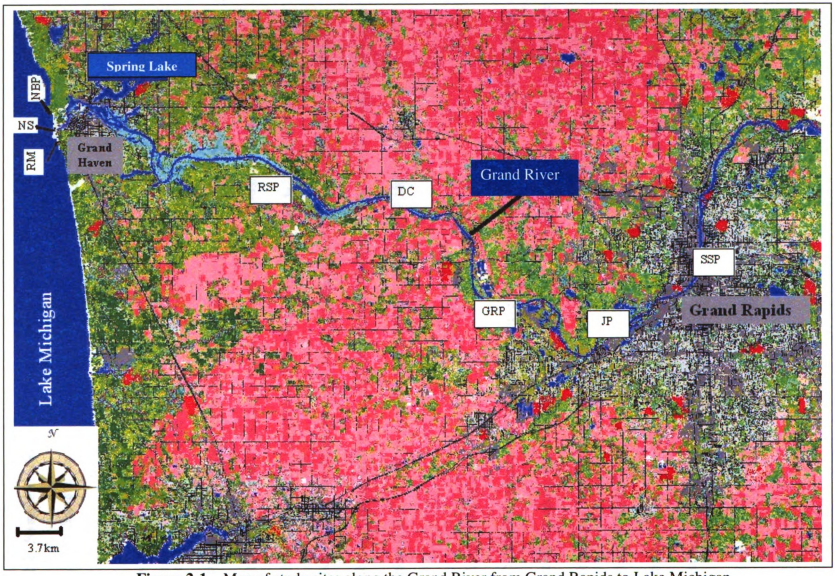


**Figure 2-1**. Map of study sites along the Grand River from Grand Rapids to Lake Michigan.

## 2.2 Sample Collection

### *2.2.1 Surface water*

Samples were collected from April 2005 to August 2006. Sites were visited twice a month during the spring/summer months (April -August) and monthly from September to March. Two one-litre surface water samples were collected from the shore line. Physical water parameter measurements taken during sample collection included water temperature, ambient air temperature, pH, and turbidity. Qualitative weather observations were recorded such as cloudiness, rain, snow fall, and whether or not the lake or river had large waves or ice accumulation. Bottles were placed in a cooler with ice, and transported back to the laboratory at Michigan State University where they were placed at 4°C until processed. Samples were processed within 24 hours.

### *2.2.2 Sediment*

Sediment samples were collected by inverting a sterile Whirlpak bag, and scooping the sediment along the submerged region up-to four inches deep where the water breaks with the shore. Samples were taken from three points, one foot apart and than pooled into a sterile Whirlpak bag. Samples were placed in a cooler with ice and transported back to the lab where they were placed at 4°C until processed. All samples were processed within 24 hours of collection.

## 2.3 Enumeration of water samples

Table 2-2 lists the various types of micro-organisms studied and the various types of media used to quantify the bacteria and coliphage.

**Table 2- 2.** Types of analysis performed on both surface water and sediment

| Analysis | Media | Incubation | Volumes of water or sediment slurry Assayed / plate or tray | Reference |
|---|---|---|---|---|
| **Fecal Coliforms** | mFC | 44.5°C | 1-200 mL (water)<br>0.5-50 mL (sediment) | EPA standard method 9222 D |
| ***E. coli*** | Colilert | 37°C | 1-100 mL (water)<br>1-100 mL (sediment) | Eckner (1998) |
| ***Enterococci*** | Enterolert | 41°C | 1-100 mL (water)<br>1-100 mL (sediment) | Eckner (1998) |
| ***C. perfringens*** | mCP | 45°C | 1-200 mL (water)<br>0.5-50 mL (sediment) | Bisson and Cabelli (1979) |
| ***Coliphage*** | TSA | 37°C | 2 mL | EPA standard method 1601 |

Water samples were filtered through a 0.45 μm membrane filter (PALL Life Sciences 47 mm sterilized) according to EPA specifications. Selective agar media were used to quantify fecal coliforms (mFC) and *C. perfringens (mCP).* Fecal coliform agar were prepared as follows: 5.2 g mFC agar (Difco267720, SanJose, CA), was added to 100mL nano-pure water, boiled with agitation for 1 minute, and adjusted to a pH to7.4±0.2. The media was placed in a 55°C water bath until media was cooled to 55°C before adding 1.0 mL of 1% rosolic acid solution (0.01 g rosolic acid + 1.0 mL of 0.2M NaOH). The selective agar used for *C. perfringens* was prepared as follows: 7.11 g mCP (Acumedia 7477A, Lansing, MI), was added to 100 mL nano-pure water, boiled until mixed, adjusted to a pH of 7.6±0.2, and autoclaved for 20 minutes. After autoclaving, the media

was cooled to 55°C water bath (until warm to touch) and the following additives were subsequently added: 0.04 g D-cycloserine, 0.0025 g Polymyxin B sulphate, 0.2 mL 4.5% ferric chloride solution, 2.0 mL of 0.5% Phenolphthalein diphospate solution and 0.006 g Indoxy β-D glucoside. Afterwards, the media was pipetted into 47 mm Petri plates and allowed to solidify. Water samples were filtered through membrane filters using vacuum filtration. Filters were placed on each of the respective selective media, mFC plates were incubated at 44.5°C in a water bath and mCP plates were incubated in a 45°C incubator in an anaerobic chamber. All plates were incubated for 24 hours. Blue colonies on the mFC plates were counted as fecal coliforms, and yellow colonies which turned pink on mCP media upon exposure to ammonium hydroxide gas were counted as *C. perfringens*.

In order to measure *E .coli* and *Enterococci*, 100 mL samples (diluted when necessary) were assayed in a Quanti-Tray/2000 (WQT-2K) using the following IDEXX (Westbrook, Maine) methods: Colilert (98-21375-00) and Enterolert (98-20705-00) system. The powdered reagent (Colilert for *E. coli* & Enterolert for *Enterococci*) was placed in a sterile container and than 100 mL of the sample were added. If samples needed to be diluted, Phosphate Buffer Water was used as the diluent. The samples were than mixed until the reagent was dissolved and than dispensed into the Quanti-Tray/2000. Colilert samples were placed in a 36.5°C incubator for 24 hours while the Enterolert samples were placed in a 41°C incubator for 24 hours. After incubation, the Quanti-Tray/2000 tray was placed in a dark room and exposed to a 365 nm long range ultra violet light. All fluorescing wells were counted and enumerated by using a most

probable number chart which was provided with the IDEXX system and results are quantified as most probable number (MPN) of *E. coli* and Enterococci.

Coliphage viruses were assayed according to an EPA method using a double agar layer method (EPA 1602 section 11.3 double agar method). An overnight culture was prepared the day before by adding 0.5mL of host bacteria (*E. coli* C3000 -ATCC 15597) to 4.5 mL of TSB and incubating at 37 °C for 24 hours. A fast growth was prepared by taking 1.0 mL of overnight culture and adding it to 45 mL of sterile TSB in a 50 mL sterile centrifuge tube and incubating at 36±1 for 4 hours until culture was visibly turbid. The fast growth host preparation was placed in 4°C to slow replication until use. Samples were first filtered in 10 mL syringes (BD ref 309604) using an MILLEX®HA filter (0.45 µm) and MILLEX®GV filter (0.22 µm). In a test tube containing 2.5 mL of melted TSB with 1.5% agar (kept at 49°C until used) 2 mL of sample and 0.3mL of host bacteria were added. Sample, host and media were mixed by rolling the test tube between the palms of the hands. The sample was than poured onto plates containing a bottom agar of Tryptic Soy Agar (40 g TSA [Difco-236920] and 1L of distilled water). The plates were allowed to solidify, were inverted and incubated at 37 °C for 24 hours. Plaques in the monolayer of the host bacteria were counted.

Bacterial colonies were counted and standardized to colony forming units (CFU) / 100 mL for surface water while coliphage was standardized to plaque forming units (PFU) / 100 mL.

**2.4 Enumeration of sediment samples**

The following protocol was developed to enumerate sediment samples. Seventy-five grams of wet sediment were placed in a sterile container and diluted by adding 675 mL of Phosphate Buffered Water (PBW). The container was capped and sealed and mixture was vortexed for 2 minutes (with a vigorous hand shake after one and two minutes). Any dilutions were performed using PBW. Immediately, the resulting suspension was collected without allowing anytime for settling and assayed using the same procedures described above for the surface water samples [membrane filtration for fecal coliforms and *C. perfringens*, Quanti-Tray 2000 for *E. coli* (Colilert) and *Enterococci* (Enterolert)].

In order to determine the amount of bacteria or virus per gram of sediment to allow for comparison among the sites, dry weight was determined. To determine soil moisture content, 10 g of wet sediment was placed in an aluminium foil boat and baked for a minimum of 48 hours at 105°C (done in duplicate). Before measuring the dry weight, sediment samples were cooled and placed in a desiccation chamber for one hour to cool before being weighed again. The weight of the aluminium boat was subtracted from the total dry weight. Dry sediment weight was divided by the total wet weight of sediment used to obtain percent dry weight. The average of the two duplicate samples was recorded.

The resulting colony forming units were multiplied by a factor of 10 (to take into account original 10 fold dilution), divided by 75 g (to get colonies per g wet weight), than divided by dry weight to wet weight ratio (per cent dry weight) to obtain colonies per

gram dry weight of sediment. Colonies were standardized to CFU/ 100 g dry weight (dw) sediment. Coliphage was standardized likewise as PFU / 100 g dw.

Soil nutrient, particle and organic matter analyses were performed on two samples of sediment from each site (winter 2005 and summer 2006) at the Michigan State University Soil and Plant Nutrient Laboratory. Sediment classification was performed using a hydrometer method and Phosphorous by bray-p1 method (Brown *et. al.*, 1998). Ammonium acetate extractable method was used to determine potassium, calcium and magnesium. Organic matter was determined using the "loss un-ignition method" where the organic matter was burned off and than subsequently weighed (Brown *et. al.*, 1998).

## 2.5 Statistical analysis

Non-detects were assigned a value of 0, and a value of 1 was added to all data points. This allowed non-dectects to be included in the analyses. Results were then $\log_{10}$ transformed. ANOVA and correlation analysis were performed using the program MATLAB version 6.5 (Natick, MA). Statistical differences in means of indicator bacteria and coliphage virus between sites were evaluated using Tukeys test from the SPSS v. 12 (Chicago, IL). If the p value was greater than 0.05, than relationship was deemed not significant, anything less than 0.05 was deemed significant. A box plot containing 50 and 95% confidence intervals and means was generated and plotted showing indicator concentrations along a spatial gradient.

Images in this thesis are presented in colour. Box plot graphs, land use images and weather figures in the appendix best viewed in colour and are thus presented as such.

In order to examine seasonality amongst sediment and water samples, four time periods were considered for analysis and were grouped as Spring (March 20-June 20), Summer (June 21-August 22), Fall (August 23-December 20) and Winter (December 21-March 19). An ANOVA was run to determine if differences existed among the different indicators and Tukeys test was used to determine significance among seasonal groups.

Regression analysis between sediment and surface water, between indicator species in surface water, indicators in sediment were evaluated using $R^2$ values and p values. When examining the effect of upstream sediment on water quality at the immediate downstream site, only samples taken on the same day, and all sites were sampled were included (n=11 for each site). Same day analysis were used to minimize changes in sampling conditions such as rainfall, weekly water changes and any significant changes in input loading which may occur. The uppermost sediment site was compared to the water samples downstream. For example, SSP sediment was compared to JP water; JP sediment was compared to GRP water etc. For the beaches, RSP sediment was compared to each individual beach site. For abbreviation classifications, please see Table 2-1.

Precipitation data for the duration of the study period was obtained from the National Oceanic & Atmospheric Administration from the "local climatological data"

(Dept. of Commerce). The precipitation recorded the day before the sample was taken data came from Grand Rapids, MI and Muskegon, MI rain gauges and than averaged. Grand River discharge data was obtained from the USGS surface-water daily statistics page from the Grand River at Grand Rapids, MI sites (USGS 04119000) from April 13$^{th}$, 2005 to August 28, 2006 (USGS, 2006). Coordinates of the gauge are latitude 42°57'52" and longitude 85°40'35". The gage datum is 585.7 ft above sea level and covers a drainage area of 4,900 mi$^2$. The average daily mean flow was measured and calculated by USGS. Values used were from the same days that the samples were collected. Data obtained was used to determine if flow of the river had any correlation to fecal indicator bacteria and coliphage.

## 2.6 References

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## 3.0 Results

Eight sites along the Grand River were examined for fecal indicators fecal coliforms, *E. coli*, Enterococci, *C. perfringens* and coliphage. Five sites were parks located along the banks of the Grand River, one beach at the mouth of the Grand River which emptied into Lake Michigan, and two beaches on either side of the river mouth (directly along the coast of Lake Michigan). Reaches of the river were broken down into 3 groups: Upper (two eastern most site including SSP and JP), Middle (three parks including GRP, DC and RSP), and Lower (the three beaches including NS, RM and NBP). Site groupings are displayed below in Table 3-1.

**Table 3-1.** Site classifications and number of total samples taken at each site throughout the course of the study.

| REACH | SITE | PARK OR BEACH | # WATER SAMPLES | # SEDIMENT SAMPLES |
|---|---|---|---|---|
| Lower | North Beach Park (NBP) | Beach | 21 | 21 |
| Lower | North Shore Pier (NS) | Beach | 20 | 19 |
| Lower | Rosy Mound (RM) | Beach | 19 | 19 |
| Middle | Grand River Park (GRP) | Park | 20 | 20 |
| Middle | Deer Creek Park (DC) | Park | 22 | 20 |
| Middle | Riverside Park (RSP) | Park | 19 | 19 |
| Upper | Sixth Street Park (SSP) | Park | 20 | 11 |
| Upper | Johnson Park (JP) | Park | 17 | 16 |

The overarching goal of this study was to determine the quality of surface water in the Grand River and to determine the level of microbiological contamination in the sediments of the same river. Sites were chosen based on areas where public recreation takes place, and sampling was conducted during spring, summer, fall and winter.

A one way ANOVA was performed on each indicator in sediment and surface water to determine if any significance existed amongst the samples. Significant differences were found amongst the sites for all indicators in water samples ($p < 0.05$), and four out of five indicators in sediment (coliphage in sediment $p > 0.05$). This analysis indicated that there are differences within the indicator results worth exploring and that further analysis can be undertaken. Results will be presented in this section.

At each site, for each fecal indicator, the geometric mean of the raw data was determined for both surface water and sediment as shown in Table 3-2 (surface water) and Table 3-3 (sediment). For surface water, SSP in the upper reach of the river had the highest geometric average for fecal coliforms and Enterococci, and JP (also in the upper portion of the river) had highest geometric for *E. coli* while GRP and DC (in the middle reach of the river had the highest for *C. perfringens* and coliphage respectively. Fecal coliform geometric averages ranged from 11.30 CFU / 100 mL at the beaches to 185.19 CFU / 100 mL at the parks. Geometric averages for *E. coli* ranged from 7.88 CFU / 100 mL at the beaches to 68.03 CFU / 100 mL at the parks. Enterococci geometric averages ranged from 3.15 CFU / 100 mL at the beaches to 52.95 CFU / 100 mL at the parks. Geometric averages for *C. perfringens* ranged from 2.13 CFU / 100 mL at the beaches to 27.90 CFU / 100 mL at the parks. Coliphage geometric averages ranged from 0.173 PFU / 100 mL at the beaches to 5.45 PFU / 100 mL at the parks.

**Table 3-2.** Geometric means of fecal indicators in surface water reported as CFU or PFU / 100 mL

| SITE | FECAL COLIFORMS | *E. COLI* | ENTEROCOCCI | *C.PERFRINGENS* | COLIPHAGE |
|---|---|---|---|---|---|
| NBP(L) | 11.30 (<1 – 69.14) | 8.78 (<1-104.35) | 3.15 (<1-55.6) | 2.13 (<0.31-11.33) | 0.271 (<10) |
| NS(L) | 23.25 (2-806)) | 10.92 (2-1356.65) | 5.92 (<1-1161.6) | 13.36 (1.5-29.0)) | 1.21 (<10-110) |
| RM(L) | 12.80 (2-84.67) | 7.88 (<1-255.75) | 4.14 (<1-195.1) | 3.23 (<0.66-14.67) | 0.173 (<10-20) |
| RSP(M) | 44.72 (10-908) | 23.45 (8.3-677) | 14.61 (1-25.293.4) | 20.94 (7.67-25.66) | 2.48 (<10-70) |
| DC(M) | 94.08 (0.6-2300) | 58.77 (14.8-1592) | 45.36 (<4-1381.8) | 18.72 (<1-88.33) | 5.45 (<10-850) |
| GRP(M) | 180.72 (66.6-348.33) | 56.46 (15.1-305.6) | 45.96 (<10-200.1) | 27.90 (<1-105.83) | 3.52 (<10-60) |
| JP(U) | 115.21 (20-545.45) | 68.03 (16-479.05) | 45.17 (5.7-801.6) | 26.66 (8.3-29.06) | 2.22 (<10-60) |
| SSP(U) | 189.95 (<1-2750) | 64.67 (23.5-754.2) | 52.95 (9.3-3032.7) | 20.38 (7-128.13) | 3.24 (<10-30) |

*Note that bracket contains the range of actual concentrations found in surface water*
*Reaches defined as (L) Lower, (M) Middle and (U) Upper. See Table 3-1 for n values*

**Table 3-3.** Geometric means of fecal indicators in sediment reported as CFU or PFU / 100 g dw

| SITE | FECAL COLIFORMS | *E. COLI* | ENTEROCOCCI | *C. PERFRINGENS* | COLIPHAGE |
|---|---|---|---|---|---|
| NBP(L) | 1.84 (<0.1-39.88) | 1.30 (<0.15-28.63) | 0.231 (<1.16-5.52) | 0 (<0.001-1.18) | <15 |
| NS(L) | 27.48 (<0.15-5895.17) | 90.14 (<15-2072.67) | 9.772 (<15-622.66) | 5.169 (<0.23-1205.06) | 0.31 (<15-164.37) |
| RM(L) | 72.73 (<0.1-945.45) | 56.57 (<15.15-2658.11) | 12.543 (<15.15-525.35) | 2.37 (<0.10-235.76) | <15 |
| RSP(M) | 8836.28 (365.41-767263.4) | 1784.58 (213.17-296533.6) | 8570.575 (224.14-231612.2) | 9000.44 (<156.25-242290.75) | 0.161 (<18-16.09) |
| DC(M) | 13638.68 (389.8-1081458) | 4025.71 (378.63-4951068) | 34794.56 (990.09-3148012) | 6426.51 (63.52-456033.1) | 0.324 (<1-273) |
| GRP(M) | 8921.006 (<1-1996008) | 2302.503 (<1-1783510) | 13519.37 (870.18-1965247) | 21134.98 (60.93-563399.4) | 0.697192 (<17-220.17) |
| JP(U) | 14196.72 (1474.38-115666.30) | 2624.24 (262-50771.1) | 3907.791 (<172.9-60682) | 17547.01 (1691.3-168960.5) | 0.5337 (<211-1436.43) |
| SSP(U) | 41681.94 (<1-1024668) | 14802.09 (750.1-167912) | 5224.882 (<1280-139231.7) | 17916.78 (2316.89-130395.1) | <1 |

*Note that bracket contains the range of actual concentrations found in surface water*
*Reaches defined as (L) Lower, (M) Middle and (U) Upper. See Table 3-1 for n values*

For sediment, SSP had the highest geometric average for fecal coliforms and *E. coli*, DC had the highest for Enterococci while GRP had highest for both *C. perfringens* and Coliphage. Fecal coliform geometric averages in sediment ranged from 1.84 CFU / 100 g dw at the beaches to 41681.94 CFU / 100 g dw at the parks. Geometric averages for *E. coli* ranged from 1.30 CFU / 100 g dw at the beaches to 14802.09 CFU / 100 g dw at the parks. Enterococci geometric averages ranged from 0.231CFU / 100 g dw at the beaches to 34794 CFU / 100 g dw at the parks. Geometric averages for *C. perfringens* ranged from <0.15 CFU / 100 g dw at the beaches to 21134 CFU / 100 g dw at the parks. Coliphage geometric averages ranged from <15 PFU / 100 g dw at the beaches to 0.697 PFU / 100 mL at the parks.

Analysis was performed to classify the type of sediment found at each site during late November and early December. Results are displayed in Table 3-4. For all sites, the sediment type was Sand with the exception of GRP which was classified as Sandy Loam. Sites DC and GRP had high levels of Phosphorous (33-40 ppm). At this level, soil can support plant and vegetation growth. Highest organic content was also found at site GRP. Sediment samples at the 3 beach sites were larger grained and easily separated in the water column. Samples at RSP, DC, GRP were observed to be "sticky" and sediment particles were hard to separate from the water. Sediment samples from September 14$^{th}$ were analyzed and generally showed the same classification, however DC did shift to a sandy-loam from sand and GRP was classified as sand rather than sandy-loam as before (Table 3-5). Sites in the middle reaches showed increases in Phosphorous with DC and GRP as the two sites with above optimum levels of Phosphorus for plant growth. All

sites collected in the fall showed increases in % organic matter except for RM and JP which stayed the same. These results show the changing nature of spring and summer conditions on the sediment classification. Table 3-8 shows the geometric physical parameters of the water samples. Parameters are arranged by site and by season and therefore displayed in section 3.2 where seasonality is explored. Date per sample are also displayed in Appendix D.

**Table 3-4.** Sediment classification and particle composition taken November/December 2005

| Site | Phosphorous (ppm) | pH | % Organic | %Sand | %Silt | %Clay | Classification |
|---|---|---|---|---|---|---|---|
| NBP (L) | 2 | 8.3 | 0 | 97.6 | 0 | 2.4 | Sand |
| NS (L) | 2 | 8.5 | 0.1 | 97.6 | 0 | 2.4 | Sand |
| RM (L) | 2 | 7.7 | 0.1 | 97.6 | 0 | 2.4 | Sand |
| RSP (M) | 14 | 7.9 | 0.4 | 98.1 | 0.5 | 1.4 | Sand |
| DC (M) | 33 | 8.1 | 0.5 | 97.1 | 1 | 1.9 | Sand |
| GRP (M) | 40 | 7.9 | 2.4 | 72.6 | 13.7 | 13.7 | Sandy Loam |
| JP (U) | 24 | 8.2 | 0.2 | 95.6 | 0.5 | 3.9 | Sand |
| SSP (U) | 15 | 8.1 | 1 | 97.5 | 0.5 | 2.4 | Sand |

***Reaches defined as (L )Lower, (M) Middle and (U) Upper.***

**Table 3-5**. Sediment classification and particle composition from samples taken September 14th, 2006.

| Site | Phosphorous (ppm) | pH | % Organic | %Sand | %Silt | %Clay | Classification |
|---|---|---|---|---|---|---|---|
| NBP (L) | 4 | 8.0 | 0.2 | 97.6 | 0 | 2.4 | Sand |
| NS (L) | 4 | 8.1 | 0.2 | 97.6 | 0 | 2.4 | Sand |
| RM (L) | 4 | 8.3 | 0.1 | 98.4 | 0 | 1.6 | Sand |
| RSP (M) | 10 | 7.9 | 1.7 | 88.4 | 8.9 | 2.7 | Sand |
| DC (M) | 44 | 7.9 | 1.5 | 82.4 | 6.2 | 11.4 | Sandy Loam |
| GRP (M) | 24 | 7.9 | 1.5 | 94.2 | 3.6 | 2.2 | Sand |
| JP (U) | 25 | 8.3 | 0.2 | 99.3 | 0 | 0.7 | Sand |
| SSP (U) | 15 | 8.6 | 1.5 | 96.8 | 1.8 | 1.4 | Sand |

*Reaches defined as (L ) Lower, (M)Middle and (U)Upper.*

In order to determine how the relationship of physical water parameters to fecal indicator bacteria in surface water and sediment, pearson correlations were run and are displayed in Table 3-6, all significant correlations occurred with a $p<0.05$. Fecal coliform in surface water showed a positive correlation with the amount of precipitation the day before sampling with R=0.369, however fecal coliform in sediment samples only showed a significant positive correlation with water temperature (R=0.22). For *E. coli*, water temperature showed a significant negative relationship in surface water and a positive relationship with *E. coli* in sediment. This suggests that *E. coli* may be growing in sediment but dies off with warmer temperature in the water column. For rainfall, *E. coli* had a positive relationship with rainfall (R=0.471) in the surface water but a significant negative correlation with sediment (R=-0.22). This suggests that more rain increases *E. coli* are in the water column from new inputs and less are in the sediment. This may indicate loss of *E. coli* from the sediment to the water column during periods of increased rainfall.

Enterococci in surface water showed a significant positive relationship with rainfall (R=0.351) and a very slight but significant negative correlation (-0.199) with flow indicating a possible dilution affect. Sediment samples were not found to significantly correlate with any of the physical water parameters. Only rainfall showed a significant relationship with *C. perfringens* in surface water (R=0.29) and in sediment a significant negative correlation was found with turbidity in the water column. This may be an indirect measure of loss form the sediments as turbidity increased that was not captured

during water sampling. Coliphage virus in surface water showed a significant positive correlation with flow (R=0.122), but a significant negative correlation with pH (R=-0.497) and water temperature (R=-0.433). However, no correlation was found for coliphage virus in the sediment with any of the physical parameters.

**Table 3-6.** Pearson correlation coefficient (R) for fecal indicator bacteria and coliphage in surface water and sediment at river sites against physical water parameters (α=0.05)

| *Indicator* | *Water Temperature* | *Turbidity* | *pH* | *Flow* | *Rainfall* |
|---|---|---|---|---|---|
| Fecal coliform | | | | | |
| Water | 0.063(96) | 0.049(96) | 0.021(98) | -0.12(99) | 0.369(99)* |
| Sediment | 0.293(83)* | 0.048(84) | 0.090(85) | -0.008(86) | -0.178(86) |
| *E. coli* | | | | | |
| Water | -0.307(97)* | 0.065(97) | -0.125(99) | -0.109(100) | 0.471(100)* |
| Sediment | 0.229(85)* | -0.171(85) | 0.124(87) | -0.086(88) | -0.221(88)* |
| Enterococci | | | | | |
| Water | 0.027(97) | 0.072(97) | 0.008(99) | -0.199(100)* | 0.351(100)* |
| Sediment | 0.029(84) | 0.008(84) | -0.025(86) | 0.115(86) | 0.071(86) |
| *C. perfringens* | | | | | |
| Water | 0.005(97) | -0.011(97) | 0.06799(99) | -0.011(100) | 0.290(100)* |
| Sediment | 0.080(85) | -0.281(84)* | 0.171(86) | 0.183(87) | -0.089(87) |
| Coliphage | | | | | |
| Water | -0.433(98)* | 0.129(98) | -0.497(100)* | 0.295(101)* | 0.122(101) |
| Sediment | 0.054(86) | -0.086(85) | 0.081(87) | -0.032(88) | 0.127(88) |

* significance found at α=0.05
(n)=number of samples
Rainfall=average rainfall (inches) day before sample

A regression / multiple regression was performed using factors (water temperature, turbidity, pH, flow, and rainfall) to further examine relationships to the indiator. Significance was determined using the pearson correlation test from Table 3-6. Basic regression was used when only one factor was found to be significant according to Table 3-6. When more then one factor was found to be significant, a multiple regression was run. Results from the ANOVA showed a $p < 0.05$ for all indicator regression models tested in surface water and in sediment for fecal coliform, *E. coli*, Enterococci, and coliphage. No significance was found for models run for *C. perfringens* in surface water ($p=0.07$) and sediment ($p=0.074$). Models were not run on Enterococci and Coliphage in sediment as none of the water parameters were found to show a significant correlation with these indicators in the sediment substrate. Results for $R^2$ were low for all indicator species with the highest being $R^2 = 0.252$ for coliphage in surface water and $R^2 = 0.119$ for *E. coli* in sediment. These results are displayed in Table 3-7. The values in Table 3-7 show water temperature and rainfall can explain 11.9% of the *E. coli* in the sediment whereas temperature, pH and flow can explain 25% of the coliphage numbers in the water column.

**Table 3-7.** $R^2$ values for Indicator species for river sites predicted by Precipitation, pH, Water temperature, turbidity and flow.

| *Indicator* | *Water Temperature* | *Turbidity* | *pH* | *Flow* | *Rainfall* | *Regression($R^2$)* |
|---|---|---|---|---|---|---|
| Fecal coliform | | | | | | |
| Water | | | | | x | 0.043 |
| Sediment | x | | | | | 0.109 |
| *E. coli* | | | | | | |
| Water | x | | | | x | 0.044 |
| Sediment | x | | | | x | 0.119 |
| Enterococci | | | | | | |
| Water | | | | x | x | 0.073 |
| Sediment | | | | | | NR |
| *C. perfringens* | | | | | | |
| Water | | | | | x | 0.021(NS) |
| Sediment | | x | | | | 0.024(NS) |
| Coliphage | | | | | | |
| Water | x | | x | x | | 0.252 |
| Sediment | | | | | | NR |

NR=regression model not run
NS=model not significant
x denotes parameter used in regression model

## 3.1 Spatial Analysis of Surface water and sediment

A land use map was generated using Arc View 3.2 of Ottawa County and Grand Rapids (Appendix A). The lower reach of the river (at the mouth of the river) contains both high and low intensity residential area with sand dunes and evergreen or decidous forests. The middle reach of the river is dominated by pastures and farms used for crops. Small farms with cows were also visually observed near site DC. As the study area transitions from middle reach to the upper reach, increased amounts of high intensity urban residential areas are observed close to the Grand River, as well as an increase in roads and transportation routes. Upper reach also contains some crop land but further away from the river.

### *3.1.1 Surface water*

A spatial comparison found that there were significant differences between some sites. Sites along the river were significantly different from beach sites (when $p<0.05$). Beach sites were found to be statistically lower in contamination compared to the upper and middle reaches of river sites. While sediments at beaches on average had higher concentrations than surface water, differences were less than $\log_{10}$. However, sediments at the park sites had generally 2-3 $\log_{10}$ higher concentrations of bacteria than in surface water. Because coliphage only had 9 positive samples in sediment, a relationship could not be adequately determined. Figures 3-1 to 3-5 (pages 82 to 86) are box plots showing the mean concentrations of fecal indicators and $50^{th}$ and $95^{th}$ percentiles along the Grand River and on Lake Michigan. All "a" graphs depict water samples, while sediment is shown in the "b" graphs. The x-axis displays the reach of the river, broken

up into sites as was described in Table 3-1. The y-axis shows the concentration of each indicator on a log scale as CFU/100 mL surface water or CFU/100 g dw sediment. In general, sediment samples showed a higher degree of variability as opposed to surface water samples.

Fecal coliforms

Figure 3-1a shows fecal coliforms in surface water and sediment. No significant differences were found among fecal coliform concentrations among the three beach sites NBP, NS and RM. A significant difference was found between RSP & NBP and RSP & RM. No significance was found between RSP and the beach site at the mouth of the river. Sites east of RSP (DC, GRP, JP and SSP) all showed significantly higher amounts of fecal coliforms than the three beach sites but no difference was noted amongst the river sites. Upper and middle reaches of the river showed higher concentrations of fecal indicators in both surface water and sediment. North Beach Park (NBP) was the only site which had higher concentrations of fecal coliforms in surface water than sediment samples on a 100 mL to 100 g dw basis. Figure 3-1a does show some variability in the water samples.

*E. coli*

Figure 3-2a shows the concentration of *E. coli* in surface water. No significant differences were observed among the three beach sites (NS, RM and NBP). A significant difference was found between RSP and NBP. All sites east of RSP (upper and middle reaches) had significantly higher concentrations of *E. coli* than the beaches, however no

significant differences between RSP, DC, GRP, JP and SSP (upper and middle reaches). No significant differences were observed between park concentrations of *E. coli*. Middle reach of the river showed increased levels of variability.

Enterococci

Figure 3-3a shows the concentration of Enterococci in surface water. No significant differences were observed among beach sites, and none among the park sites. All parks (upper and middle reaches) had significantly higher concentrations of Enterococci than the beach NBP, however one park (RSP) was not statistically different when compared to beach sites NS and RM.

*C. perfringens*

The concentration of *C. perfringens* in surface water is shown in Figure 3-4a. The two farthest beaches NBP and RM did not show significant differences in concentrations of *C. perfringens* amongst each other, however both beaches were found to have significantly lower concentrations of *C. perfringens* than the beach located at the mouth of the river into Lake Michigan (NS). The only park to have significantly higher concentrations of *C. perfringens* than NS was GRP. No significance was found between GRP and the other park sites and among park sites in general (upper and middle reaches). The least amount of variability occurred in the water samples for *C. perfringens* compared to all other indicators.

Coliphage

Site DC had significantly higher concentrations of coliphage than the two beaches north and south of the Grand River mouth (NBP and RM). No other significant differences were observed. However, only 54 samples of 164 samples tested positive for coliphage. Coliphage concentrations are displayed in figure 3-5a.

### *3.1.2 Sediment*

Fecal coliforms

Figure 3-1b shows the concentration of fecal coliforms in sediment samples. Out of all the sites, NBP had the lowest concentration of fecal coliforms found in the sediment and had significantly less fecal coliforms than all sites except NS. NS and RM did not show significant differences from each other. All river sites (upper and middle reaches) had significantly higher concentrations of fecal coliforms in sediment than the beach sites. Sediment samples showed high levels of variability at all three reaches of the river.

*E. coli*

Concentrations of *E. coli* in sediment samples are shown in figure 3-2b. Site NBP was found to have significantly less concentrations of *E. coli* than all other sites in sediment. No significant differences were observed between beaches NS and RM. All sites in the upper and middle reaches of the study area (parks) had significantly higher concentrations of *E. coli* compared to beach sediments (in lower reaches). No significance was observed among sediments at the park sites. Variability was shown

along all reaches of the river in sediment, sediment samples had higher levels of variability compared to *E. coli* in water samples.

Enterococci

Enterococci exhibited the similar trends in sediment as *E. coli,* however Enterococci were found to have slightly higher concentrations in sediment compared to *E. coli* in sediment (however this trend was reversed in surface water). Site NBP was found to have significantly fewer concentrations of Enterococci as shown in figure 3-3b, than all other sites in sediment. No significant differences were observed between beach sites (NS and RM). All parks (upper and lower reaches) had significantly higher concentrations of Enterococci compared to beach sediments in the lower reach of the study area. No significant differences were observed among sediment samples among the park sites.

*C. perfringens*

Figure 3-4b shows concentration of *C. perfringens* in sediment samples. There was a clear difference in *C. perfringens* concentrations between parks and beaches. No significant difference was observed among beaches (sites in the lower reaches), and no significant differences were observed amongst the park sediments (among upper and lower reaches of the study area). However, parks (upper and middle reaches) had significantly higher concentrations of *C. perfringens* compared to the three beach sites (lower reaches). Sediment samples had a larger range of variability compared to water samples, especially in the middle reach of the river.

Coliphage

As shown in figure 3-5b, only a few samples tested positive for coliphage, 6 samples out of 148 tested positive for coliphage in the sediment. No significance was observed among any of the sediment samples ($p=0.709$). However, the majority of the positive samples (5 of 6) were found in the middle reaches of the river. Coliphage was present in sediment at NS (June 19, 2005), RSP (June 21, 2005), DC (July 27, 2005), GRP (September 28, 2005 & April 25, 2006) and JP (May 1, 2006).

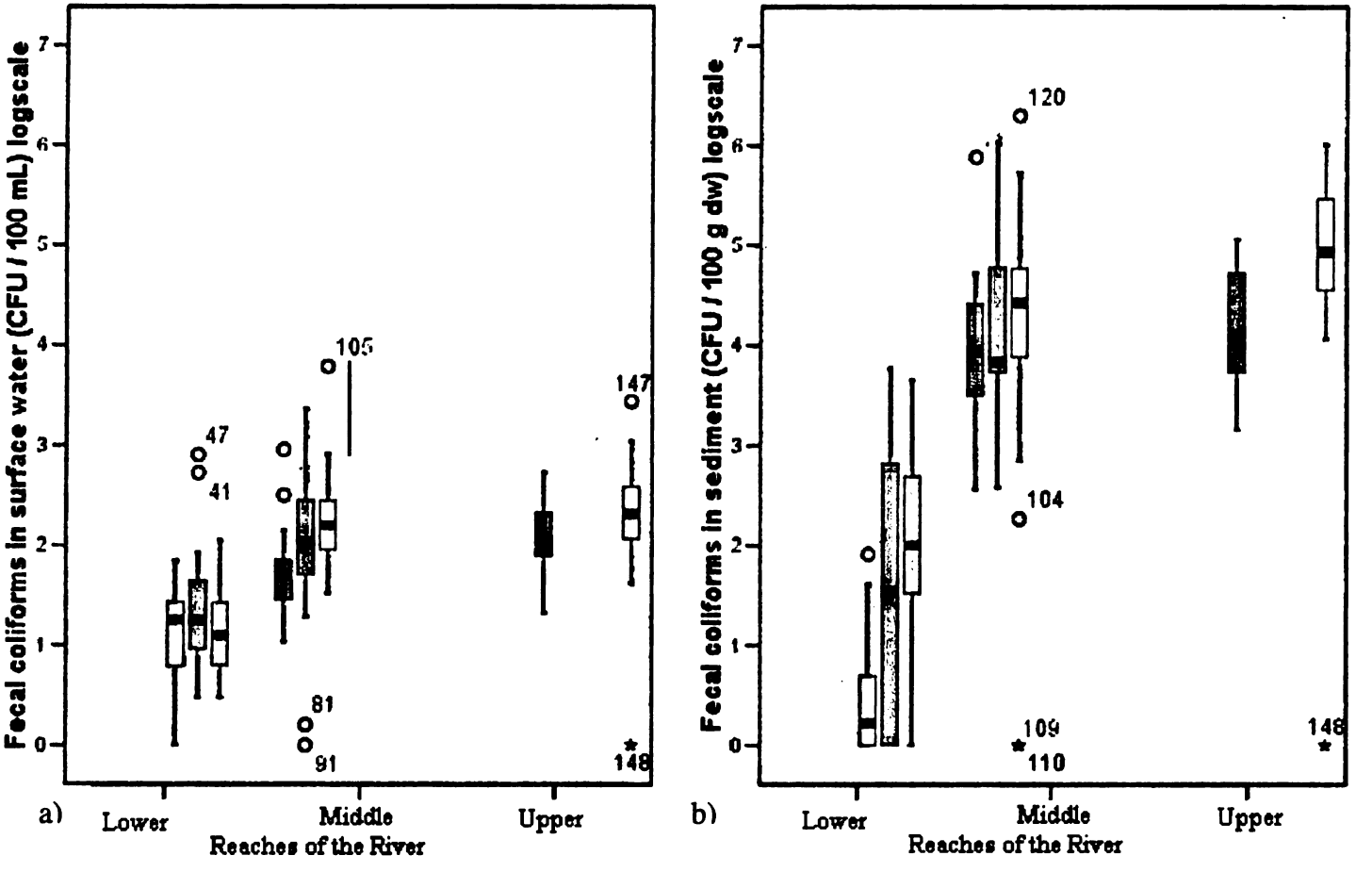



**Figure 3-1**: Concentrations of fecal coliforms along the Grand River in a) surface water and b) sediment NBP NS RM RSP DC GRP JP SSP

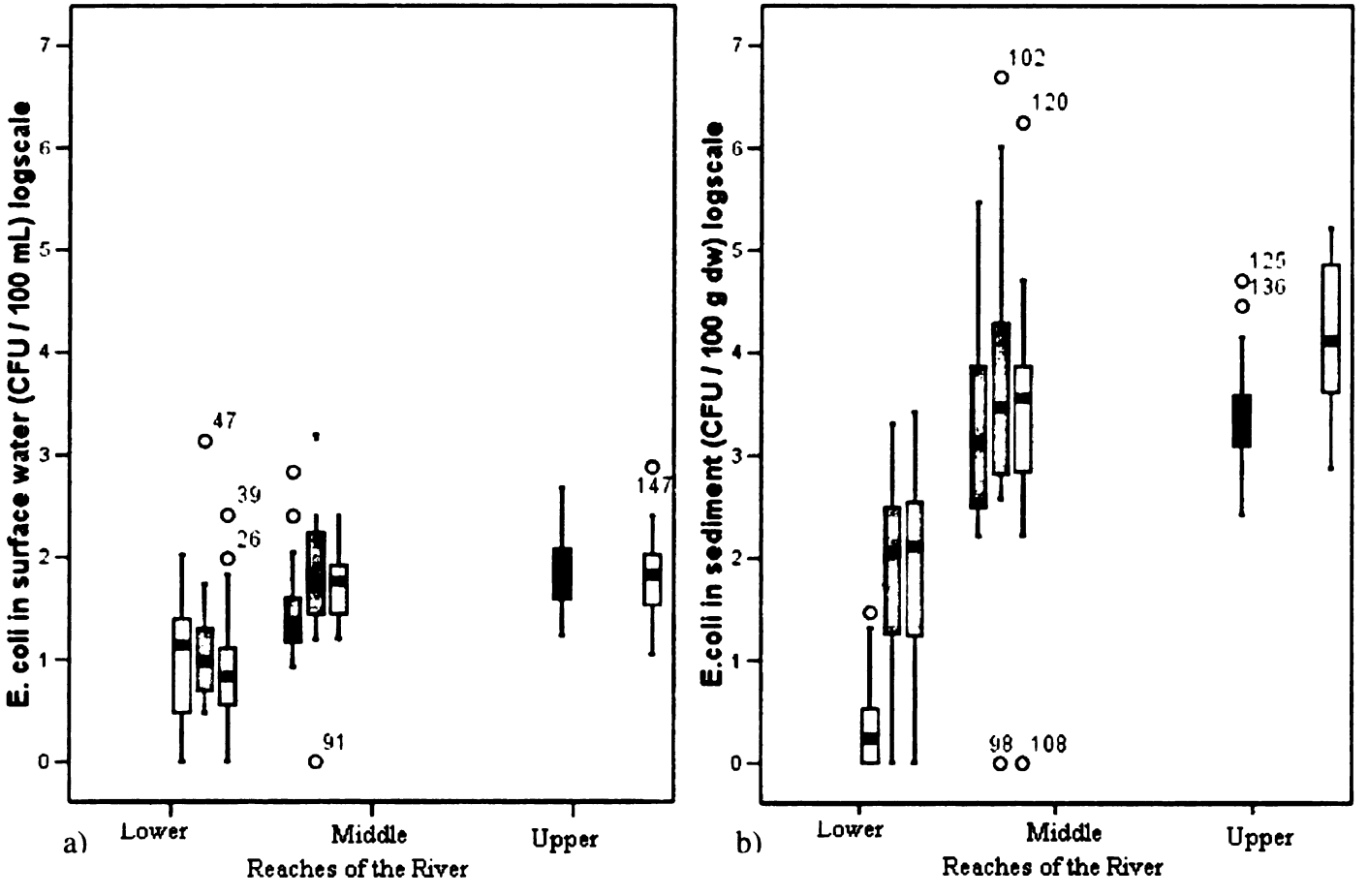



**Figure 3-2**: Concentrations of *E. coli* along the Grand River in a) surface water and b) sediment NBP NS RM RSP DC GRP JP SSP

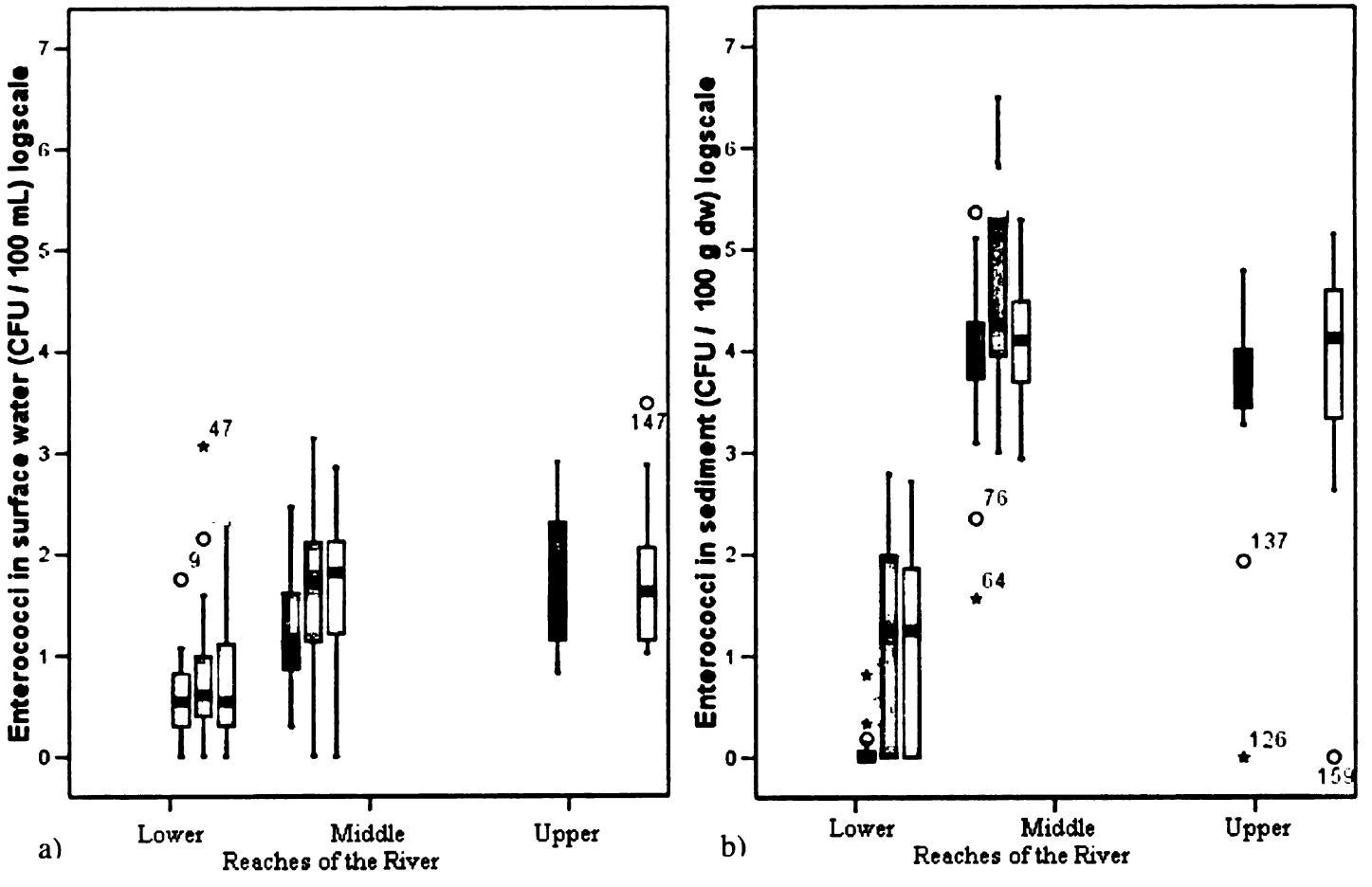



**Figure 3-3**: Concentrations of Enterococci along the Grand River in a) surface water and b) sediment NBP NS RM RSP DC GRP JP SSP

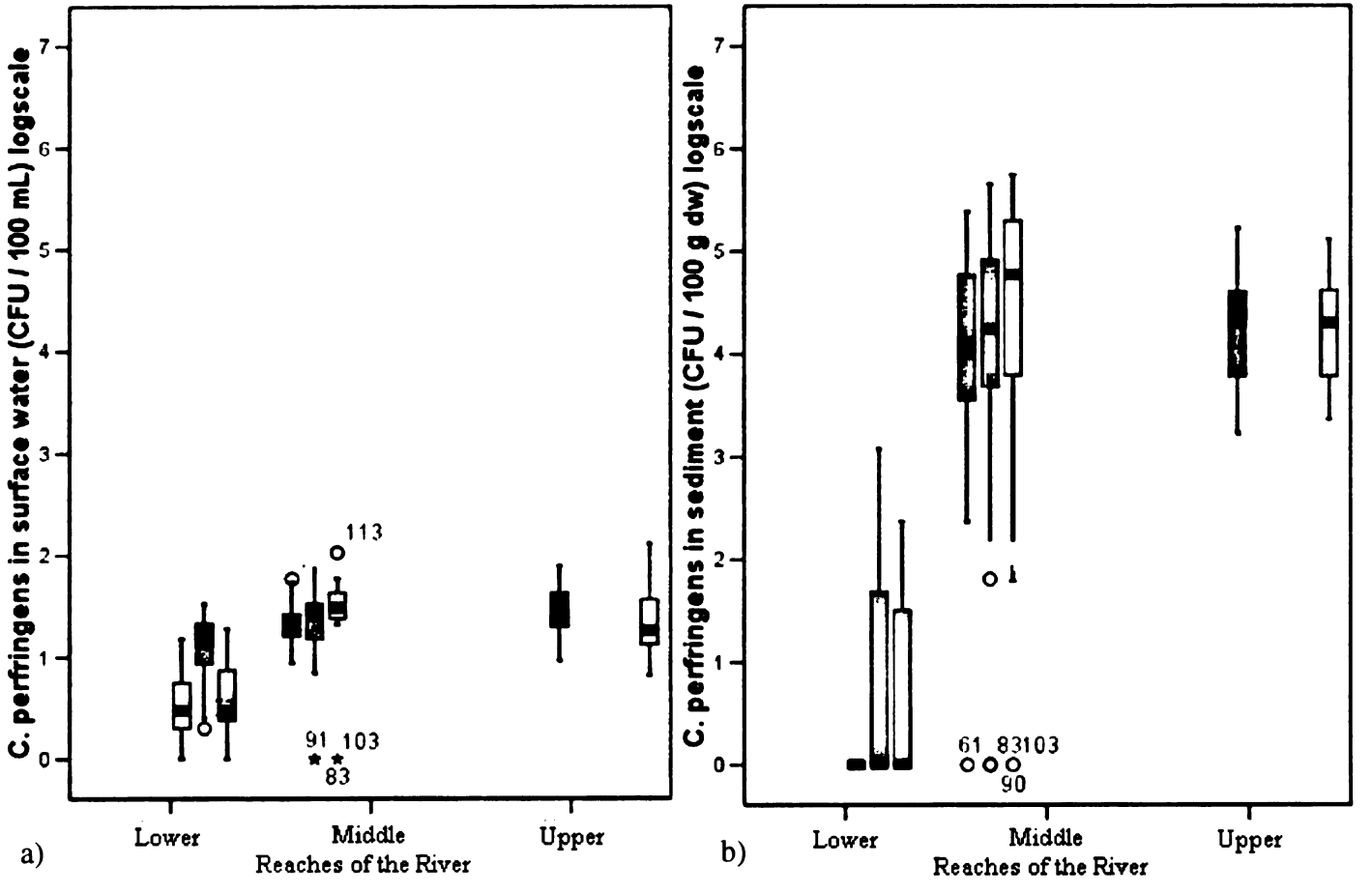



**Figure 3-4**: Concentrations of *C. perfringens* along the Grand River in a) surface water and b) sediment NBP NS RM RSP DC GRP JP SSP

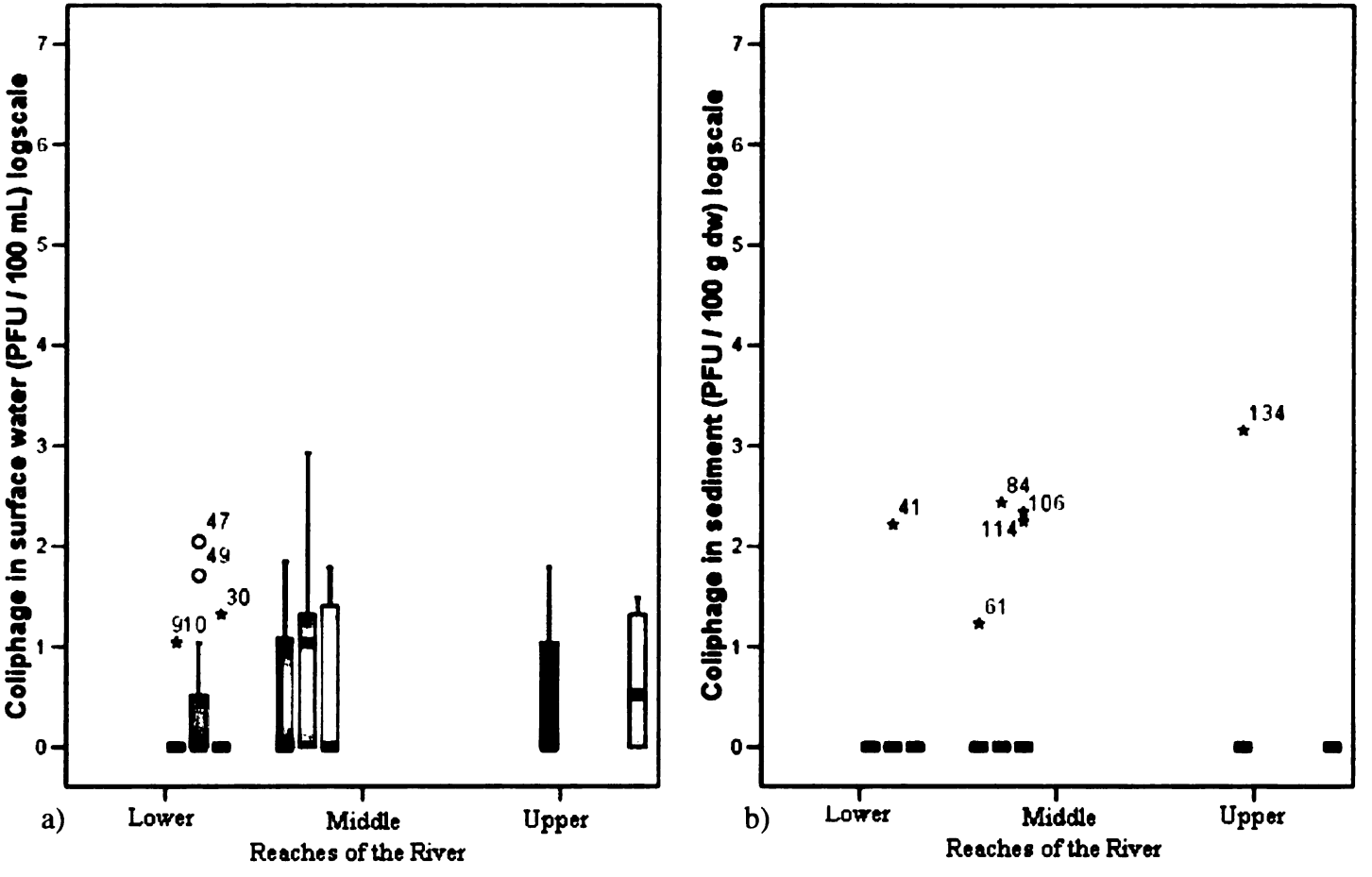



**Figure 3-5**: Concentrations of coliphage along the Grand River in a) surface water and b) sediment NBP NS RM RSP DC GRP JP SSP

### 3.2 Temporal analysis of surface water and sediment

Four time periods were considered for analysis and were grouped as Spring (March 20-June 20), Summer (June 21-August 22), Fall (August 23-December 20) and Winter (December 21-March 19).

Precipitation was averaged from two gages (Muskegan and Grand Rapids airport), both gages had high correlation with each other ($R=0.85$, $p<0.05$). A summary of the averages for pH, water temperature, and precipitation is shown in Table 3-8. The pH of the water was generally consistent ranging from 8.19 to 8.61. The two warmest seasons were summer and fall. Flow was measured at one site using the USGS flow gauge located near site SSP. Seasonal geometric average of flow is shown in Table 3-9 and the monthly mean for rainfall is shown in Table 3-10. Highest seasonal flow was observed at spring, while the lowest occurred in fall. Highest monthly rainfall occurred in July of 2005 in single month. However the highest period of rainfall occurred between a three month period occurred between November 2005 and January 2006.

**Table 3-8.** Geometric mean of the physical water parameters per site over the course of the four seasons

| Site | Season | pH | Water Temperature (°C) | Turbidity (NTU) |
|---|---|---|---|---|
| NBP | | | | |
| | Spring | 8.61 | 5.15 | 3.84 |
| | Summer | 8.55 | 15.20 | 2.96 |
| | Fall | 8.50 | 20.72 | 3.32 |
| | Winter | 8.56 | 1.55 | 2.26 |
| RM | | | | |
| | Spring | 8.43 | *0.90* | 9.11 |
| | Summer | 8.53 | 18.06 | 2.49 |
| | Fall | 8.53 | 21.15 | 2.39 |
| | Winter | 8.51 | 2.71 | 2.65 |
| NS | | | | |
| | Spring | 8.53 | 3.34 | 6.21 |
| | Summer | 8.55 | 13.86 | 6.60 |
| | Fall | 8.52 | 20.50 | 4.98 |
| | Winter | 8.49 | *6.30* | 6.22 |
| RSP | | | | |
| | Spring | 8.33 | 10.00 | 9.56 |
| | Summer | 8.52 | 20.33 | 7.28 |
| | Fall | 8.60 | 22.75 | 9.98 |
| | Winter | 8.19 | *-0.90* | 5.33 |
| DC | | | | |
| | Spring | 8.24 | *6.24* | 15.34 |
| | Summer | 8.31 | 20.45 | 10.92 |
| | Fall | 8.22 | 19.35 | 6.32 |
| | Winter | 8.27 | *1.14* | 17.69 |
| GRP | | | | |
| | Spring | 8.22 | *4.62* | 9.00 |
| | Summer | 8.49 | 20.16 | 6.57 |
| | Fall | 8.41 | 20.07 | 5.96 |
| | Winter | 8.24 | *2.30* | 7.61 |
| JP | | | | |
| | Spring | 8.27 | 10.20 | 10.46 |
| | Summer | 8.41 | 19.84 | 5.91 |
| | Fall | 8.51 | 19.70 | 5.72 |
| | Winter | 8.29 | *0.14* | 7.27 |
| SSP | | | | |
| | Spring | 8.33 | 9.24 | 11.38 |
| | Summer | 8.42 | 21.12 | 7.48 |
| | Fall | 8.56 | 16.87 | 9.54 |
| | Winter | 8.34 | *0.01* | 6.11 |

**Table 3-9.** Geometric average of seasonal flow

| Season | Flow (cu ft/s) |
|---|---|
| Spring | 5704.833 |
| Summer | 2971.293 |
| Fall | 1408.345 |
| Winter | 3868.762 |

**Table 3-10.** Average monthly rainfall during the months of the study

| Month | Rainfall (in.) |
|---|---|
| 2005 April | 0 |
| 2005 May | 0.1 |
| 2005 June | 0 |
| 2005 July | 0.61 |
| 2005 August | 0 |
| 2005 September | 0 |
| 2005 October | 0 |
| 2005 November | 0.240 |
| 2005 December | 0.035 |
| 2006 January | 0.357 |
| 2006 February | 0 |
| 2006 March | 0 |
| 2006 April | 0.149 |
| 2006 May | 0.088 |
| 2006 June | 0.025 |
| 2006 July | 0.005 |
| 2006 August | 0 |

Note: that n=37 for number of rainfall dates used

Seasonal means of indicator species in surface water and sediment are displayed in Table 3-11. Higher colony or plaque counts occurred usually in the winter season for water samples and in spring and summer for sediment samples.

**Table 3-11.** Mean concentration of fecal indicator species over time in water samples and sediment samples

| | | **Mean water CFU/100 mL** | **# water sample** | **Mean sediment CFU/100 g dw)** | **# sediment sample** |
|---|---|---|---|---|---|
| Fecal coliform | | | | | |
| | Spring | 36.47 | 54 | 615.46 | 47 |
| | Summer | 66.02 | 62 | 2374.65 | 61 |
| | Fall | 74.32 | 28 | 295.80 | 27 |
| | Winter | 79.73 | 14 | 217.22 | 10 |
| *E. coli* | | | | | |
| | Spring | 17.17 | 55 | 220.75 | 47 |
| | Summer | 26.51 | 63 | 1152.13 | 63 |
| | Fall | 64.54 | 28 | 277.91 | 27 |
| | Winter | 55.48 | 14 | 71.40 | 10 |
| Enterococci | | | | | |
| | Spring | 13.16 | 55 | 479.62 | 47 |
| | Summer | 18.13 | 63 | 670.35 | 61 |
| | Fall | 32.73 | 28 | 268.72 | 27 |
| | Winter | 24.84 | 14 | 263.57 | 10 |
| *C. perfringens* | | | | | |
| | Spring | 18.50 | 55 | 785.60 | 46 |
| | Summer | 10.37 | 63 | 501.42 | 61 |
| | Fall | 12.80 | 28 | 165.31 | 27 |
| | Winter | 17.14 | 14 | 246.72 | 10 |
| Coliphage | | | | | |
| | Spring | 2.45 | 55 | 1.48 | 45 |
| | Summer | 1.98 | 64 | 1.14 | 63 |
| | Fall | 4.18 | 28 | 1.20 | 28 |
| | Winter | 15.03 | 14 | 1.00 | 11 |

### *3.2.1 Surface water*

Fecal coliforms

There were no significant differences in fecal coliform concentrations throughout the year in surface water ($p>0.05$). A gradual increase in concentrations was observed and peaked during the winter season. Figure 3-6 shows the concentration of fecal coliforms over the four seasons.

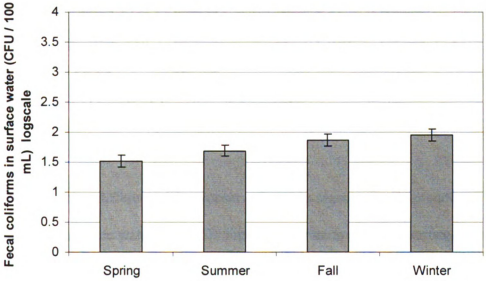



**Figure 3-6.** Seasonal trends of fecal coliforms in surface water.

*E. coli*

No significant differences were observed between spring and summer among *E. coli* concentrations. Fall (mean 64.53 CFU/100) and winter (mean 55.48 CFU/100 mL) showed significantly higher concentrations than spring (mean 17.17 CFU/100 mL). Fall had significantly higher concentrations of *E. coli* than summer (mean 26. Winter was

only significantly higher than spring. Figure 3-7 shows the gradual increase of *E. coli* as the year progresses.

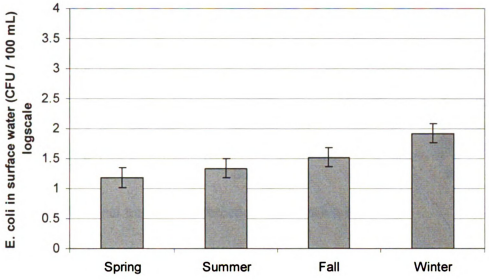



**Figure 3-7.** Seasonal trends of *E. coli* in surface water.

Enterococci

No significant differences were observed among seasons for Enterococci concentrations in surface water ($p>0.05$). Figure 3-8 illustrates how Enterococci concentrations peaked during both the summer and winter months.

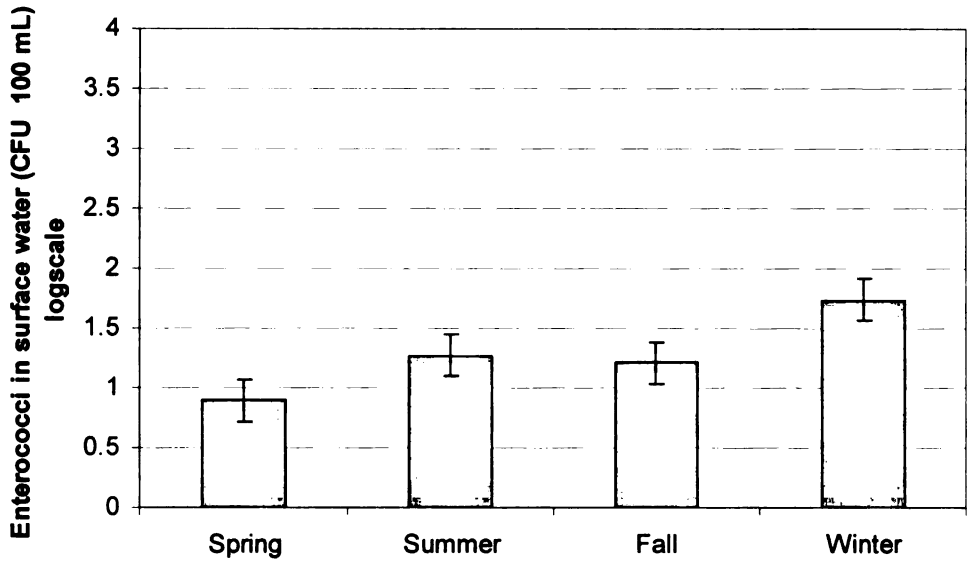



**Figure 3-8**: Seasonal trends of Enterococci in surface water.

*C. perfringens*

Significance was found among the means of the different seasons ($p<0.05$). Spring was found to have significantly higher concentrations of *C. perfringens* in surface water compared to summer, but not fall and winter. Concentrations gradually decrease from spring to summer than fall before slightly increasing in winter. Peaks in both spring and winter are shown in Figure 3-9.

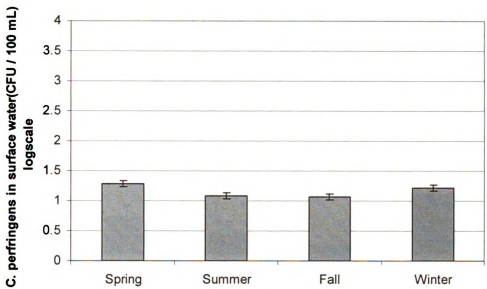



**Figure 3-9**: Seasonal trends of *C. perfringens* in surface water.

Coliphage

Significance was found among surface water in surface water for coliphage ($p<0.05$). Winter showed significantly higher concentrations of coliphage compared to all seasons. Concentrations peaked in winter and steadily decreased until late summer. Figure 3-10 shows the seasonal trend of coliphage over the year.

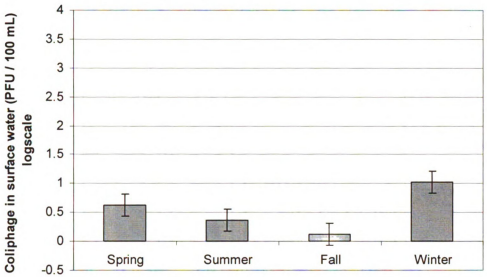



**Figure 3-10**: Seasonal trends of Coliphage in surface water.

### *3.2.2 Sediment*

No significance was found amongst seasons in any of the indicator species in sediment samples ($p>0.09$). General trends for fecal coliform, *E. coli* and Enterooccci include a gradual increase in indicator species, peaking in fall. However, a decline is observed in winter. Spring was the peak season for *C. perfringens* in sediment, while winter was the peak season for coliphage. Seasonal trends are displayed in figure 3-11.

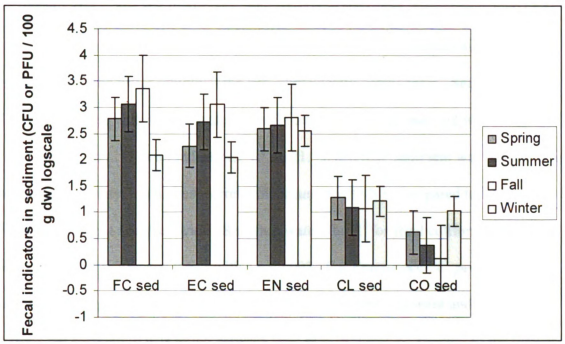



**Figure. 3-11**: Seasonal trends in fecal indicators in sediment in fecal coliforms, *E. coli*, Enterococci, *C. perfringens* and coliphage.

### 3.3 Relationship between surface water and sediment

A regression analysis was performed on surface water and corresponding sediment samples for fecal coliforms, *E. coli, Enterococci*, *C. perfringens* and Coliphage virus. The $R^2$ values were 0.444 (fecal coliform), 0.391 (*E. coli*), 0.331 (*Enterococci*), 0.441 (*Clostridium)* and 0.128 (Coliphage). Correlation graphs for each indicator species are displayed in Figure 3-12. The strongest correlation was found for *C. perfringens* and fecal coliforms when examining sediment and water. Regression analyses was also performed on river sediment and river water samples for each indicator according to seasonality. The highest $R^2$ value between water and sediment was 0.625 for *C. perfringens* which occurred in summer followed by $R^2$=0.38 for fecal coliform in winter ($p<0.05$). These results are shown in Table 3-12.

In order to test the significance of upstream sediment on downstream surface water, sediment for each indicator species from the immediate upstream site was regressed with the water sample of the site immediately downstream. Sediment from SSP was matched with surface water from JP, JP sediment was matched with GRP surface water etc... For all indicators, $R^2$ <0.21. When RSP sediment was compared to beach water quality, the highest correlation occurred with NS, compared to RM and NBP. For RSP sediment analyzed with NS surface water, the $R^2$ for fecal coliform and *E. coli* was found to be 0.706 and 0.675 with a $p < 0.002$. For Enterococci, *C. perfringens* and coliphage, no significance was found ($p > 0.05$) between RSP sediment and NS surface water. No correlation was found between RSP sediment and the beaches to the right and left of the river mouth (NS) and $p > 0.10$.

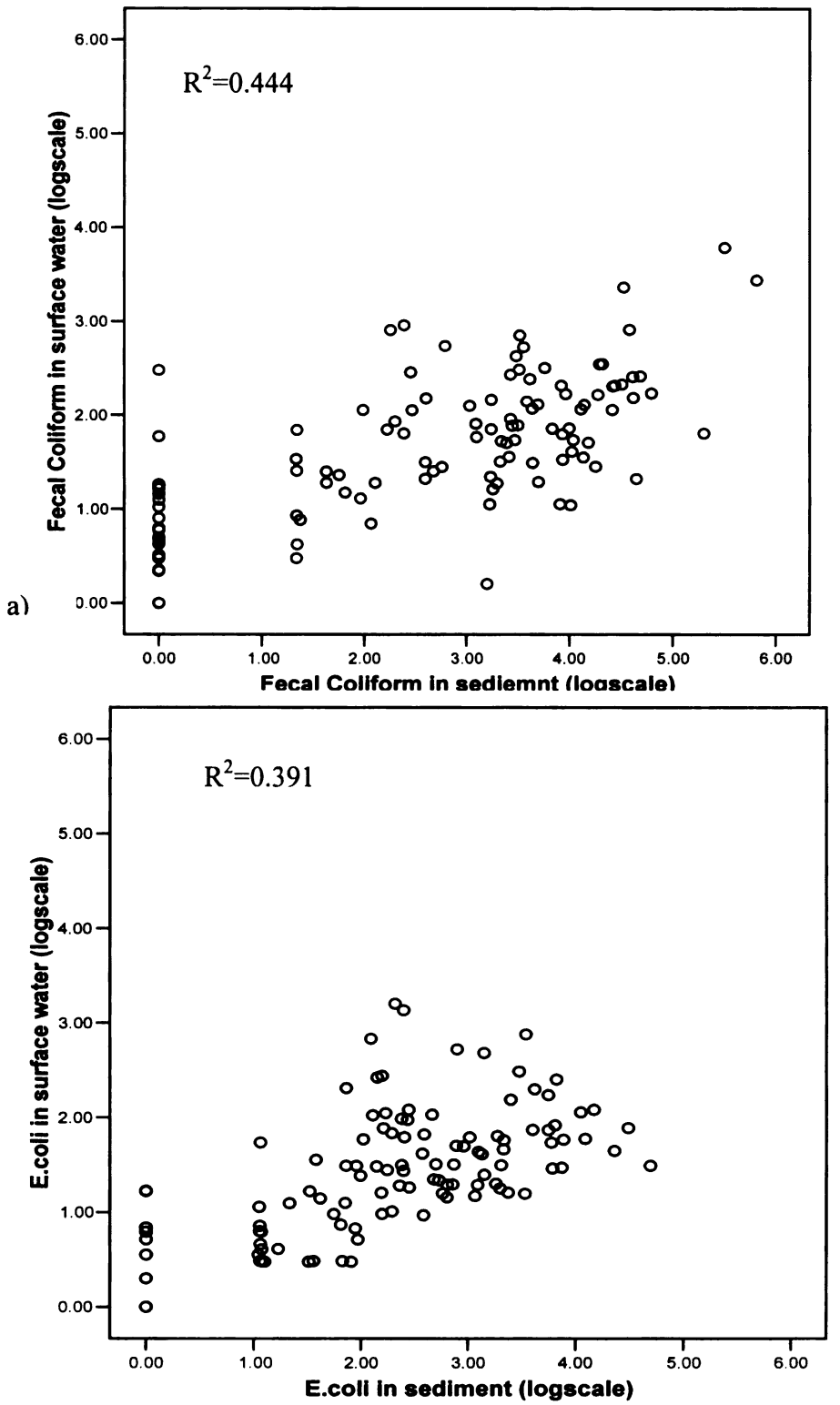
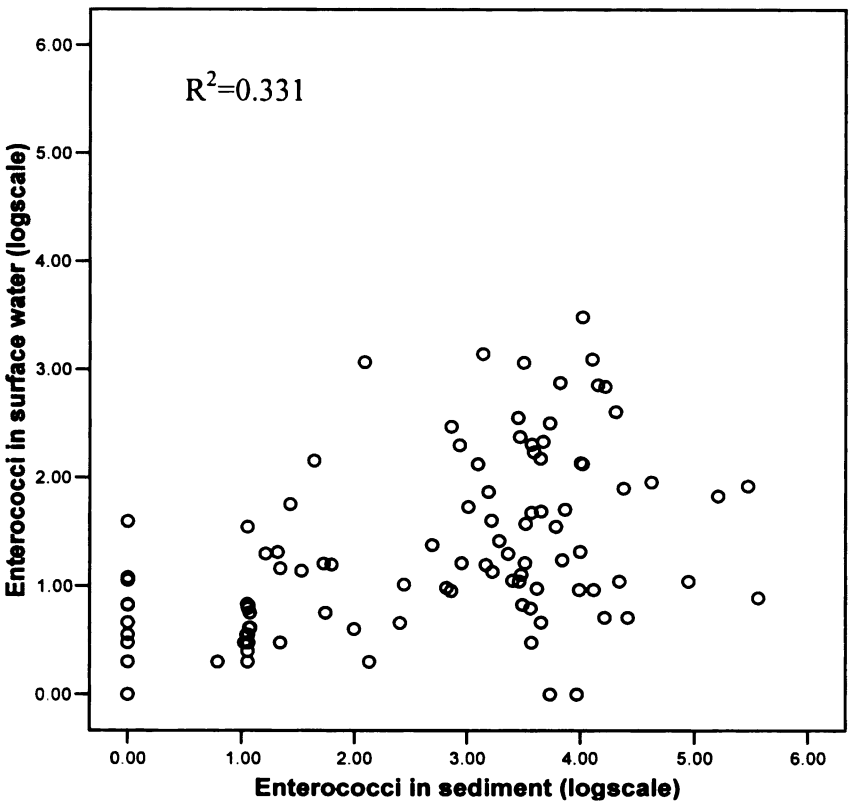



**Figure 3-12**. Correlation graphs of indictor species in surface water and sediment for a) fecal coliforms, b) *E. coli*, c) *Enterococci*, d) *Clostridium* and e) coliphage

c)




d)

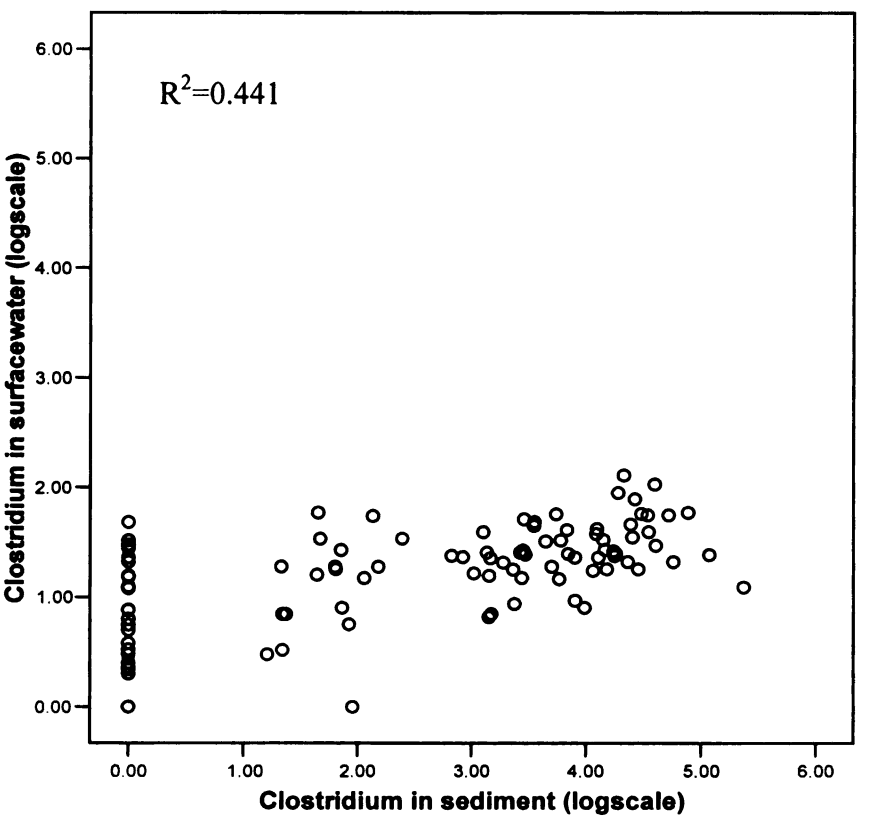



**Figure 3-12** cont'd.

e)

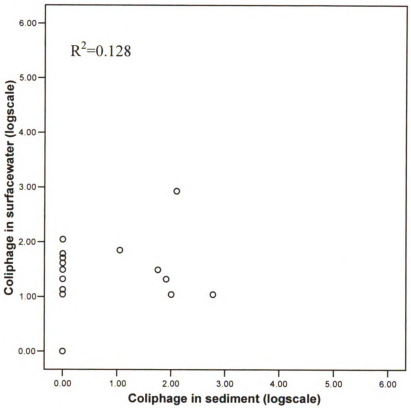



**Figure 3-12** cont'd

**Table 3-12.** Regression values between water and sediment concentrations for each indicator species broken down by seasons.

| | **Fecal coliforms** | ***E. coli*** | **Enterococci** | ***C. perfringens*** | **Coliphage** |
|---|---|---|---|---|---|
| **Spring** | .352(13)* | .228(13) | .036(13) | .062(13) | .056(12) |
| **Summer** | .310(30)* | .255(32)* | .028(30) | .625(30)* | .008(31) |
| **Fall** | .066 (24) | .056(25) | .016 (25) | .136 (26) | .147(26)* |
| **Winter** | .380(13)* | .000(12) | .005(13) | .053(13) | N.A |

Note: Values in brackets represent number of samples, * represents p value <0.05

Regression analysis was performed on each indicator in relationship to the other four indicators in surface water. Similar analysis was also performed on indicators in sediment. Highest regression in water was found between fecal coliforms and *E. coli* ($R^2$=0.647) followed by *Enterococci* and *E. coli* ($R^2$=0.617). The same trend was found in sediment: fecal coliforms and *E. coli* ($R^2$=0.597) followed by *Enterococci* and *E. coli* ($R^2$=0.551). Results are shown in Table 3-13 for $R^2$ in water and in Table 3-13 for sediment.

**Table 3-13.** Regression of each indicator species in relation to other fecal indicator species surface water (values represent $R^2$).

| | Fecal coliforms | *E. coli* | *Enterococci* | *C. perfringens* | Coliphage |
|---|---|---|---|---|---|
| Fecal coliforms | x | 0.647 | 0.488 | 0.385 | 0.083 |
| *E. coli* | 0.647 | x | 0.617 | 0.282 | 0.165 |
| *Enterococci* | 0.488 | 0.617 | x | 0.243 | 0.178 |
| *C. perfringens* | 0.385 | 0.282 | 0.243 | x | 0.044 |
| Coliphage | 0.083 | 0.165 | 0.178 | 0.044 | X |

**Table 3-14.** Regression of each indicator species in relation to other fecal indicator species in sediment (values represent $R^2$).

| | Fecal coliforms | *E. coli* | *Enterococci* | *C. perfringens* | Coliphage |
|---|---|---|---|---|---|
| Fecal coliforms | x | 0.597 | 0.502 | 0.391 | 0.034 |
| *E. coli* | 0.597 | x | 0.551 | 0.400 | 0.039 |
| *Enterococci* | 0.502 | 0.551 | x | 0.448 | 0.022 |
| *C. perfringens* | 0.391 | 0.400 | 0.448 | x | 0.009 |
| Coliphage | 0.034 | 0.039 | 0.022 | 0.009 | x |

**3-4 Combined Sewer Overflow samples**

Two CSO samples were examined for fecal indicators. The first event occurred on February 16th, 2006. Total combined CSO discharge was 1.457 million gallons and occurred between 2:40pm and 7:48pm. Discharge occurred due to rain and snowmelt, with a total precipitation amount of 1.67 inches occurring between 11:45am and 7:02pm. The duration of the overflow sampled (Site A) was 5 hours and 8 minutes where 1.443 million gallons of raw sewage was discharged into a creek leading to the Grand River.

A CSO discharge pipe is located at the intersection of Ionia and Stevens (Grand Rapids) where it leaves the waste water treatment plant and at this point the sample is on route to being released into the environment (at this point the pipe is underground in the city). This point is called Site A (Ionia & Stevens). Low concentrations of fecal indicators were found at this point. Site B is the point where the CSO effluent (mixture of dilute raw sewage and storm water) is emptied into the Grand River, the pipe can be seen from the banks of the Grand River. However at the actual discharge into the Grand River [Site B - Goodrich outfall (located on bank of Grand River)] high levels of the fecal indicators were found. Site B is 1.2 miles downstream from SSP (the eastern most site for the study). Site C (Freeman Drive) was approximately 1 mile downstream from site B and 3 miles upstream from study site JP. Table 3-15 shows the concentration of indicators found in the surface water and Table 3-16 shows concentrations in sediment. No sediment sample was obtained from the sewer sample at site A. Similar trends were found in sediment as was found for surface water. The outfall showed higher concentrations of fecal coliforms, *E. coli* and Enterococci. However, *C. perfringens* was

found in higher concentrations downstream at site C compared to site B. Water samples had bacterial concentrations ranging between <1 to $5.75x10^4$ CFU/ 100 mL and coliphage ranged between <6.7 to 326.67 PFU/ 100 mL. Sediment samples had bacterial concentrations ranging between 184 to $5.69x10^5$ CFU/ 100 mL. Coliphage was not detected in the sediment samples.

**Table 3-15**. Concentration of fecal indicator bacteria and virus in surface water as CFU or PFU / 100 mL after February 16th, CSO.

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage | Turbidity (ntu) |
|---|---|---|---|---|---|---|
| A | 3 | <1 | 6.8 | <1 | <6.7 | 4.4 |
| B | $5.75x10^4$ | 22950 | 4970 | 1600 | 326.67 | 24 |
| C | 1975 | 1335 | 1040 | 666.67 | <6.7 | 17 |

**Table 3-16**. Concentration of fecal indicator bacteria and virus in sediment as CFU or PFU / 100 g dw after February 16th CSO

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage |
|---|---|---|---|---|---|
| A | No sample | No sample | No sample | No sample | No sample |
| B | $5.70x10^5$ | $1.39 x10^5$ | 36308.05 | $2.21 x10^5$ | <14.33 |
| C | $3.51 x10^5$ | 184.36 | 737.46 | $4.71 x10^5$ | <23.89 |

The second CSO overflow occurred between July 17th and July 18th 2006. Total combined volume of the CSO for the city of Grand Rapids that day was 25.331 million gallons starting at 10:31pm until 7:49am July 18th. Total precipitation amount was 2.91 inches of rain, rainfall started 10:12pm July 17th and ended 2:10am July 18th. The duration of the overflow where samples were collected (Site A) was 2 hours and 17 minutes starting at 10:42pm July 17th, 1.52 million gallons of raw sewage was discharged. The results of discharge at Site A are displayed in Table 3-17 (surface water) and Table 3-18 (sediment). No sediment sample was obtained from the sewer sample at

site A. Similar trends were found in sediment as was found for surface water. Water samples had bacterial concentrations ranging between 583.33 to 4.55 x$10^5$ CFU/ 100 mL and coliphage ranged between 300 to 2160 PFU/ 100 mL. Sediment samples had bacterial concentrations ranging between 249079.3 to 8284736.58 CFU/ 100 mL. Coliphage ranged between <10 to 16.23 PFU/ 100 mL in sediment samples.

**Table 3-17**. Concentration of fecal indicator bacteria and virus in surface water as CFU or PFU / 100 mL from the July 18th CSO.

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage | Turbidity (ntu) |
|---|---|---|---|---|---|---|
| A | 2.72 x$10^5$ | 33200 | 10140 | 5950 | 2160 | 85 |
| B | 4.55 x$10^5$ | 12210 | 17220 | 583.33 | 300 | 330 |
| C | 3.30 x$10^5$ | 24235 | 28680 | 822.73 | 460 | 31 |

**Table 3-18**. Concentration of fecal indicator bacteria and virus in sediment as CFU or PFU / 100 g dw from July 18th CSO.

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage |
|---|---|---|---|---|---|
| A | No sample | No sample | No sample | No sample | No sample |
| B | 8.29 x$10^6$ | 2.49 x$10^5$ | 2.14 x$10^6$ | 3.11 x$10^5$ | <10 |
| C | 3.09 x$10^7$ | 7.68 x$10^6$ | 1.51x x$10^6$ | 6.87 x$10^5$ | 16.23 |

Two surface water samples were taken at the Goodrich outfall (Site B) on non-CSO discharge days. These results are shown in Table 3-19. There is a 100 to 1000 fold decrease in fecal indicator bacteria from the first CSO discharge and a sample taken 39 days later.

**Table 3-19.** Concentrations of fecal indicator bacteria and virus at Site B (Goodrich overflow point in surface water).

| Date | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage | Turbidity (ntu) |
|---|---|---|---|---|---|---|
| **2/16/2006** | 57500 | 22950 | 4970 | 1600 | 326.67 | 24 |
| 3/28/2006 | 122.7 | 52 | 15 | 76.2 | <10 | 15.8 |
| 5/08/2006 | | 19.45 | 76.1 | | <10 | 4.6 |
| **7/18/2006** | $4.55 \times 10^5$ | 12210 | 17220 | 583.33 | 300 | 330 |

*Note that CSO events occurred on 2/16/2006 and 7/18/2006 and middle two days were when no rainfall occurred previously.*

### 3.5 Site Rankings

The geometric average of all fecal indicators at each site was determined, and on that basis, each site was ranked in terms of which site had the highest levels of fecal indicator relative to each other. Each site was given a rank between one and eight. The rankings were summed up and the site with the lowest rank (being 1) classified as the most contaminated site. This was performed for both surface water (Table 3-20) and sediment (Table 3-21). For surface water the SSP site was found to be the most contaminated in surface water, while GRP had the highest level of fecal indicator bacteria in the sediment. The beaches and RSP maintained the same rankings and were consistently the least contaminated for both surface water and sediment.

**Table 3-20.** Relative ranking of sites for highest to lowest amount of contamination in surface water.

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage | Summed Score | Overall Rank |
|---|---|---|---|---|---|---|---|
| NBP(L) | 8 | 7 | 8 | 8 | 7 | 38 | 7 |
| NS(L) | 6 | 6 | 6 | 6 | 6 | 30 | 5 |
| RM(L) | 7 | 8 | 7 | 7 | 8 | 37 | 6 |
| RSP(M) | 5 | 5 | 5 | 3 | 4 | 22 | 4 |
| DC(M) | 4 | 3 | 3 | 5 | 1 | 16 | 3 |
| GRP(M) | 2 | 4 | 2 | 1 | 2 | 11 | 1 |
| JP(U) | 3 | 1 | 4 | 2 | 5 | 15 | 2 |
| SSP(U) | 1 | 2 | 1 | 4 | 3 | 11 | 1 |

Note: L (Lower reach), M (Middle reach), and U (Upper reach) Site with highest geometric mean given a value of one and site with lowest amount given an eight. Site given a 1 indicates it is the most contaminated.

**Table 3-21.** Relative ranking of sites for highest to lowest amount of contamination in sediment.

| Site | Fecal coliforms | *E. coli* | Enterococci | *C. perfringens* | Coliphage | Summed Score | Overall Rank |
|---|---|---|---|---|---|---|---|
| NBP(L) | 8 | 8 | 8 | 8 | 7 | 39 | 7 |
| NS(L) | 7 | 6 | 7 | 6 | 4 | 30 | 5 |
| RM(L) | 6 | 7 | 6 | 7 | 7 | 33 | 6 |
| RSP(M) | 5 | 5 | 3 | 4 | 5 | 23 | 4 |
| DC(M) | 3 | 2 | 1 | 5 | 3 | 14 | 2 |
| GRP(M) | 4 | 4 | 2 | 1 | 1 | 12 | 1 |
| JP(U) | 2 | 3 | 5 | 3 | 2 | 15 | 3 |
| SSP(U) | 1 | 1 | 4 | 2 | 7 | 15 | 3 |

Note: L (Lower reach), M (Middle reach), and U (Upper reach) Site with highest concentration of indicators given a value of one and site with lowest amount given an eight. Site given a 1 indicates it is the most contaminated.

### 3-6 Indictor Violations

Throughout the study, single samples which exceeded various criteria and standards were noted. Most violations occurred in the upper and middle reaches of the

study sites and occurred in the Grand River. Results are displayed in Table 3-22. Because Michigan currently does not have a standard for *C. perfringens*, results were compared to Hawaii's freshwater criteria of 50 CFU/100 mL. For *E. coli* and Enterococci criteria and standards, see Table 1-2.

There were more US EPA violations when using the Enterococci than *E. coli*, 38 versus 13 with only 12 if using the *C. perfringens*. The middle reaches of the river were violation 27 times versus 19 in the upper reaches if considering the EPA criteria. The higher contamination risk is also reflected in the rankings. See the above table (Table 3-20 and 3-21).

**Table 3-22.** Number of indicator violations in the upper, middle and lower reaches of the Grand River and Lake Michigan study sites

| | ***E. coli*** | **Enterococci** | ***C. perfringens*** |
|---|---|---|---|
| US EPA Criteria | 13/157 [5 upper, 6 middle and 2 lower] | 38/157 [14 upper,21 middle, and 3 lower] | NA |
| Michigan standard | 7/157 [2 upper, 4 middle and 1 lower] | NA | NA |
| Hawaii fresh water criteria | NA | NA | 12/158 [6 upper, 6 middle and 0 lower] |

Note: NA means not applicable. N=157 samples collected. For EPA and Michigan standards see Table 1-2).

## 4.0 Discussion

Surface water quality has always been a concern for both recreational users and beach managers in the state of Michigan due to the long coastline along the Great Lakes. Traditionally, only surface water has been measured for fecal indicator organisms such as fecal coliforms and *E. coli* which are used in Michigan for measuring safety. Studies undertaken by EPA however, evaluated a number of indicators and found that Enterococci had a higher correlation with swimming associated gastroenteritis rates at marine and fresh water bathing beaches. Enterococci had a correlation coefficient with regards to recreational sickness of 0.75 followed by *E. coli* (0.52) and *Klebsiella* (0.32), *Einterobacter / Citrobacter* (0.26), total coliform (0.19), *C. perfringens* (0.19), *P. aeruginosa* (0.19), and fecal coliforms (-0.01) (EPA, 1986). Many states including Michigan, are not requiring the monitoring of recreational water for Enterococci.

This study examined the Grand River and beaches along Lake Michigan influenced by the Grand River watershed using routine and alternative indicators. In addition to *E. coli* and fecal coliforms (which may show evidence of re-growth under warmer conditions) and Enterococci coliphage and *C. perfringens* were also used as a measure of water quality. By using a variety of indicators species, on multiple occasions in this study, Enterococci were found in higher concentrations than *E. coli* in surface water. This represents a potential health risk to people who use the Grand River as a place for water recreation. Based on the results of this study, a broad spectrum of indicators should be used in Michigan, and water quality should not just be limited to traditional indicators such as fecal coliforms and *E. coli*.

Using multiple types of indicators, several studies have shown how surface run-off, human sewage, animal waste and grazing pastures impact surface water quality (Jiang *et. al.*, 2001; Mallin, 2000; Burkholder *et. al.*, 1997; Fischer and Endale, 1999; Gary *et. al.*, 1983). Limited research has also shown that sediment is a source of fecal indicator bacteria and once bound to the sediment particles, can become re-suspended into the water column through sediment disturbance by wave action and rain events (An *et. al.*, 2002; Whitman and Nevers, 2003; Davies *et. al.*, 1995; Shiaris *et. al.*, 1987; Pettibone *et. al.*, 1996). However, not much is known about the role that sediment plays in water quality degradation. One of the goals of this study was characterization of the Grand River in Michigan in regards to microbial pollution in sediments as the source of problems in the water column. This study suggests that sediments should be more routinely examined to better characterize pollution loading to surface waters

It is not surprising that as this study examined the spatial differences in fecal indicator concentrations along the Grand River and Lake Michigan in both parks and beaches using traditional and alternative indicators (fecal coliforms, *E. coli*, Enterococci, *C. perfringens* and Coliphage), the River showed higher concentrations of fecal indicator species, specifically in the sediment samples than beach sites in Lake Michigan. The upper reach of the river is near the city of Grand Rapids, MI which has a population of 197,800 according to the US Census of 2000. Grand Rapids also has 77,960 total occupied housing units that are connected to the requiring sewage disposal system. During periods of high rain, wastewater treatment plants may not be able to handle the

increased volume of water, thereby resulting in numerous CSO events. During this study, Grand Rapids experienced 42 CSO events (24 of which either directly or indirectly discharged into the Grand River), and 31 SSO events as shown in (Table 4-1). For seasonal breakdown of fecal indicator species in water and sediment samples over seasons see Table 3-11). Volumes of raw diluted sewage were discharged, specifically on July 17$^{th}$, 2006 where over 23 million gallons of raw sewage were discharged directly into the Grand River over a 9 hour and 28 minute time period and 2.91 inches of precipitation.

Samples from CSO events discharging into the Grand River included both partially treated sewage and diluted raw sewage. These inputs contained high concentrations of fecal bacterial indicators ($2.3x10^4$ to $4.55x10^5$ CFU/100 mL). Coliphage were seen as good indicators of the recent impact as numbers were low generally in the river, but in the discharges to the river, they ranged between 327-460 PFU/100 mL.. It was difficult to sample and thus compare CSO events relative to one another as the total volume of discharge were different, and the amount of rainfall triggering the CSO was also different. Often these CSOs occurred in the Grand River and were reported and there was not sufficient time to sample. However, the sampling in this study showed that very little difference was found throughout the river and this was consistent over time. It is likely that the Grand River and beaches are similar to the national data showing that within the US, beach closures associated with CSO and SSO are prevalent. In 2003, out of 3214 closures, 272 closures were specifically due to CSO and SSO events.

**Table 4-1.** Average monthly CSO sewage discharge directly entering the Grand River, [no intermediary creek or river]. (MDEQ, 2006).

| Month of Discharge | Average amount of sewage (in million gallons) |
|---|---|
| May 2005 | 0.027 |
| June 2005 | 0.431 |
| July 2005 | 0 |
| August 2005 | 0.013 |
| September 2005 | 0.021 |
| October 2005 | 0 |
| November 2005 | 0.031 |
| December 2005 | 0 |
| January 2006 | 0.001 |
| February 2006 | 0.014 |
| March 2006 | 0.006 |
| April 2006 | 0 |
| May 2006 | 0.157 |
| June 2006 | 0.0625 |
| July 2006 | 11.92 |
| August2006 | 0.119 |

One of the single CSO samples tested showed lower concentrations of bacteria than expected at one of the sewer/manhole. This may have been due to large volumes of water from rain being diverted through the sewer system from impervious surface runoff at that location at that time etc. However samples at the outfall showed much higher concentrations indicating that water quality was compromised at discharge sites. While CSO events may contribute to water impairment, Murray *et. al.*. (2001) found that there was no correlation between CSO sites and water quality impairment when looking at fecal coliform and fecal streptococci bacteria. They cited that data depicted "a strong influence of upstream water and rural runoff "on water quality of the Rouge River located in south-eastern Michigan. This study of the Grand River, found that while CSOs

contribute to short term peaks of water quality impairments, over time those numbers decrease as shown in Table 3-18. However, these events are also depositing sediment and debris which settles to the bottom with microbes attached, and are seeding the sediments. Even though CSO events do not occur on a day to day basis, it still represents a chronic problem that influences water quality at the beach.

According to the city of Grand Rapids waste water treatment fact sheet (2007), Grand Rapids waste water collection system covers a geographical area of approximately 201.6 square miles, and of that, 5.37 miles is a combined sewer system. Compared to the late 1960's, where 12.6 billion gallons of overflow was produced, in 2006, the total of CSO water released was 0.0323 billion gallons. Long term water quality and sediment monitoring are not available, and improvements in water quality can not be demonstrated. While the overall volume has decreased, there remains an impact from untreated sewage entering Michigan natural water bodies. In the state of Michigan in 2006, 19.8 billion gallons of combined sewage was released by 31 communities, of which Grand Rapids was responsible for 50.276 million gallons (0.25%) which ended up in the Grand River. The average concentration of *E. coli* found in the two CSO samples (February 16 and July18th, 2006) discharging in the river in this study was 17,580 CFU/100 mL. If Grand Rapids was responsible for 50.276 million gallons, the number of *E. coli* entering the Grand River would be approximately $3.33 \times 10^{13}$ CFU / year from CSOs.
The city of Grand Rapids is however, required to eliminate combined sewer overflows by December 31$^{st}$, 2019, until then, CSOs will be an important input of microbial pollution to the Grand River.

There are other sources of fecal inputs to the river. In the upper and middle reaches of the Grand River many farms and pastures dominate the landscape. Many farms use manure as a fertilizer for vegetable crops. If manure is not dried long enough, some fecal indicators and pathogens may remain viable and attach onto manure particles. In the event of heavy precipitation, the water can wash surface soil and manure into the Grand River and creeks which feed into the Grand River. However, this study did not demonstrate any differences in water or sediment quality along the 77.3 km (48 mile) stretch of the river related to land use.

The second objective of this study was to examine the impact of seasonal changes on the concentration of fecal indicators. Not all indicators showed seasonal differences in water (only fecal coliforms and Enterococci did with slightly lower numbers in water) and no indicators showed seasonal differences in sediment. Thus the numbers in sediment seemed to reach an equilibrium. Many seasonal studies of recreational waters are done in warmer areas where recreation occurs year round. Shibata *et. al.*. (2004) looked at seasonal differences of fecal indicators (Enterococci, *E. coli*, fecal coliform, total coliform and *C. perfringens*) between wet and dry seasons in Florida. Only total coliforms showed a seasonal difference, the other indicators did not show significant difference between wet and dry season, even though the physical parameters (rainfall, temperature, salinity and pH) differed greatly.

In mid-Michigan, the most obvious climatic change by season is temperature. In this study the following was observed with regards to seasonality and water / sediment

quality. It was reported by National Climatic Data Center (2006b) that between September and November 2005, and between December 2005 to February 2006 the conditions in Michigan was more wet (by 15-30%) in both of these time periods compared to the 1971-2000 average (shown in Appendix E). Soil moisture is important in influencing pathogen survival, with survival being more likely under moist conditions (Crane and Moore, 1986. and Entry *et. al.*., 2000). When *E. coli* was placed in dry soil for 14 days and then moistened, re-growth was shown (Chandler and Craven, 1980). Considering that the climate during the study period was wetter than average, a potential for *E. coli* re-growth may be possible after periods of desiccation even in the soils that may have run off into the Grand river. Sediment moisture was found to facilitate pathogen survival (Byappanahalli *et. al.*, 2003) with higher concentrations of *E. coli* being found with higher moisture. Fecal coliforms, Enterococci and *E. coli* showed a trend of peaking in winter with the lowest temperatures and the lowest concentrations occurring in spring (during thawing) before starting to increase in summer and fall. The highest volumes of CSOs were in the summer then spring followed by fall and finally winter. These lower concentrations in spring may indicate a washing effect due to the extra volume coming in during the spring melt.

In Michigan, some forms of recreation do occur in the fall and winter, and include boating and fishing, both of which can result in direct contact with water. While most CSO events occurred in the summer and spring, winter temperatures stabilized the system and the fecal indicators over winter easily. Due to the constant freeze–thaw cycle, while fresh snowfall and rain may make an immediate impact to the water system,

melting snow can take time to reach the mouth of the river from various elevated areas in the water shed.

The only indicator which showed a dramatic change with temperature was the coliphage virus. A much greater die-off was observed in the summer and fall months. Higher concentrations of coliphage occurred when water temperatures ranged between -0.9°C and 6.3°C. However, coliphage were rarely found to survive in sediment samples. This indicates that coliphage viruses are very sensitive to temperature changes, and would be a good indicator used for determining whether or not recent contamination has taken place in the Grand River.

Currently, some farms in Michigan apply manure on top of snow during the winter. However, it is not known if this was the case in the Grand River watershed. One reason for increased indicators in the water column may be due to winter application of manure onto fields during the winter months. Because Michigan winters are variable, manure applied on top of snow may enter the water column during periods of snow melt as there is constant freeze and thaw weather events occurring in Michigan and other states and provinces in the mid-west USA and in Canada. Currently no law prevents winter manure applications for smaller animal operations. In the Michigan Department of Agriculture does mention that winter manure application should be avoided, but if "necessary" solid waste can only be applied to slopes 6% or less and liquid manure applied to slopes 3% or less. Stronger language is needed to minimize this potential

source of pathogens and / or clear guidelines as to what constitutes "necessary" needs to be established.

Due to a possible dilution affect and length of transportation time, numbers of coliphage virus may have been too low to be used as a consistent indicator of pollution at the beaches of Lake Michigan. In a survey of literature by John and Rose (2005), it was observed that coliphage viruses had a higher inactivation rate when temperatures increased, especially between 11-30°C where inactivation rates ranged between 0.03-2.5 log/day compared to 0-0.1 log/day at temperatures between 0-10°C. This trend is reflected in this study, as higher concentration of coliphage occurred during cooler periods. The advantage of using coliphage in the Grand River is that it is less likely to re-grow in the environment compared to indicators such as *E. coli* and Enterococci. Thus it is recommended that coliphage be used in the river, but further studies would be needed to demonstrate this indicator usefulness at the beaches in the lake.

Trends for *C. perfringens* were different in surface water compared to fecal coliform, *E. coli*, Enterococci and coliphage. A peak concentration was found in spring related to flow or rain. As mentioned previously, *C. perfringens* does not re-grow in the environment, which is why it is used as an indicator in warmer climates. Because it is a spore forming bacteria, it can represent older pollution. Our results found that *C. perfringens* significantly decreased from spring to summer, and stayed similar between summer and fall as flows and CSOs increased.

*Sediment*

Concentrations in sediments of the Grand River appear to be stable throughout the year due to the protective aspects of the sediment; however the sediments which were sand in nature at the beach sites had very low concentrations of bacteria which could have been influenced by wave action suspending bacteria to water column than being exposed to ultraviolet radiation from sunlight. Surface water was much less turbid at the beaches compared to the park surface water, therefore less particles to deflect and absorb ultraviolet rays in the shallow water - sand interface. Enhanced water clarity increases light transmittance (Mcisaac, 1996) through surface water and this can be seen to occur when there are zebra mussels. Lake Michigan has an abundance of zebra mussels, including areas at the beach study sites.

In this study, sediments were found to be a reservoir and source for the fecal bacteria throughout the year. Fecal coliforms and *E. coli* were less tightly bound to sediments where as Enterococci and *C. perfringens* were more tightly bound to sediment particles. This was highlighted when analyzing rainfall, it was observed that gram negative bacteria, which have lipids in their cell walls had a higher negative correlation between values in sediment and rainfall. It was found by Jamison *et. al.*., (2005) that *E. coli* re-suspension was limited to the rising limb of the storm hydrograph and only a finite amount of *E. coli* was available for re-suspension during rain events. Concentrations in the sediment bed were replenished afterwards, possibly due to fresh inputs from surface runoff (Jamison *et. al.*., 2005). Gerba and Bitton (1984) stated that rainfall "mobilizes previously retained bacteria and viruses" and promotes transportation to water bodies.

Physical sediment properties were characterized and revealed that the river samples had higher concentrations of Phosphorus (10-44 ppm) compared to beaches (2-4 ppm). In a study that looked at surface water and ground water from a natural areas, it was found that when phosphorus was added, there was an increase in microbial growth (Miettinen *et. al.*., 1997). However, the addition of other nutrients such as nitrogen, Potassium, Magnesium and Calcium did not significantly affect microbial growth. In another study Carrillo *et. al.*. (1985) found that both fecal coliforms and *E. coli* were found to be positively correlated (R= 0.668and 0.469) with phosphate concentrations in the Mameyes River (Puerto Rico). Rivers studied in the UK were found to have the highest soluble reactive phosphorus during low-flow conditions, with concentrations diluted as the flow increases (Jarvie *et. al.*., 2006). The Grand River has many winding turns with sections of high flow and sections of slower moving water. The highest concentration of phosphorus found in sediment in this study occurred in the middle reach of the study site where the river slows compared to the upper and lower reaches. Unfortunately in this river, there is only one river gauge, better monitoring of flow will be important to modeling water quality in this system.

This river also has the middle third dominated by lands used for agriculture and housing projects which dump sewage into the river. This may be another reason that microbial concentrations such as *E. coli* as well as phosphorous are high in the middle reach. In a study which looked at sediments and seawater, it was found that *E. coli* survived longer in the sediment which was attributed to having higher organic and

nutrient content then the surface water (Gerba and McLeod, 1976). High nutrient content (phosphorus) and organic content in the sediment in parks (Table 3-4 and Table 3-5) allow for good growing conditions and accumulation for bacteria. When sediment is disturbed or re-suspended it can affect downstream water quality.

Electrostatic and hydrophobic forces are generally recognized as important in bacterial adhesion (Salerno, Logan and Velegol, 2004, McNamara, Lemke and Leff, 1997)). Because the bacteria have a charge associated with them (+ or -), the charge may also influence sediment to water interactions. Soil properties such as particle size, cation-exchange capacity, and clay content (Gerba and Bitton, 1984) can also influence retention of fecal indicator bacteria to sediment particles. The higher the clay content, the more negative the charge on of the sediment particles. Even though many studies have been done examining the physical characteristics of bacteria, "mechanisms governing the adhesion of bacterial cells onto sediment grains are not fully understood" (Redman *et. al..*, 2004). Rheinheimer (1985) concluded that most aquatic bacteria are gram-negative while soil bacteria were predominantly gram-positive. Our results found that the gram negative bacteria (*E. coli*) had higher concentrations in the water column and is most likely to be re-suspended from sediments to the water column during rainfall events. Also, gram positive bacteria concentrations are slightly higher in sediment samples in the middle and upper reaches of the Grand River.

McNamara *et. al.*. (1997) found that 44% of hydrophobic bacteria and 17% of hydrophilic bacteria were gram-positive bacteria (Enterococci and *Clostridium*). A

difference in enzymatic activity was observed between the two types of bacteria (McNamara *et. al.*., 1997). However, studies have shown that when bacterial cells and sediment granules with high quartz content both had negative charges, a rate of bacterial deposition was observed (Redman *et. al.*., 2006). This phenomenon may be due to the secondary energy medium encountered by the bacteria as it approaches the quartz particle. Hahn and Melia (2004) describe that the cell which is unable to overcome the repulsion after the initial attraction may be bound to the particle. In the case of coliphage, it was only found in 6 (upper and middle reaches of the river) of 148 sediment samples and 54 of 161 surface water samples. Based on the results, it seems the coliphage does not survive very long in sediment or encounters obstacles which prevent it from reaching the sediment substrate

Sediment is a source of fecal pollution in the Grand River. Sediment cleanup methods currently employed include dredging, capping and doing nothing. Dredging requires removing the contaminated sediment to an offshore site for further remediation or landfill. The main drawback is the initial re-suspension when the sediment is removed (Thorma *et. al.*, 1993). Grimes (1975) found fecal coliform concentrations to significantly increase in the vicinity of dredging. When a small lake in Sweden was dredged, bacterial concentrations were found within the top 5cm of sediment and at the same concentration as before dredging (Cronberg, Gelin and Larsson, 1975). However, it was found that the water quality improved after 2km from the dredge site (Grimes, 1980).

Capping involves covering contaminated sediment with layers of additional substrate materials such as gravel, rocks, and or synthetic materials. Generally capping methods are used when contamination of sediments consists of nutrients such as phosphorus and metals (Berge *et. al.*, 2004; Kim *et. al.*, 2007; Eek *et. al.*, 2007). The materials used can rocks, clean sediment, calcite (limestone), sand and gypsum granules (O'Conner , 1984; Berg *et. al.*, 2004; Park et. Al., 2007) depending on what the major contamination in the sediment is. It was suggested that the capping layer be one meter deep J(O'Conner, 1984) to prevent benthic organisms from burrowing and disturbing the capping material. Clean sand was used to cover sediment with heavy concentrations of sewage sludge in Hiroshima Bay and dredging was used remediate nutrients in Osaka Bay (Kuroda and Fujita, 1982). Hiroshima Bay had decreased nutrients while Osaka Bay remained unchanged (O'Conner, 1984). When sand material was compared with cohesive silty material, both when exposed to severe storms, kept contamination from the water, but the silt cap was severely eroded (O'Conner, 1984). The idea is to prevent the contaminated sediment from being re-suspended into the water column by layering it with other materials. This method can only be used if the source of pollution has been determined and prevented from reoccurring, if the bottom of the water body or river can support the additional amount of substrate. Initial issues with capping could involve re-suspension of contaminants when placing the first substrate layer over the sediment bed. Capping is not always 100% affective. Future issues regarding sediment capping could arise if there are changes to the hydrology of the system, or there is redirected water flow / discharge to the capped area there is the potential for the added substrate to be disrupted or washed thereby re-suspending the contaminants.

The final alternatives are do nothing and simply monitor to see if the contaminants will degrade or get diluted out. At this point, both dredging and capping would not be an ideal solution as they are expensive and the deposition of microbial fecal pollution is ongoing. Fresh sediment would be easily re-contaminated. For the Grand River, efforts should be directed at minimizing inputs of pollution to the river and minimizing contamination from CSOs and agricultural run-off.

Beach sand can be dealt with using proper grooming techniques or sand remediation. Grooming can occur using a mechanical groomer or hand raking the sand. It was found that by using a mechanical grooming machine, *E. coli* counts were significantly higher then that of hand raking and doing nothing (Kinzelman *et. al.*, 2003). Depth of sand grooming and sand moisture were found to be important (Kinzelman *et. al.*., 2004). Concentrations of *E. coli* in unleveled and deeper groomed sand was lower in visibly moist sand but not in dry sand. However, grooming techniques were found to reduce the number of poor water quality advisories due to dry weather effects by 30% (Kinzelman *et. al.*, 2004). Grooming is a type of tilling method which can aerate and remoisten sand. This process can provide bacteria with protection from heat desiccation and UV (Kinzelman *et. al.*, 2003). By raking the sand before recreation occurs and long enough for the surface of the sand to be exposed to UV, pathogens can be eliminated. Grooming methods should be designed to minimize standing water on beaches. Sand remediation can occur by removing contaminated sand and replacing with clean sand. Contaminated sand can be trucked to a landfill site or moved to a treatment facility. This method can prove quite costly and may only be an expensive temporary solution. Sand remediation is generally used for beaches with chronic sand contamination problems or

when large scale contamination occurs such as sewage and oil spills. All parks and beaches in this study had birds and waterfowl within the vicinity. Beaches along Lake Michigan have many gulls and subsequently gull feces in sand, beach sand grooming and maintenance should be carefully examined and tested before proceeding.

Indicators such as *E. coli* and Enterococci, (particularly Enterococci) have proven to be highly correlated with recreational illness compared to other indicators. This study suggests that 10 to 80% of fecal bacteria are coming from the sediments. It has been suggested that 1 g of sediment containing $10^4$ MPN / 100 g Enterococci is a similar exposure "as ingesting 100 mL of water at the health limit" (Lee *et. al.*., 2006). Sediments in the Grand River have high enough concentrations to pose risks to recreational users who use the river for full bathing recreational activities. This study supports only secondary uses of the Grand River. During high flow events with large sediment loads emerging from the river swimming should be restricted and signs posted indicating reasons for restricted full bathing.

*Economic concerns for Grand River and Lake Michigan parks and Beaches*

Optimal recreational opportunities are often based on factors such as aesthetics (cloudiness, visibility, day length), physical climatic conditions (wind, air quality, ultra violet radiation) and thermal climatic conditions (de Fereitas, 2003). For beach recreational opportunities, such as Grand Haven, MI along Lake Michigan, communities

rely on the summer season for water recreation to bolster the economy. In summer, daylight is longer, allowing for prolonged amounts of recreation to take place, warmer water temperatures allow for increased amounts of time spent swimming, water and jet skiing. In order for the full economic potential of recreational beaches and parks to be realized, one must factor in water quality. Fecal coliform, *E. coli*, Enterococci, coliphage and *Clostridia* can be used to address water safety and contamination. Yet the state of Michigan uses only one indicator, *E. coli.*

In the event of beach closures due to high fecal bacteria indicators, there can be severe economic repercussions to the community, and result in negative attitudes towards beach managers for not "fixing" the problem. When social norm curves were generated measuring acceptability with beach closures due to bacterial contamination, the threshold of acceptability was exceeded after 7 days of closures (Smyth, Watzin and Manning, 2007). But not closing the beach may have severe health implications and the current indicator system is not adequate to address this trade-off. Some beaches also are host to multiple large scale sporting events, such as boating competitions and triathlons. In a study performed by Van Asperen *et. al.* (1998), they found that among endurance athletes, triathletes (those who biked, swam and ran) were twice as likely to develop symptoms of gastroenteritis the week following exposure from fresh waters which met European bathing standards compared to the non-swimming athletes (who ran, biked, and than ran again). Thus also indicating that current water quality standards used are not adequate to protect public health. Closures of beaches result in cancellation of large scale events which economically benefit the community; it can also impact local businesses.

Businesses such as hotels, government revenue, cottage rentals, restaurants and small business involved in selling or renting recreational equipment can lose large amounts of money. Thus there is a tendency to protect the economic safety of the community as compared to the public health safety.

Economic impact of swim closures were examined by Rabinovici *et. al.* (2004) using existing water quality and visitor data from Lake Michigan taken from 1998 to 2001. The authors found that based on information from Indiana Dunes National Lakeshore system, beach closures can cause an average economic loss of up to $35 000 per day (ranging between $1274 -$37,030) for those who want to swim. The authors feel that an efficient standard would be set when the fecal indicator bacteria concentrations where the gains from closing the swim area exceed the recreation value lost such as 423 *E. coli* cfu/100 mL and perhaps never. Dwight *et. al.*, (2005) estimated gastrointestinal illness (GI) burden for two beaches in southern California. The estimated that the economic burden is approximately $36.58 per gastrointestinal illness, $76.76 per acute respiratory disease, ear ailment was $37.86, and eye irritation $27.31. Values were adjusted to 2001 dollar value. These two beaches were estimated to generate approximately 36,778 GI plus 38,000 other illnesses per year. Based on the number of expected illness, the public health burden was estimated to be $3.3 million per year for these two beaches (Dwight *et. al.*, 2005) but are likely to be conservative as number of GI's were derived from a risk model. Other studies found the cost per illness to range between $218 to $2198 US according to 2001 dollars and adjusted for inflation (Scott *et. al.*, 2000; Frühwirth *et. al.*, 2001, Fleisher *et. al.* 1998).

Generally, closures are implemented 24 hours after a high fecal indicator bacteria reading. The 24 hour lag is due to time needed in collecting, processing and to analyze the samples. Tests such as IDEXX's colilert and enterolert require at minimum 18 hours of incubation before a reading can be taken. This is in essence a retrospective assessment of the water quality. It in reality is telling beach managers today what they should have been doing yesterday. Because there is a delay of usually 24 hours between sample taken and closure of beaches, the actual fecal indicator levels may not be harmful, thereby resulting in a needless closure, and loss of economic income. This type of unnecessary closure occurred on 14 out of 118 days that were monitored (Rabinovichi *et. al.*. 2004). This means roughly $490 000 was unnecessarily lost if you multiplied the average loss by the 14 days when unnecessary closures were issued. This value could be higher, considering days not included in the study were not factored in.

In 2005, two beaches in this study were found to exceed the 300 CFU/100 mL Michigan standards for *E. coli* in a single sample surface water (Table 4-2). This shows that beaches are susceptible to water impairments, and factors such as wind, rain and river water quality may be factors worth investigating.

**Table 4-2.** State water quality exceedences at beaches along Lake Michigan at sites also studied in this project (MDEQ, 2006).

| Park | Date | *E. coli* (CFU/100 mL) |
|---|---|---|
| Grand Haven State Park (state designation for our study site "North Shore" | 7/18/2005 | 311.66 |
| Rosy Mound Recreational Area | 6/21/2005 | 371.25 |
| Rosy Mound Recreational Area | 7/18/2005 | 708.31 |

and this highlights the need for further study into sediment role in the river and it's impact at the beach with regards to microbial pollution. Sediments are a reservoir of microbial pollution and must be taken into account when examining water quality. Currently, no standard method exists for analyzing fecal indicator bacteria in sediments as exists for other pollutants. Further monitoring of sediments for fecal indicator bacteria is imperative, without this data appropriate standards/guidelines could not be established in setting safety levels for monitoring. By combining sediment and surface water data, predictive models can be established to help better predict water quality and reduce the amount of unnecessary beach closures while at the same time, improving the understanding the dynamics of the river watershed system.

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## 5.0 Summary

Hot spots have been identified along the Grand River near CSOs and sewage discharge points as sites containing high concentrations of fecal indicators. These sites predominate in the upper reaches of the river and at Deer Creek. It is likely that sediments are a source of microbial pollution and can impact water quality given optimum climatic conditions (for example *E. coli).* Sediment contamination should be considered when making water quality decisions as re-suspension of sediment can be due to heavy precipitation, runoff or river discharge causing suspension of those microbes.

When examining both surface water and sediment, higher concentrations were found in the upper and middle reaches of the river compared to the lower reach indicating a large dilution affect occurring in the river system. While the Grand River is likely contributing to Lake Michigan beach quality, the concentration is diluted. Other sources such as bird feces and tourist garbage must be also taken into account when identifying sources of pollution.

In general, most indicators had highest concentrations in surface water during the winter and spring seasons. However, fecal indicator bacteria in sediment showed no statistical significance when analyzed for seasonality. Generally, declines in fecal indicator concentrations were observed sediment during the winter season. This shows that some species are able to survive the freeze thaw cycles found during Michigan winter seasons and/or there are additional inputs to the river during the fall / winter seasons.

Results were assessed for correlation between sediments and surface water for fecal coliforms, *E. coli,* Enterococci, *C. perfringens* and coliphage. The $R^2$ values for the aforementioned ranged between 0.444 and 0.331 for the bacterial indicators. While sediment quality was not directly related to the water column above it, it is a reservoir of fecal contaminants downstream. For *E. coli*, both rainfall and water temperature showed a significant relationship in both surface water and sediment. For rainfall, *E. coli* had a positive relationship with rainfall and a negative correlation with sediment. This may indicate loss of *E. coli* from the sediment to the water column during periods of increased rainfall.

When making decisions regarding issuing beach closures and advisories, the concentration of *Enterococci spp.* should be considered if it is shown to violate EPA criteria more than *E. coli*. Because *E. coli* and Enterococci can re-grow in the environment under warmer conditions, alternative microbial indicator species should be tested for. One such indicator is the spore forming C. *perfringens;* it requires anaerobic growth conditions and will persist in the environment. It is a good indication of past pollution levels. Another indicator, coliphage, is very sensitive, and does not persist long in the environment, therefore making it a good indicator of fresh pollution. Both *C. perfringens* and coliphage virus do not to grow in the environment, and would prevent over estimating extent of pollution.

## 5.1 Conclusions

1. Upper and middle reaches of the study sites has significantly higher concentrations of fecal indicator species

2. There are no seasonal differences in surface water for fecal coliforms and Enterococci, but exist for *E. coli*, *C. perfringens* and coliphage

3. No seasonal differences exist in sediment samples for any of the indicator species

4. On a per gram dry weight to mL volume, sediments have 2-3 $\log_{10}$ higher oncentrations of indicator species than surface water

5. Sediment samples are not related to surface water directly above

6. Riverside Park sediments correlated with North Shore surface water concentrations for fecal coliforms and *E. coli*

## 5.2 Management Recommendations

1. Implement a no body contact with surface water for atleast 24 hours after 1 inch of rain or CSO event

2. Mandatory testing for Enterococci for beaches and bathing sites

3. Build prediction models for different seasons

4. Separate raw sewage from surface water (CSO separation)

5. Development criteria and standards for fecal indicators in sediment and beach sand

6. Future work on examining re-suspension of indicator species

7. Mandate farms have vegetative cover on land before applying manure spreads on farm land to minimize surface run-off into rivers and lakes

8. Store manure for atleast 6 months before spreading onto land.

9. Prohibit liquid or dry manure from being placed on snow or on snow covered ground. Must be injected directly into ground during that time period.

10. Post advisories on beaches which have closed due to fecal indicator bacteria exceedances posting date and by how much it exceeded state laws

11. Post signs for good beach etiquette (do not throw garbage on the beach, encouraging defecation and urination in public beach waters, do not feed gulls)

12. Increase testing around parks and beaches with farms and combined animal farming operations

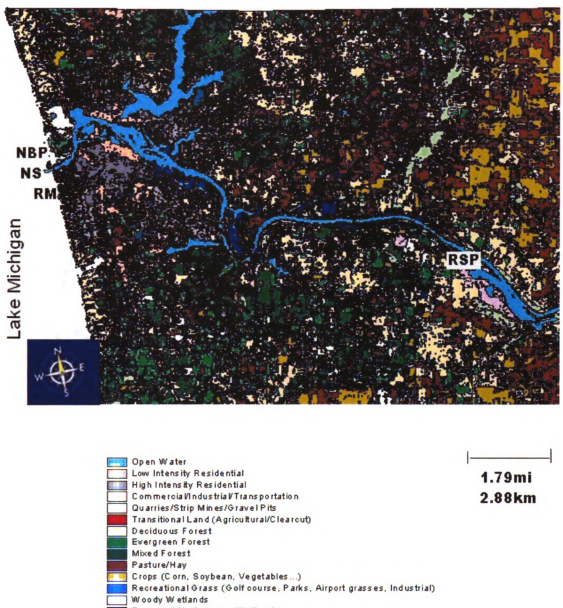
13. Farms and CAFO's be required to have an emergency plan in case of sewage spill or discharge

## Appendix A Important Journal article findings

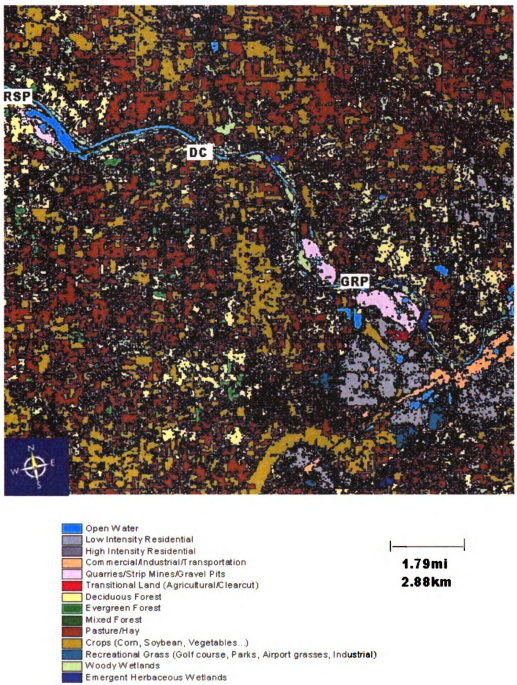
| Reference | Microbe & water type | Year/season | Finding | Water body |
|---|---|---|---|---|
| Weiskel *et. al.*, 1996 | FC (fresh) | 1986-86 | Low of $0.49x10^9$ FC/day in Aug. to $217x10^9$ FC/ day in Dec. from waterfowl | Buzzards Bay (MA) |
| Burkholder *et. al.*, 1997 | FC (fresh) | 1995 | $10^2$-$10^3$ cfu / 100 mL water $\geq 10^4$ cfu / 100 mL slurry sediment | New River (NC) |
| Esham and Sizemore 1998 | FC (saline) | Jan –Dec 1992 | cfu inc. 22-34°C FC inverse r/w salinity | Creeks (se Carolina) |
| Jiang *et. al.* 2001 | Coliphage (fresh) | Feb-Mar 1999 | 5.3 to 3332 PFU/litre of freshwater | Creeks and rivers beween malibou and Mexican border (CA) |
| Farag *et. al.*, 2001 | EC (fresh) | Summers of 96&97 | Cascade + for elk, deer, human, avian, canine, rodent, and human coliforms; Garnett - pres | Cascade Creek Garnett Creek |
| Shiaris *et. al.*, 1987 | FC (saline) | June 5, 1985 (sed) Oct.15&17, 1984 (water) | sediments 2 to 4 orders > water column; Sediment 200 – 60, 000 cfu/100mL slurry | Savin Hill Cove (MA) |
| Ahn *et. al.*, 2005 | FC (saline) | Feb. 18-Mar 3, 2004 | concentrations exceeding CA ocean bathing water standards by up to 500% ; pollution limited to <5 km from river outlet | Santa Ana River (CA) |
| Munawar *et. al.* 2001 | FC, EC (fresh) | 1991 | highest bacterial abundance in Lake Erie; lowest concentration Georgian Bay and Lake Superior. | Great Lakes Basin |
| McLellan and Salmore, 2003 | EC (fresh) | June-September 2001. | Offshore counts (10–150m from shore) levels did not exceed 235 CFU/100 ml in more than 5%; beach samples exceeded that mark in 66% of the samples | Lake Michigan (WI) |
| Marsalek *et. al.*, 1996 | EC (fresh) | | St. Mary's River 4-162 cfu /100 mL, St. Clair River 62-5130 cfu /100 mL Detroit River 392-1929 cfu / 100 mL | Great Lakes Basin (Windser, Sarnia) |
| Irvine *et. al.* (2005) | FC (fresh) | May, June, July September 2000 | 5-450 cfu/100 mL during rain; concentrations peaked 1 to 24 hours after rain events at concentrations of approx.1000 – 54000 cfu/100 | Buffalo River |

**Appendix B Land use maps**

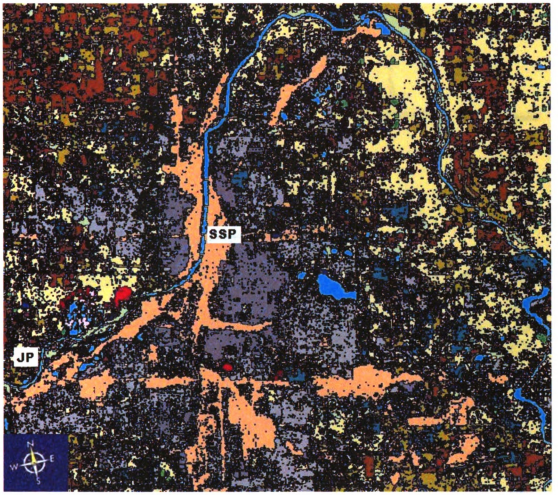
**1992 Land use map of mouth of the Grand River**



**1992 Land use of the middle reach of Grand River study site**



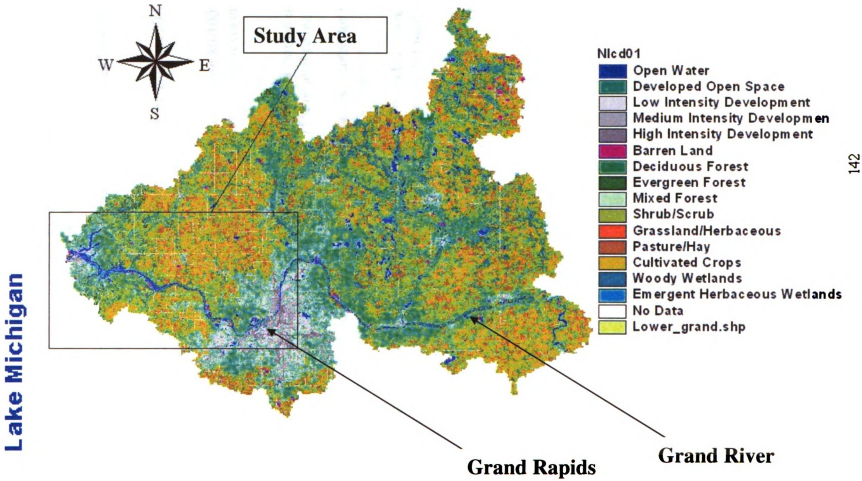
**1992 Land use of the upper reaches of Grand River study sites**




- Open Water
- Low Intensity Residential
- High Intensity Residential
- Commercial/Industrial/Transportation
- Quarries/Strip Mines/Gravel Pits
- Transitional Land (Agricultural/Clearcut)
- Deciduous Forest
- Evergreen Forest
- Mixed Forest
- Pasture/Hay
- Crops (Corn, Soybean, Vegetables...)
- Recreational Grass (Golf course, Parks, Airport grasses, Industrial)
- Woody Wetlands
- Emergent Herbaceous Wetlands

1.79mi
2.88km

# Lower Grand River Watershed
# Landuse 2001



Notes for Land use map classification

Developed open space – includes areas with a mix of constructed and vegetation (such as front lawn) and impervious surfaces account for less then 20 of total cover

Low intensity development- Impervious surfaces are between 20-49% of the total cover and area is mainly single family housing units

Medium intensity development- Impervious surfaces are between 50-79% of the total cover and area is mainly single family housing units

High intensity development- Impervious surfaces are between 80-100% of the total cover and area includes single family housing units, apartment complexes and commercial and industrial buildings

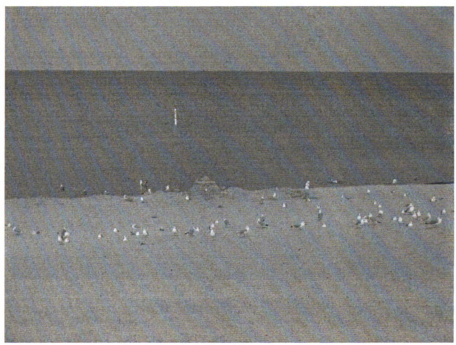
Barren land- mainly sand dunes, strip mines, gravel pits and earthen material accumulations. Vegetation accounts for less then 15% of total cover.

Pasture/Hay- Areas of grasses, legumes or grass-legumes planted for ruminants or hay crops. Pasture vegetation accounts for more then 20% of cover.

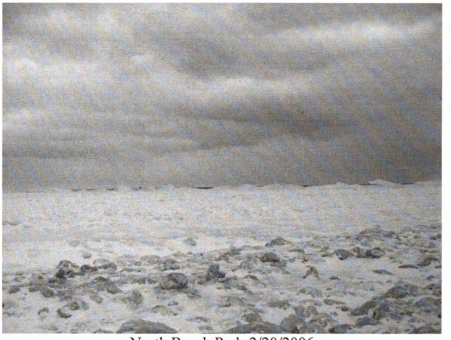
Cultivated crops- Areas used for annual crop production such as corn, soybeans, vegetables, tobacco. Crop vegetation is greater then 20% of the total vegetation and land is being actively tilled.

**Appendix C Site Photographs**

Rosy Mound Beach-6/25/2006



North Beach Park-8/28/2006



North Beach Park-2/20/2006
Ice build up along shoreline preventing access to water.

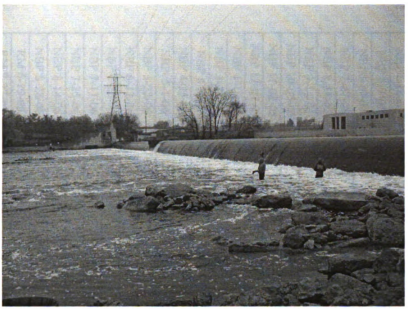
North Shore Beach-2/20/2006


Riverside Park-5/21/2006
Flooding of the boat launch area

Riverside Park-8/28/2006

Johnson Park - 8/28/2006

Johnson Park – 12/14/2006



Sixth Street Park – 11/16/2005

Sixth Street Park – 12/14/2005

## Appendix D Raw data used for analysis

| Sample ID | FC water | FC sed | EC water | EC sed | EN water | EN sed | CL water | CL sed | CO water | CO sed | pH | Temp. °C | Turbidity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NBP4-14-05 | . | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 | 8.19 | . | 2.2 |
| NBP 6-7-05 | 1.27 | 1.61 | 1.4 | 1.47 | 0.55 | 0 | 0.48 | 0 | 0 | 0 | . | . | 2.2 |
| NBP6-19-05 | 1.05 | 1.91 | 0.48 | 0.05 | 0.75 | 0.19 | 0.52 | 0 | 0 | 0 | 8.15 | 12 | 11 |
| NBP 7-27-05 | 1.32 | 0.92 | 1.14 | 0.24 | 0.48 | 0.07 | 0 | . | 0 | 0 | 8.56 | 24 | 2.6 |
| NBP 8-24-05 | 1.5 | 0.84 | 1.5 | 0.66 | 0.61 | 0.07 | 0 | 0 | 0 | 0 | 8.41 | 19.1 | . |
| NBP 9-14-05 | 1.81 | 0.69 | 1.45 | 0.58 | 0.83 | 0.07 | 0.58 | 0 | 0 | 0 | 8.55 | 22.2 | 3 |
| NBP 10-05-05 | 1.36 | 0.28 | 1.49 | 0.34 | 0.55 | 0.07 | 0.34 | 0 | 0 | 0 | 8.55 | 20.8 | 12 |
| NBP 11-02-05 | 0.34 | 0 | 0 | 0 | 0.3 | 0 | 0.3 | 0 | 0 | 0 | 8.52 | 10.3 | 0.5 |
| NBP 12-01-05 | 1.85 | 0.57 | 2.02 | 0.49 | 1.75 | 0.16 | 0.85 | 0 | 1.04 | 0 | 8.55 | 1.2 | 4.5 |
| NBP 1-10-06 | 1.28 | 0.23 | 1.22 | 0 | 0.8 | 0.07 | 0.8 | 0 | 1.04 | 0 | 8.61 | 0.3 | 5.1 |
| NBP 3-21-06 | 0 | 0 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8.8 | 2.3 | 3.6 |
| NBP 4-3-06 | 0.85 | 0.45 | 0.48 | 0.31 | 0 | 0 | 1.18 | 0 | 0 | 0 | 8.68 | 6.4 | 6.1 |
| NBP 4-25-06 | 1.02 | 0 | 0.79 | 0 | 0.66 | 0 | 1.09 | 0 | 0 | 0 | 8.8 | 9.3 | 4.5 |
| NBP 5-1-06 | 0.35 | 0 | 0.8 | 0.07 | 0.4 | 0.07 | 0.4 | 0 | 0 | 0 | 8.73 | 10 | 4.9 |
| NBP 5-22-06 | 0.51 | 0 | 0.3 | 0 | 0.3 | 0 | 0.48 | 0 | 0 | 0 | 8.6 | 9.7 | 0.8 |
| NBP 6-12-06 | 0.8 | 0 | 0.48 | 0.18 | 0.82 | 0 | 0.48 | 0 | 0 | 0 | 8.41 | 11.9 | 2.5 |
| NBP 6-26-06 | 1.22 | 0 | 1.55 | 0.21 | 1.08 | 0 | 0.3 | 0 | 0 | 0 | 8.4 | 17.6 | 0.4 |
| NBP 7-11-06 | 1.36 | 0 | 1.14 | 0.61 | 0.3 | 0 | 0.3 | 0 | 0 | 0 | 8.7 | 22.5 | 18 |
| NBP 7-25-06 | 1.52 | 0 | 1.21 | 0.53 | 0.4 | 0 | 0.98 | 0 | 0 | 0 | 8.9 | 21.6 | 3.9 |
| NBP 8-14-06 | 1.62 | 1.5 | 1.26 | 1.32 | 0.9 | 0.81 | 0.54 | 0 | 0 | 0 | 8.6 | 22.3 | 2.8 |
| NBP 8-28-06 | 0.78 | 0.52 | 0.79 | 0.52 | 0.89 | 0.33 | 0.3 | 0 | 0 | 0 | 8.4 | 19.4 | 1.2 |
| RM 6-19-05 | 1.05 | 3.44 | 0.48 | 2.13 | 0.55 | 1.25 | 0.36 | 0 | 0 | 0 | 8.11 | 22 | 2 |
| RM 7-27-05 | 1.45 | 2.97 | 1.01 | 2.51 | 0.48 | 0 | 0.48 | 0 | 0 | 0 | 8.59 | 25 | 2.8 |
| RM 8-24-05 | 1.28 | 2.27 | 0.87 | 1.97 | 0.3 | 0 | 0.48 | . | 0 | 0 | 8.39 | 21.5 | . |
| RM 9-14-05 | 1.93 | 2.52 | 1.83 | 2.51 | 1.54 | 1.26 | 0.48 | 0 | 0 | 0 | 8.56 | 22 | 2.8 |
| RM 10-05-05 | 2.05 | 2.68 | 1.99 | 2.59 | 1.2 | 2.01 | 0.4 | 0 | 0 | 0 | 8.49 | 20.9 | 2.3 |
| RM 11-02-05 | 0.7 | 0 | 0.55 | 1.24 | 0.3 | 0 | 0.4 | 0 | 0 | 0 | 8.48 | 10.6 | 1 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RM 12-01-05 | 0.69 | 0 | 0.55 | 0 | 0.66 | 0 | 0.7 | 0 | 0 | 0 | 8.52 | 1.7 | 6 |
| RM 1-10-06 | 0.62 | 1.54 | 0.61 | 1.42 | 0.48 | 1.25 | 0.52 | 1.54 | 0 | 0 | 8.54 | 1.1 | 3.1 |
| RM 3-21-06 | 0.88 | 1.51 | 0 | 0 | 0 | 0 | 0.85 | 1.51 | 1.32 | 0 | 8.5 | -1.3 | 7.9 |
| RM 4-3-06 | 1.18 | 2 | 0.84 | 0 | 1.14 | 1.72 | 1.28 | 2.37 | 0 | 0 | 8.1 | 1.8 | 11.8 |
| RM 4-25-06 | 0.65 | 0 | 0.55 | 0 | 0.3 | 0 | 1.2 | 0 | 0 | 0 | 8.7 | 8.4 | 8.1 |
| RM 5-1-06 | 0.48 | 1.55 | 0.48 | 1.77 | 0.3 | 1.26 | 0.9 | 2.08 | 0 | 0 | 8.6 | 12.9 | 6.2 |
| RM 5-22-06 | 1.1 | 1.53 | 0.79 | 1.25 | 0.61 | 1.25 | 1.08 | 0 | 0 | 0 | 8.5 | 13.7 | 4.7 |
| RM 6-12-06 | 0.93 | 1.54 | 0.83 | 2.16 | 1.3 | 1.42 | 1.28 | 2.01 | 0 | 0 | 8.44 | 13.1 | 3.3 |
| RM 6-26-06 | 1.41 | 2.58 | 1.22 | 2.82 | 0 | 0 | 0.3 | 0 | 0 | 0 | 8.4 | 20.5 | 0.95 |
| RM 7-11-06 | 1 | 0 | 0.71 | 2.72 | 0.3 | 2.1 | 0.3 | 0 | 0 | 0 | 8.8 | 20.9 | 0.85 |
| RM 7-25-06 | 1.16 | 2.71 | 1 | 2.11 | 1.09 | 2.71 | 0.3 | 0 | 0 | 0 | 8.8 | 20.7 | 3.4 |
| RM 8-14-06 | 1.59 | 2.98 | 2.41 | 2.83 | 2.29 | 2.56 | 0.6 | 0 | 0 | 0 | 8.6 | 20.4 | 2.8 |
| RM 8-28-06 | 1.51 | 3.66 | 1.29 | 3.42 | 0.67 | 2.72 | 0 | 0 | 0 | 0 | 8.6 | 21 | 1.8 |
| NS 6-19-05 | 2.73 | 3.77 | 1.49 | 2.17 | 2.15 | 1.9 | 1.48 | 1.24 | 0 | 2.22 | 7.92 | 21.7 | 7.7 |
| NS 7-27-05 | 1.51 | 3.52 | 0.71 | 2.17 | 0.6 | 2.19 | 1.01 | 0.13 | 0 | 0 | . | 13 | 7.1 |
| NS 8-24-05 | 1.4 | 1.84 | 0.86 | 1.26 | 0.3 | 0 | 1.18 | 0 | 0 | 0 | 8.62 | 21 | . |
| NS 9-14-05 | 1.34 | 3.44 | 1.17 | 3.28 | 0.66 | 0 | 0.75 | 2.13 | 0 | 0 | 8.42 | 18.9 | 4.2 |
| NS 10-05-0 | 1.11 | 2.17 | 1.1 | 2.06 | 0.48 | 1.54 | 0.88 | 0 | 0 | 0 | 8.52 | 21.7 | 5.9 |
| NS 11-02-0 | 0.48 | 0.04 | 0.48 | 1.25 | 0.3 | 0 | 0.4 | 0 | 0 | 0 | 8.5 | 19.1 | 3.5 |
| NS 12-01-0 | 2.91 | 0 | 3.13 | 2.6 | 3.07 | 2.28 | 1.44 | 0 | 2.05 | 0 | 8.28 | 10.4 | 7 |
| NS 1-10-06 | 1.84 | 1.54 | 1.74 | 1.26 | 1.6 | 0 | 1.52 | 0 | 1.04 | 0 | 8.71 | -0.7 | 9.8 |
| NS 2-21-06 | 1.77 | 0 | 1.44 | 0 | 1.42 | 0 | 1.34 | 0 | 1.71 | 0 | 9.2 | 0.9 | 6.4 |
| NS 3-21-06 | 1.26 | . | 0.71 | . | 0 | . | 0.88 | . | 1.04 | . | 7.8 | 2.2 | 6.7 |
| NS 4-3-06 | 1.53 | 0 | 1.1 | 1.55 | 0.83 | 0 | 1.28 | 1.55 | 0 | 0 | 8.3 | 6.8 | 6.3 |
| NS 4-25-06 | 0.78 | 0 | 0.66 | 1.25 | 0.48 | 0 | 1.36 | 0 | 0 | 0 | 8.9 | 9.2 | 5.5 |
| NS 5-1-06 | 0.63 | 0 | 0.61 | 1.24 | 0.75 | 1.24 | 1.2 | 1.83 | 0 | 0 | 8.78 | 10.7 | 5.8 |
| NS 5-22-06 | 1.25 | 0 | 1.06 | 1.27 | 0.83 | 1.27 | 1.32 | 0 | 0 | 0 | 8.4 | 11.3 | 6.1 |
| NS 6-12-06 | 1.16 | 0 | 0.98 | 1.92 | 1.16 | 1.51 | 1.26 | 1.98 | 0 | 0 | 8.88 | 11.7 | 7 |
| NS 6-26-06 | 0.9 | 2.82 | 0.48 | 3 | 0 | 2.79 | 1.1 | 3.08 | 0 | 0 | 8.8 | 17.8 | 6.1 |
| NS 7-11-06 | 1 | 2.39 | 0.71 | 2.4 | 0.3 | 2.09 | 0.3 | 0 | 0 | 0 | . | . | . |
| NS 7-25-06 | 1.93 | 0 | 1.51 | 2.25 | 0.61 | 0 | 1.22 | 0 | 1.04 | 0 | . | . | . |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NS 8-14-06 | 0.87 | 3.26 | 0.61 | 3.32 | 0.61 | 0 | 1.33 | 0 | 0 | 0 | . | . | . |
| NS 8-28-06 | 1.24 | 2.83 | 0.98 | 3 | 0.55 | 2.79 | 0.98 | 3.08 | 0 | 0 | . | . | . |
| RSP 6-21-0 | . | 3.52 | 1.21 | 2.38 | 2.23 | 3.78 | . | 0 | 1.85 | 1.23 | 8.35 | 23.2 | 8.7 |
| RSP 8-03-05 | 1.4 | 2.93 | 0.98 | 2.45 | 0.48 | 3.82 | 1.37 | 3.18 | 0 | 0 | 8.7 | 27 | 10 |
| RSP 08-18- | . | 2.56 | . | 2.22 | . | 4.32 | 1.53 | 2.7 | 0 | 0 | 8.49 | 23.7 | 12 |
| RSP 09-28-05 | 1.74 | 3.72 | 1.48 | 2.4 | 1.31 | 1.56 | 1.39 | 3.73 | 0 | 0 | 8.5 | 18.7 | 6.5 |
| RSP 10-05-05 | 1.85 | 3.43 | 1.35 | 2.87 | 1.1 | 3.67 | 1.43 | 3.64 | 1.04 | 0 | 8.55 | 20.8 | 12 |
| RSP 11-02-05 | 2.96 | 2.63 | 2.83 | 2.33 | 2.47 | 3.1 | 1.74 | 2.37 | 1.04 | 0 | 8.28 | 10.1 | 3.9 |
| RSP 12-01-05 | 2.5 | 3.94 | 2.4 | 4 | 2.37 | 3.64 | 1.32 | 3.46 | 1.13 | 0 | 8.29 | 2.1 | 3.57 |
| RSP 1-10-06 | 1.86 | 4.14 | 2.05 | 2.53 | 1.55 | 4.09 | 1.42 | 4.54 | 1.61 | 0 | 8.1 | 1.5 | 8.9 |
| RSP 2-21-06 | 2.14 | . | 1.74 | . | 1.25 | . | 1.26 | . | 1.49 | 0 | 8.1 | -1 | 6.5 |
| RSP 3-21-06 | 1.04 | 4.71 | 1.3 | 3.96 | 0.71 | 5.11 | 0.94 | 4.07 | 1.61 | . | 8.3 | 4 | 15 |
| RSP 4-3-06 | 1.55 | 4.52 | 1.29 | 3.47 | 1.7 | 4.25 | 1.55 | 4.79 | 0 | 0 | 8.3 | 18.1 | 16.2 |
| RSP 4-25-06 | 1.61 | 4.31 | 1.16 | 3.09 | 0.79 | 3.84 | 1.17 | 4.05 | 0 | 0 | 8.4 | 13.8 | 3.6 |
| RSP 5-1-06 | 1.77 | 3.86 | 1.28 | 3.12 | 0.71 | 4.97 | 1.4 | 5.01 | 1.04 | 0 | 8.48 | 13.9 | 3.7 |
| RSP 5-22-06 | 1.8 | 4.34 | 1.2 | 3.94 | 0.98 | 4.03 | 1.77 | 5.3 | 0 | 0 | 8.2 | 15.5 | 5.7 |
| RSP 6-12-06 | 1.45 | 4.49 | 1.47 | 4.11 | 1.69 | 3.89 | 1.26 | 4.69 | 0 | 0 | 8.72 | 21 | 8.1 |
| RSP 6-26-06 | 1.21 | 3.48 | 0.97 | 2.81 | 0.3 | 2.35 | 1.26 | 4.41 | 0 | 0 | 8.6 | 22.8 | 8.2 |
| RSP 7-11-06 | 1.28 | 3.7 | 0.93 | 3.25 | 0.92 | 4.17 | 1.15 | 4.07 | 0 | 0 | 8.7 | 24.8 | 10.5 |
| RSP 7-25-06 | 2.15 | 5.88 | 1.82 | 5.47 | 1.45 | 4.58 | 1.22 | 4.99 | 0 | 0 | 8.6 | 24.2 | 8.5 |
| RSP 8-14-06 | 1.54 | 4.11 | 1.17 | 3.81 | 0.93 | 5.36 | 1.15 | 4.76 | 0 | 0 | 8.65 | 23.7 | 11 |
| RSP 8-28-06 | 1.69 | 4.72 | 1.15 | 3.57 | 0.93 | 4.19 | 1.19 | 5.38 | 0 | 0 | 8.7 | 23.5 | 9.6 |
| DC 4-14-05 | 0.2 | 3.69 | 1.25 | 3.55 | 0.66 | 4.15 | 1.41 | 3.92 | 0 | 0 | 8.38 | . | 10 |
| DC 5-23-05 | 2.16 | 3.73 | 1.7 | 3.38 | 0.89 | 6.05 | 1.41 | . | 0 | . | 8.37 | . | 11 |
| DC 6-21-05 | 1.29 | 4.04 | 1.26 | 2.79 | 2.98 | . | 0 | 0 | 2.93 | 0 | 7.95 | 21.4 | 11 |
| DC 7-27-05 | 2.52 | . | 1.87 | 4.07 | 1.92 | 5.79 | 1.52 | 4.11 | 0 | 2.44 | 8.88 | 28 | . |
| DC 8-18-05 | 2.1 | 3.3 | 1.76 | 4.16 | 3.06 | 3.78 | 1.43 | 2.13 | 0 | 0 | 8.38 | 22.67 | 4.8 |
| DC 8-31-05 | 2.46 | 2.59 | 1.62 | 2.71 | 0.95 | 3 | 1.53 | 1.81 | 0 | 0 | 8.36 | 22.4 | 16 |
| DC10-19-05 | 1.71 | 4.55 | 1.77 | 4.46 | 1.13 | 3.59 | 0.85 | 3.55 | 1.04 | 0 | 7.89 | 11.4 | 3.4 |
| DC 11-16-05 | 2.85 | 3.82 | 2.72 | 3.2 | 3.09 | 4.4 | 1.52 | 4.45 | 2.05 | 0 | 8.08 | 6.3 | 6 |
| DC 12-14-05 | 2.32 | . | 2.44 | . | 1.77 | . | 1.53 | . | 1.32 | 0 | 8.52 | -0.7 | 12.3 |

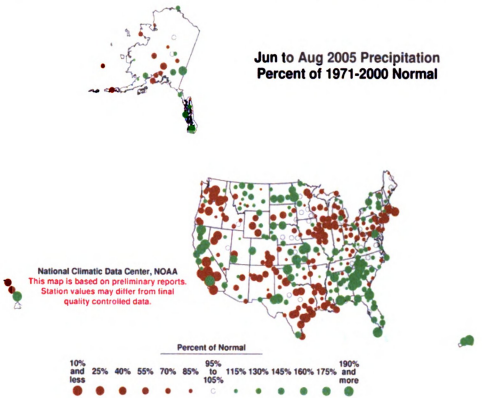
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DC 1-30-06 | 3.36 | 4.79 | 3.2 | 2.58 | 3.14 | 3.4 | 1.68 | 0 | 1.79 | 0 | 8.23 | 3.5 | 75 |
| DC 2-21-06 | 0 | . | 0 | . | 0 | . | 0 | . | 0 | . | 8.1 | -0.9 | 40.5 |
| DC 3-21-06 | 1.56 | 3.7 | 1.51 | 3.16 | 0 | 4.26 | 1.09 | 5.66 | 1.32 | 0 | 8.1 | 6 | 14.2 |
| DC 4-3-06 | 2.33 | 5.03 | 2.19 | 3.92 | 1.83 | 5.72 | 1.95 | 4.8 | 1.71 | 0 | 8.3 | 13.5 | 21.7 |
| DC 4-25-06 | 1.74 | 4.78 | 1.46 | 4.54 | 1.3 | 4.11 | 1.47 | 5.35 | 1.49 | 0 | 8.3 | 13.3 | 6.8 |
| DC 5-1-06 | 1.89 | 3.85 | 1.79 | 2.81 | 1.31 | 4.4 | 1.61 | 4.24 | 0 | 0 | 8.19 | 13.4 | 10 |
| DC 5-22-06 | 1.72 | 3.73 | 1.2 | 3.16 | 1.24 | 4.23 | 1.76 | 4.13 | 1.04 | 0 | 8.2 | 15.5 | 7.9 |
| DC 6-12-06 | 1.7 | 3.8 | 1.44 | 2.81 | 2.12 | 4.43 | 1.36 | 4.32 | 1.04 | 0 | 8.53 | 21 | 10.5 |
| DC 6-26-06 | 1.96 | 3.77 | 1.23 | 0 | 1.21 | 3.3 | 1.4 | 3.81 | 1.04 | 0 | 8.2 | 22.1 | 12 |
| DC 7-11-06 | 2.16 | 4.25 | 1.89 | 3.65 | 1.88 | 4.26 | 1.36 | 5.16 | 0 | 0 | 8.3 | 22.5 | 12 |
| DC 7-25-06 | 2.83 | 6.03 | 2.24 | 6.02 | 1.92 | 5.88 | 1.26 | 4.97 | 0 | 0 | 8.2 | 23.5 | 14 |
| DC 8-14-06 | 1.79 | 4.96 | 2.26 | 4.42 | 1.65 | 6.5 | 1.18 | 4.86 | 0 | 0 | 8.1 | 20.8 | 4.6 |
| DC 8-28-06 | 2.86 | . | 2.27 | 6.69 | 2.59 | 5.03 | 1.18 | 5.09 | 1.04 | 0 | 8.4 | 22.5 | 8.4 |
| GRP 6-22-05 | 2.42 | 5.03 | 1.65 | 4.71 | 2.28 | . | 0 | 0 | 1.61 | 0 | . | . | . |
| GRP 8-03-05 | 2.05 | 2.27 | 1.39 | 2.28 | 1.67 | 3.85 | 1.38 | 3.11 | 0 | 0 | 8.5 | 27.5 | 9.5 |
| GRP 8-31-05 | 3.79 | 5.73 | 1.82 | 2.82 | 1.87 | 3.42 | 1.65 | 3.78 | 0 | 0 | 8.34 | 21.1 | 5.1 |
| GRP 9-28-05 | 2.39 | 3.86 | 1.87 | 3.85 | 1.41 | 3.53 | 1.39 | 4.09 | 1.04 | 2.25 | 8.3 | 18.4 | 3.7 |
| GRP 10-19-05 | 2.18 | 2.85 | 1.77 | 2.27 | 1.38 | 2.94 | 1.59 | 3.35 | 0 | 0 | 8.4 | 12 | 6.7 |
| GRP 11-16-05 | 2.91 | 4.72 | 2.39 | 0 | 2.85 | 4.29 | 1.77 | 1.79 | 1.49 | 0 | 8.21 | 6.9 | 10 |
| GRP 12-14-05 | 1.77 | 0 | 2.49 | 4.15 | 1.96 | 5.29 | 1.41 | 3.81 | 1.79 | 0 | 8.31 | -0.2 | 2.75 |
| GRP 1-30-06 | 2.48 | 0 | 2.31 | 2.22 | 2.13 | 4.37 | 1.59 | 4.91 | 0 | 0 | 8.2 | 4.7 | 16 |
| GRP 2-21-06 | 2.06 | 4.62 | 1.79 | 3.52 | 1.04 | 3.97 | 1.58 | 4.59 | 1.49 | 0 | 8.1 | -0.3 | 5.2 |
| GRP 3-21-06 | 1.53 | 4.45 | 1.21 | 3.89 | 0 | 4.25 | 1.39 | 5.59 | 0 | 0 | 8.2 | 6.7 | 10.35 |
| GRP 4-3-06 | 2.54 | 4.83 | 2.3 | 4.16 | 2.3 | 4.11 | 2.03 | 5.13 | 0 | 0 | 8.3 | 13.7 | 25.9 |
| GRP 4-25-06 | 2.22 | 4.4 | 1.29 | 3.29 | 0.96 | 4.55 | 1.76 | 4.92 | 1.32 | 2.34 | 8.3 | 11.6 | 4.7 |
| GRP 5-1-06 | 2.49 | 3.94 | 1.98 | 2.86 | 2.12 | 3.52 | 1.66 | 4.81 | 0 | 0 | 8.33 | 13.7 | 8.33 |
| GRP 5-22-06 | 1.86 | 4.49 | 1.29 | 3.3 | 0.96 | 4.48 | 1.32 | 5.25 | 0 | 0 | 8.6 | 17.9 | 11 |
| GRP 6-12-06 | 2.11 | 4.52 | 1.76 | 3.71 | 2.17 | 4.02 | 1.32 | 4.74 | 0 | 0 | 8.62 | 21 | 5 |
| GRP 6-26-06 | 2.12 | 3.91 | 1.51 | 2.91 | 0.98 | 3.03 | 1.62 | 4.31 | 1.32 | 0 | 8.4 | 21.4 | 6.8 |
| GRP 7-11-06 | 1.83 | 4.22 | 1.59 | 3.63 | 1.88 | 5.07 | 1.53 | 5.75 | 0 | 0 | 8.6 | 24.3 | 7 |
| GRP 7-25-06 | 2.4 | 6.3 | 1.81 | 6.25 | 1.94 | 5.19 | 1.45 | 5.7 | 1.71 | 0 | 8.4 | 25.1 | 3.7 |

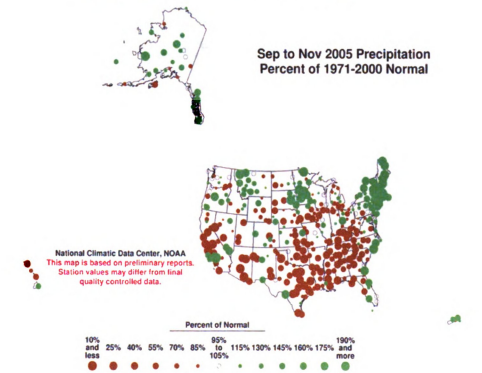
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GRP 8-14-06 | 1.83 | 4.82 | 1.31 | 3.81 | 1.75 | 4.5 | 1.34 | 5.5 | 1.32 | 0 | 8.4 | 22.2 | 6.8 |
| GRP 8-28-06 | 2.22 | 4.05 | 1.67 | 3.61 | 1.77 | 4.1 | 1.43 | 5.35 | 0 | 0 | 8.5 | 23 | 5.5 |
| JP 6-22-05 | 1.9 | . | 1.54 | . | 2.9 | . | 1.48 | . | 1.71 | . | 8.27 | 22 | 12 |
| JP 8-24-05 | 2.43 | 3.65 | 2.44 | 2.42 | 2.5 | 3.95 | 1.69 | 3.78 | 0 | 0 | 8.67 | 22.1 | . |
| JP 9-28-05 | 2.06 | 4.63 | 1.89 | 4.71 | 1.04 | 4.56 | 1.35 | 3.38 | 0 | 0 | 8.3 | 19.2 | 5.4 |
| JP 10-19-05 | 1.89 | 3.73 | 1.8 | 3.51 | 1.05 | 0 | 1.25 | 3.6 | 0 | 0 | 8.5 | 13.1 | 4.2 |
| JP 11-16-05 | 2.63 | 3.7 | 2.08 | 2.66 | 2.33 | 3.88 | 1.51 | 3.86 | 0 | 0 | 8.3 | 6.6 | 6.3 |
| JP 12-14-05 | 1.49 | 3.89 | 1.66 | 3.58 | 1.57 | 3.76 | 0.97 | 4.15 | 1.04 | 0 | 8.35 | -1.1 | 1.9 |
| JP 1-30-06 | 2.74 | 3.17 | 2.42 | 2.53 | 2.3 | 3.31 | 1.75 | 4.92 | 1.71 | 0 | 8.32 | 3.5 | 13 |
| JP 2-21-06 | 1.91 | 3.35 | 1.89 | 2.47 | 0.83 | 3.75 | 1.2 | 3.42 | 1.79 | 0 | 8.2 | -0.5 | 18 |
| JP 3-21-06 | 2.07 | 4 | 1.34 | 3.09 | 1.21 | 3.88 | 1.89 | 4.79 | 1.32 | 0 | 8.2 | 6.2 | 33.5 |
| JP 4-3-06 | 2.55 | 4.72 | 2.24 | 4.15 | 1.9 | 4.78 | 1.75 | 5.12 | 0 | 0 | 8.2 | 12.4 | 8.55 |
| JP 4-25-06 | 2.23 | 5.06 | 1.64 | 3.37 | 1.19 | 3.44 | 1.38 | 4.52 | 1.04 | 0 | 8.4 | 13.8 | 4 |
| JP 5-1-06 | 1.32 | 5.03 | 2.68 | 3.53 | 2.55 | 3.83 | 1.43 | 4.54 | 1.04 | 3.16 | 8.41 | 13.5 | 7.2 |
| JP 5-22-06 | 1.73 | . | 1.23 | . | 1.09 | . | 1.61 | . | 0 | . | 8.2 | 15.2 | 5.9 |
| JP 6-12-06 | 2.19 | 4.91 | 2.08 | 4.46 | 2.61 | 4.6 | 1.4 | 4.55 | 0 | 0 | 8.6 | 20.5 | 4.4 |
| JP 6-26-06 | 2.15 | 3.79 | 1.7 | 3.17 | 1.2 | 1.93 | 1.22 | 3.23 | 0 | 0 | 8.4 | 22 | 4.9 |
| 1JP 7-11-06 | 1.51 | 3.85 | 1.41 | 3.43 | 1.21 | 4.01 | 1.34 | 5.23 | 0 | 0 | 8.6 | 25 | 5.2 |
| 1JP 7-25-06 | 2.44 | 4.92 | 1.84 | 3.98 | 1.62 | 4.53 | 1.49 | 4.38 | 0 | 0 | 8.4 | 23.8 | 4.4 |
| 1JP 8-14-06 | 1.98 | 4.08 | 1.35 | 3.48 | 1.03 | 3.57 | 1.65 | 4.08 | 0 | 0 | 8.5 | 22.7 | 8.3 |
| 1JP 8-28-06 | 2.05 | 4.11 | 1.71 | 3.59 | 1.48 | 3.28 | 1.04 | 4.61 | 0 | 0 | 8.6 | 23.5 | 5.7 |
| SSP 6-2-05 | 2.03 | . | 1.88 | . | 2.08 | . | 0.91 | . | 0 | . | 8.4 | 21.5 | 15 |
| SSP 6-24-05 | 2.32 | 4.06 | 1.73 | 3.92 | 2.84 | 4.5 | 2.11 | 4.61 | 1.04 | 0 | 8.48 | 24 | 6.8 |
| SSP 08-03- | 2.31 | 4.64 | 1.61 | 3.36 | 1.73 | 3.24 | 1.24 | 4.29 | 0 | 0 | 8.8 | 27.2 | 27 |
| SSP 8-31-0 | 1.81 | 5.5 | 1.49 | 4.89 | 1.04 | 5.14 | 1.28 | 3.9 | 0 | 0 | 8.35 | 22.4 | 5.1 |
| SSP 10-19- | 2.41 | 4.8 | 2.05 | 4.24 | 1.05 | 3.59 | 1.18 | 3.63 | 0 | 0 | 8.58 | 4.1 | 7.5 |
| SSP 11-16- | 3.44 | 6.01 | 2.88 | 3.74 | 3.48 | 4.21 | 1.71 | 3.66 | 0 | 0 | 8.63 | 6.3 | 5.8 |
| SSP 12-14- | 0 | 0 | 2.03 | 2.88 | 1.6 | 3.43 | 0.82 | 3.37 | 1.32 | 0 | 8.31 | -1.2 | 1.67 |
| SSP 1-30-0 | 2.46 | . | 2.06 | . | 1.96 | . | 1.22 | . | 1.32 | . | 8.31 | 2.6 | 15 |
| SSP 2-21-0 | 2.1 | . | 1.95 | . | 1.04 | . | 1.4 | . | 1.49 | . | 8.1 | -0.8 | 9.61 |
| SSP 3-21-0 | 1.62 | . | 1.42 | . | 2.04 | . | 1.04 | . | 1.32 | . | 8.3 | 4.9 | 20 |

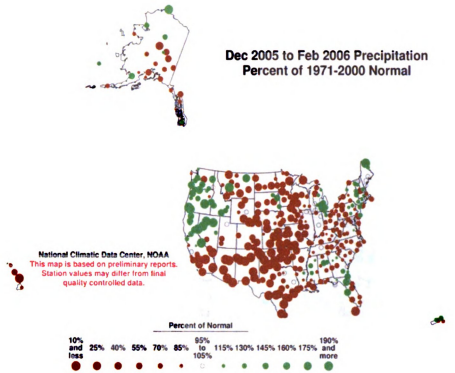
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SSP 4-3-06 | 3.02 | . | 2.4 | . | 2.19 | . | 1.8 | . | 1.32 | . | 8.3 | 11.4 | 11 |
| SSP 4-25-0 | 2.67 | . | 1.66 | . | 1.3 | . | 1.64 | . | 0 | . | 8.4 | 14.1 | 6.7 |
| SSP 5-1-06 | 3.05 | . | 1.77 | . | 1.45 | . | 1.49 | . | 0 | . | 8.42 | 14.4 | 6 |
| SSP 5-22-0 | 2.2 | . | 1.39 | . | 1.28 | . | 1.74 | . | 1.32 | . | 8.2 | 15.5 | 6.8 |
| SSP 6-12-06 | 2.22 | 4.48 | 1.92 | 4.01 | 2.87 | 4.02 | 1.36 | 4.31 | 1.04 | 0 | 8.6 | 21.7 | 7.2 |
| SSP 6-26-06 | 2.32 | 4.63 | 1.5 | 3.5 | 1.01 | 2.63 | 0.9 | 4.18 | 1.04 | 0 | 8.4 | 25 | 8.3 |
| SSP 7-11-06 | 2.36 | 5.53 | 1.95 | 4.9 | 1.64 | 4.66 | 1.13 | 4.89 | 0 | 0 | 8.6 | 25.2 | 5.5 |
| SSP 7-25-06 | 2.81 | 5.28 | 2.03 | 4.54 | 1.74 | 0 | 1.18 | 4.46 | 1.32 | 0 | 8.3 | 25.1 | 7.2 |
| SSP 8-14-06 | 1.98 | 5.06 | 1.05 | 4.84 | 1.05 | 4.64 | 1.32 | 5.12 | 0 | 0 | 8.6 | 23.3 | 8.3 |
| SSP 8-28-06 | 2.51 | 5.44 | 1.56 | 5.23 | 1.23 | 4.55 | 1.11 | 4.62 | 0 | 0 | 8.5 | 23.5 | 9.2 |

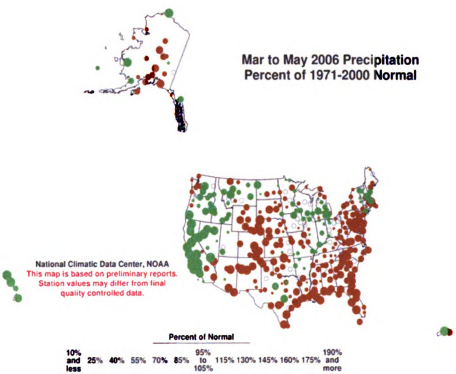
Note that concentrations for fecal indicators in water are CFU or PFU / 100 mL water + 1 and then log 10 transformed
Note that concentrations for fecal indicators in sediment are CFU or PFU / 100 g dw + 1 and then log 10 transformed

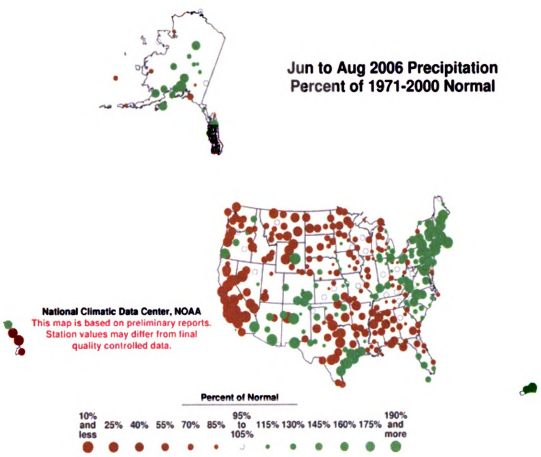
**Appendix E Seasonal changes of precipitation compared to 1971 - 2000**



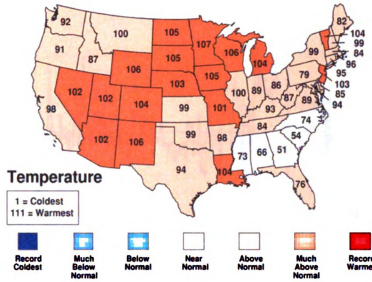

Dec 2005 to Feb 2006 Precipitation
Percent of 1971-2000 Normal
National Climatic Data Center, NOAA
This map is based on preliminary reports.
Station values may differ from final
quality controlled data.
Percent of Normal
10% and less
25%
40%
55%
70%
85%
95% to 105%
115%
130%
145%
160%
175%
190% and more


Mar to May 2006 Precipitation
Percent of 1971-2000 Normal
National Climatic Data Center, NOAA
This map is based on preliminary reports.
Station values may differ from final
quality controlled data.
Percent of Normal
10% and less
25%
40%
55%
70%
85%
95% to 105%
115%
130%
145%
160%
175%
190% and more


Jun to Aug 2006 Precipitation
Percent of 1971-2000 Normal
National Climatic Data Center, NOAA
This map is based on preliminary reports.
Station values may differ from final
quality controlled data.
Percent of Normal
10% and less
25%
40%
55%
70%
85%
95% to 105%
115%
130%
145%
160%
175%
190% and more

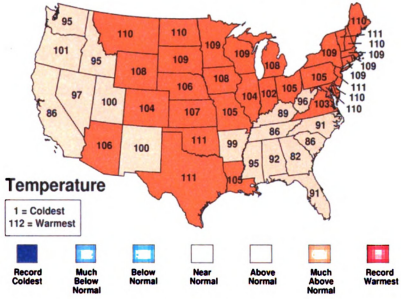
## January-December 2005 Statewide Ranks

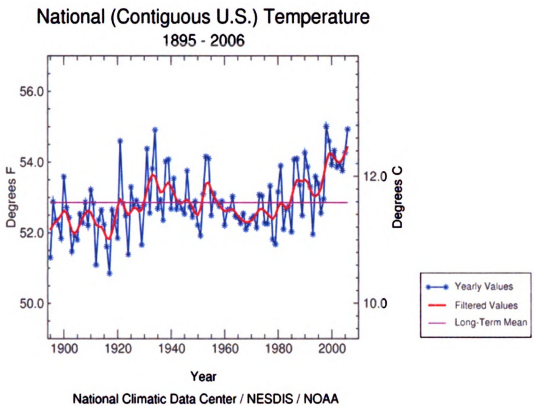
**National Climatic Data Center/NESDIS/NOAA**




## January-December 2006 Statewide Ranks

**National Climatic Data Center/NESDIS/NOAA**




National (Contiguous U.S.) Temperature
1895 - 2006
Degrees F
56.0
54.0
52.0
50.0
12.0
10.0
Degrees C
1900
1920
1940
1960
1980
2000
Year
Yearly Values
Filtered Values
Long-Term Mean
National Climatic Data Center / NESDIS / NOAA

# Shikha Singh

## Education

**Graduate Studies (MSc. candidate)** **January 2005– May 2007**
**Dept. of Fisheries and Wildlife (College of Agriculture and Natural Resources)**
**Michigan State University, East Lansing, MI.**
Research Focus:
Surface water and sediment quality in parks and beaches along the Grand River in Michigan.

- Sites are located between Grand Rapids, MI to Grand Haven and Ferrysburg MI
- Organisms studied: Fecal coliforms, *Escherichia coli*, *Enterococci sp.*, *Clostridium perfringens* and Coliphage virus
- Defended April 3rd, 2007

Relevant Courses:
Human dimension research in Fisheries and Wildlife Management, Conservation Biology (advanced topics), Environmental Engineering Project, Outreach in Fisheries and Wildlife Resource Management, Quantitative Methods Ecology & Evolution, Historical Roots of Epidemiological Thought, Investigation of Wildlife Disease Outbreaks (seminar), Shaping Future Water Policy: The Role of Science (seminar), Introduction to ArcView 3.2 and GIS

Relevant Experiences:
Teaching Assistant for FW 414 Aquatic Ecosystem Management **Fall 2006**
Course Grader for FW 414 Aquatic Ecosystem Management **Fall 2005**

**Department of Chemical & Biochemical Engineering** **Fall Term 2004**
University of Western Ontario, London, ON
Courses Taken:
Biochemical Engineering II, Chemical Engineering Thermodynamics II (audit)

**Field Course** **August & September 2003**
**Ecosystem Survey in South & Central Siberia (Russia)**
**University of Western Ontario, London, ON. & Queens University, Kingston, ON.**
Relevant experiences:
Visited and studied tundra, taiga, dessert and steppe ecosystems where we observed vegetation growth, local animals, and effects of pollution on the environment. Major areas travelled were Krasnoyarsk, Stolby National Park, Shira (Khakassia), Shushenskoye, Kyzyl, Abakan, Sayano mountain region, Yenesi River Paper topic: Impact of Russian Dams on the Environment

**Bachelor of Science** **June 2003**
**Dept. of Biology (Faculty of Science)**
**University of Western Ontario, London, ON**

## Research & Work Experience

**Research Assistant, Michigan State University** **September 2004 – December 2006**

- Membrane Filtration and IDEXX analysis of water and sediment samples
- DNA extraction of bacteria, measurement of DNA concentration (NanoDrop Spectrophotometer), PCR amplification, Gel electrophoresis
- Media preparation and bacteria growth
- **Side Projects**: PRD-1 and Rhodamine Tracer Study in Grand River (Grand Rapids, Michgian), Microbial characterization in beach sands of North Beach Park, Water quality of Lake Billings (Manton, MI) & Sediment analysis in St. Claire River (Sault Ste. Marie, Michigan)

**Research Assistant, University of Western Ontario** **May 2003 – July 2004**

- The purpose was to optimize growth conditions for *Halobacterium salinarium* in order to extract Bacterio Rhodopsin (purple membrane), a protein produced in various bacteria
- Lab organization, safety and maintenance also part of duties

**Undergraduate Researcher, University of Western Ontario** **1999 – 2002**

- Worked during the summer months between school terms
- Involved independent and unsupervised work on an assigned project involving *Vibrio fisheri* and *Escherichia coli*,
- Main focus was to immobilize bacteria onto a gel and place in a light sensing chamber to monitor parameters
- Resulted in two conference proceeding publications

## Conference Oral presentations

- South Bass Island ground water outbreak
  Michigan Environmental Health Association, 2007 Annual Education Conference
  *Invited speaker*
  March 30th, 2007

- Microbial and Pathogenic pollution in parks and beaches along the Grand River
  Michigan chapter of American Water Works Association - Sept. 13, 2006

- Microbial pollution in Beaches and Parks along the Grand River – from Grand Rapids, MI to Lake Michigan
  Fisheries and Wildlife Graduate Student Symposium- March 17th, 2006

## Lectures Given

- Ground water contamination at South Bass Island
  Monroe county public officials (May 18th) **2007**
  *Invited keynote speaker*

- Chemical and Microbial Contamination in Aquatic Ecosystems
  (4th year Aquatic Ecosystem Management) **2006**

- Public Education-Water quality Lecture Chemical and Microbial Contamination in Aquatic Ecosystems
  230 Grade 8 students-Ionia Middle School (October 31st) **2006**
  Spoke about water quality issues, contamination and economic impact

- Water and sediment quality along the Grand River, from Grand Rapids to L. Michigan
  (Graduate Student Seminar) **2005**

- Impact of Russian Dams on the Environment
  (4th year Political Biology) **2004**

- Ivan Turgenev: A study of his themes and how they portray 19th century Russia
  (3rd year Russian History) **2003**

## Publications

- Knopf, G.K, Bassi, A.S., **Singh, S.**, Fiorilli, M., and Jauda, L. 2000. "Optoelectronic biosensor for remote monitoring of toxins", OptoMechatronic Systems, Proc. Soc. Photo - Opt. Instrum. Eng., vol. 4190, pp. 9 - 19.

- Knopf, G.K., Bassi, A.S., **Singh, S.**, Macleod, R. 1999. Biosensor for the remote monitoring of airborne toxins. *SPIE The international Society for Optical Engineering, Symposium on Environmental Monitoring and Remediation Technologies II* (19-22 September), Boston, MA, pp. 185-193.

- McNinch, R, **Singh, S.** and J.B. Rose. 2007. UNESCO (United Nations Educational Scientific and Cultural Organization) encyclopaedia article: recreational waters

## Proposals

- **S. Singh** and J.B. Rose. 2007. Microbial Water Quality Assessment for Muskegon county. Muskegon County Waste Water Treatment Project (accepted)

## Poster

- **S Singh**, T. Shibata, T-T. Fong. 2007. Septic tank Impairment of Water Quality at Silver Lake Sand Dunes Recreational Area, Michigan. National Beaches Conference, October 3rd, Traverse City, MI.

- **S Singh**, M Phani Kumar & JB Rose. "Comparing bacterial indicators in surface water and sediment at Lake Michigan beaches and parks along the Grand River, MI". *American Society of Microbiologists national conference*, Toronto, Canada. May 21-25, 2007
- **S Singh** and J.B. Rose. "Presence of fecal indicator bacteria in sediment and surface water along the Grand River and beaches of Lake Michigan". Ottawa County public meeting August 19, 2006

- **S Singh,** L Liu, M Phani Kumar & JB Rose. "CSO and occurrence of faecal indicators in sediment and water along the Grand River and at the beaches of Lake Michigan". *International Water Association/World Health Organization: 13th International symposia on Health-Related Water Microbiology.* Swansea, Wales, UK. September 3-10, 2005

## Volunteer Experience

| | |
|---|---|
| **Graduate Students Organization**<br>Seminar Committee | **May 2006 – December 2007** |
| **Liason Officer**<br>**Graduate Women in Science (Mu Sigma Upsilon)** | **September 2005 - May 2006** |
| **Executive of Communications**<br>**UWO Russian Students' Union** | **September 2003 – June 2004** |
| **Lets Talk Science** | **September 1999 – August 2003** |
| **President - UWO Cause for Paws** | **September 2002 – May 2003** |
| **President - UWO Humane Society Club** | **September 2001 – May 2002** |
| **Executive of Fundraising - UWO Humane Society Club** | **September 2000 – May 2001** |

## Memberships

- American Society of Microbiologists (2005-2007)
- Graduate Women in Science (National Chapter)-Sigma Delta Epsilon (2005/06)
- Graduate Women in Science (Michigan State University)-Mu Sigma Upsilon (2005/06)
  Executive Member-Liaison to the National Chapter