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Reducing Microbial Concentrations with Stormwater Retention Ponds and Constructed Wetlands

Instructor: Vincent D. Reynolds, MBA, PE

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Performance of Stormwater Retention Ponds and Constructed Wetlands in Reducing Microbial Concentrations

By

Scott D. Struck
Ariamalar Selvakumar
Michael Borst

Urban Watershed Management Branch
Water Supply and Water Resources Division
National Risk Management Research Laboratory
Edison, NJ 08837

NATIONAL RISK MANAGEMENT RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OH 45268

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Abstract

Stormwater runoff can transport high concentrations of pathogens to receiving waters. Bacteria indicator organisms, as surrogates for pathogens, are the most often reported cause of receiving water impairments. Stormwater best management practices (BMPs) are often considered effective tools to mitigate the effects of stormwater pollutants before they appear in receiving waters. However, BMP performance for pathogen removal is not well documented. Many questions remain on the transport and fate of indicator bacteria that enter and exit stormwater BMPs.

The National Risk Management Research Laboratory (NRMRL), part of U.S. EPA's Office of Research and Development (ORD) investigated the fate of indicator organisms in the stormwater runoff entering and exiting two commonly used BMPs, constructed wetlands and retention ponds. This research used controlled-condition, pilot-scale systems that represent larger field-scale systems to determine the dominant mechanisms that influence the reduction of indicator organism concentrations. The pilot-scale work was supported by bench-scale laboratory experiments investigating the effects of single parameters such as temperature, sunlight, and salinity on indicator organism inactivation rates. Presented in this report are the results of developing techniques for creating bacterially enriched stormwater, bench-scale studies, and the pilot-scale BMP research. Bench-scale study results show that the temperature and sunlight affect the inactivation rates significantly. Results from the pilot-scale research suggest that constructed wetlands and retention ponds lower microbial concentrations in stormwater runoff. Bacteria inactivation generally followed the first-order, K-C* empirical model that acknowledges an irreducible concentration. Factors such as sunlight and temperature provide much of the inactivation in indicator bacteria, but other factors (e.g., predation, sedimentation, filtration, sorption, pH, and BOD) appear to also influence indicator bacteria concentrations. Future research validating results of the pilot-scale systems to field-scale systems should be done.

Developing microbial inactivation models to predict effluent concentrations from BMPs will help reduce the uncertainty and improve the capabilities of surface water quality models. First-order models that do not consider background concentrations or resuspension, may underestimate actual bacterial concentrations.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Sally C. Gutierrez, Director
National Risk Management Research Laboratory

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Acronyms and Abbreviations

ANOVA	Analysis of Variance
APHA	American Public Health Association
ASCE	American Society of Civil Engineers
ASTM	American Standard Testing Methods
BHI	Brain Heart Infusion
BMP	Best Management Practice
BOD	Biochemical Oxygen Demand
CFU	Coliform Forming Unit
CWA	Clean Water Act
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DQI	Data Quality Indicator
EC	*Escherichia coli*
EMC	Event Mean Concentration
EN or ENT	Enterococci
EPA	US Environmental Protection Agency
FC	Fecal Coliforms
FR	Federal Register
FS	Fecal Streptococci
HDPE	High Density Polyethylene
MDE	Maryland Department of the Environment
MDL	Method Detection Limit
MF	Membrane Filtration
MPN	Most Probable Number
MS4	Municipal Separate Storm Sewer System
MWLAP	Ministry of Water, Land and Air Protection
NPDES	National Pollutant Discharge Elimination System
NRMRL	National Risk Management Research Laboratory
NSQD	National Stormwater Quality Database
NTU	Nephelometric Turbidity Unit
ORISE	Oak Ridge Institute for Science and Education
ORP	Oxygen Reduction Potential
ppt	Parts Per Thousand
PVC	Poly Vinyl Chloride
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SM	Standard Methods
SS	Suspended Solids
TC	Total Coliforms
TMDL	Total Maximum Daily Loads

TNTC	Too Numerous To Count
TOC	Total Organic Carbon
TSS	Total Suspended Solids
QA/QC	Quality Assurance/Quality Control
USEPA	US Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultraviolet
UWMB	Urban Watershed Management Branch
UWRF	Urban Watershed Research Facility
WEF	Water Environment Federation
WQS	Water Quality Standards
YSI	Yellow Springs Instruments

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Anthony N. Tafuri, P.E., Branch Chief
Thomas P. O'Connor, Environmental Engineer
Mary Stinson, Chemical Engineer
Dr. Christopher Nietch, currently with NRMRL, WSWRD, Cincinnati, OH
Carolyn Esposito, Quality Assurance Officer

Thomas P. O' Connor and Mary Stinson performed reviews of this report. Carolyn Esposito reviewed the quality assurance project plan and this report. Mano Sivaganesan of Water Supply and Water Resources Division, Cincinnati reviewed the statistical part of this report.

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Executive Summary

USEPA's 305(b) water quality reports consistently show stormwater runoff as a leading cause of water quality impairment in the United States. Water quality standards (WQS) have been developed through states and the federal government to improve the condition of our nation's surface and groundwater. When waters of the United States do not meet the WQS, regulations have been put in place to overcome impairments. To improve or prevent further degradation of water quality, regulators rely on best practicable control technologies currently available to reduce the loading of stressors from point sources (and, at times, non-point sources). Microorganisms are a high priority stressor because of the many waterbodies that are listed as impaired.

This report documents the efforts to evaluate simple predictive relationships affecting concentrations of indicator organisms in stormwater runoff based on environmental conditions. The report begins by describing the breadth of surface water resources affected by bacterial stressors to identify needs for continued research on understanding the engineering approaches for management of point- and non-point sources associated with this stressor. In Chapter 2 factors from the scientific literature that influence bacteria indicator concentrations are reviewed. The subsequent chapters describe bench- and pilot-scale experiments that attempt to determine the dominant factors that favor a reduction in indicator bacteria concentrations. Finally, Chapter 5 contains synopses of the results of these experiments and an assessment of the first-order decay formula's ability to predict bacterial concentrations based on influent concentrations and other environmental factors. Inactivation rates for each bacteria indicator from these experiments and coefficients are given.

Combined in this report are bench- and pilot-scale data to assess first-order equations to better predict the performance of constructed wetland and retention pond best management practices (BMPs). By measuring varying physical, chemical, and biological parameters that may influence effluent indicator organism concentrations and characteristics of other stormwater parameters that are often contained in stormwater runoff factors that most influence inactivation rates were determined. The BMPs used in this research are small-scale, controlled systems (termed mesocosms) that offer a unique environment for investigating many parameters that can affect the reduction of indicator organisms.

Detailed in Appendix A is the development of methods to grow and harvest bacteria indicators to provide an enriched source to increase the concentrations in stormwater for wet weather flow research. The mesocosm research necessitated this ancillary research as a technique to establish the desired influent bacteria concentrations. The technique may prove useful to others undertaking similar research.

Chapter 1 Introduction

The Clean Water Act (CWA) of 1972 developed an ambient water quality management program to measure the condition of a waterbody and determine whether that waterbody meets the criteria associated with the designated use. By definition, this process depends on setting appropriate water quality standards (WQSs). Realistic standard setting must balance watershed conditions (hydrologic, ecological, and land use) against the corresponding need to protect human health, infrastructure, and the environment.

Where waters of the nation are not meeting established WQSs after implementing best practicable control technologies currently available to reduce the loading of stressors from point sources or other pollution control programs, the CWA requires establishing a Total Maximum Daily Load (TMDL) for each pollutant of concern. As part of the 1987 amendments to the CWA, Congress added Section 402(p) to cover point-source discharges composed entirely of stormwater. Section 402(p)(2) of the Act requires permit coverage for discharges associated with industrial activity and discharges from large (over 250,000 people) and medium (between 100,000 and 250,000 people) municipal separate storm sewer systems (MS4s). These discharges are referred to as Phase I MS4 discharges.

USEPA issued regulations on December 8, 1999 (64 FR 68722), expanding the National Pollutant Discharge Elimination System (NPDES) stormwater program to include discharges from smaller MS4s (including all systems within "urbanized areas" and other systems serving populations less than 100,000) and stormwater discharges from construction sites that disturb one to five acres, with opportunities for area-specific exclusions. This program expansion is referred to as Phase II.

Urbanization

USEPA used urbanized areas and population to define program boundaries because of the increased risk to human health in these areas through greater potential for exposure from point and non-point sources and risks associated with a greater population density. Another component of the selected boundaries is that urbanization characteristically results in a larger percentage of impervious areas that lead to larger quantities of stormwater runoff that contribute significant amounts of debris and other pollutants (e.g., litter, oils, microorganisms, sediments, nutrients, organic matter, and heavy metals) to receiving waters. USEPA identified urban stormwater runoff as one of the four leading causes of water quality impairment related to human activities in lakes and reservoirs (USEPA, 2002). Poor water quality, especially pathogen contaminated water, can cause illnesses such as gastroenteritis (characterized by vomiting, diarrhea, abdominal pain or fever) or upper respiratory (ear, nose, and throat) infections to exposed swimmers. Highly polluted water can occasionally cause serious diseases such as typhoid fever,

dysentery, hepatitis, and cholera. An epidemiological study conducted in the Santa Monica Bay adjacent to Los Angeles County, CA found higher risks of a broad range of symptoms, including upper respiratory and gastrointestinal cases for people swimming closer to storm drains, implicating stormwater runoff as the source of the illnesses (Haile *et al.*, 1999). Similarly, the Southern California Coastal Water Research Project showed that more than half of Southern California's shoreline (from Santa Barbara to San Diego) is unsafe for swimming after rainstorms because of bacteria carried to the ocean by urban runoff (Noble *et al.*, 2000).

Indicator Bacteria

To protect public health, surface waters are tested for indicators that serve as a proxy for harmful pathogens. Indicator bacteria are used because it is difficult to measure the pathogens themselves. Indicator bacteria organisms tested by public health agencies include fecal indicator bacteria such as total coliforms, fecal coliforms, fecal streptococci, *Escherichia coli* (*E. coli*), and enterococci. The concentrations of these indicators are used to determine the potential for fecal contamination and to compare to public health-based thresholds. Like the pathogens they represent, fecal indicator bacteria are found in feces from both human sources (e.g. sewer discharges, and failing septic systems) and non-human sources (e.g. pets, waterfowl, and farm animals). Historically, total and fecal coliforms with fecal streptococci have served as the preferred indicators, but there are efforts to substitute enterococci and *E. coli* for water quality monitoring because of higher correlation with gastrointestinal illness (Gray, 2000). *E. coli* and enterococci are more representative of warm blooded animal fecal contamination in water than total or fecal coliforms. They both can survive, but generally not grow outside the intestinal tract (Ashbolt *et al.*, 2001). In 1976, the USEPA recommended that states adopt a bathing WQS of fecal coliforms not to exceed 200 organisms/100 mL (USEPA, 1976). In 1986, based on the higher correlation, the USEPA recommended that states revise the recreational water quality microbial criteria to use enterococci for marine waters and *E. coli* or enterococci for freshwaters. Suggested criteria are 35 enterococci per 100 mL for marine waters and 33 enterococci per 100 mL and 126 *E. coli per* 100 mL for freshwaters (USEPA, 1986). If a single sample exceeds 235 *E. coli* per 100 mL in freshwater and 104 enterococci per 100 mL in saltwater, the USEPA recommends that a swimming area be closed, or posted until levels are lower. Several states have established policies that advisories are posted at more protective levels of indicator bacteria. Although EPA advised the states of the benefits of changing the indicators, many states continue to use the traditional indicators for a variety of reasons.

Some have questioned the relationship between indicator bacteria and human health risks associated with pathogen exposure in surface water. Few epidemiological studies have tested the health effects of exposure to waters receiving direct and recent stormwater runoff. However, Wade *et al.* (2003) quantitatively analyzed many studies conducted during the past 50 years. The studies, when analyzed collectively, provide a weight of evidence showing the regulations suggested by the USEPA for the enterococci rule (for marine waters) and *E. coli* rule (for freshwaters) are protective when considering the risks associated with recreational water contact including swimming.

Stormwater or stormwater-influenced receiving waterbodies can have indicator bacteria concentrations that greatly exceed WQS. Because elevated fecal indicator bacteria are often associated with stormwater runoff, some state and local agencies close swimming areas preemptively whenever rainfall exceeds a set amount based on site-specific studies. In a recent Water Environment Federation (WEF) report (WEF, 2006), the authors summarized 51 studies from around the world that found ranges in concentrations of total coliforms, fecal coliforms, *E. coli*, fecal streptococci, and enterococci as reported in Table 1-1. Sources of these samples included coastal waters, rivers, creeks, drainage canals, and wetlands.

Table 1-1. Range of Concentrations of Indicator Organisms Found in Varying Waterbodies

Indicator Organism	Concentration Range (per 100 mL)	Median (per 100 mL)
Total Coliforms	$1 \times 10^1 - 2 \times 10^6$	1.2×10^4
Fecal Coliforms	$1 \times 10^1 - 8 \times 10^6$	5.1×10^3
Eschericia coli (E. coli)	$1 \times 10^2 - 2 \times 10^6$	1.7×10^3
Fecal Streptococci	$1 \times 10^1 - 2 \times 10^4$	1.7×10^4
Enterococci	$1 \times 10^1 - 8 \times 10^4$	—

Source: WEF, 2006; Maestre and Pitt, 2005

When looking at impaired waterbodies in the United States, Maestre and Pitt (2005) using data from the National Stormwater Quality Database, reported nationwide median concentrations for fecal coliforms, fecal streptococci, total coliforms and *E. coli* that are found in Table 1-1. On the US EPA's 303(d) list of impaired waters, pathogen contamination is the most commonly listed source (7,742 reported impairments, Figure 1-1) and second for the number of approved TMDL allocations (2,608 TMDLs, Figure 1-2), indicating the prevalence of this stressor (USEPA, 2004). Of the 2,608 approved TMDL allocations, more than 84% are for fecal coliforms.

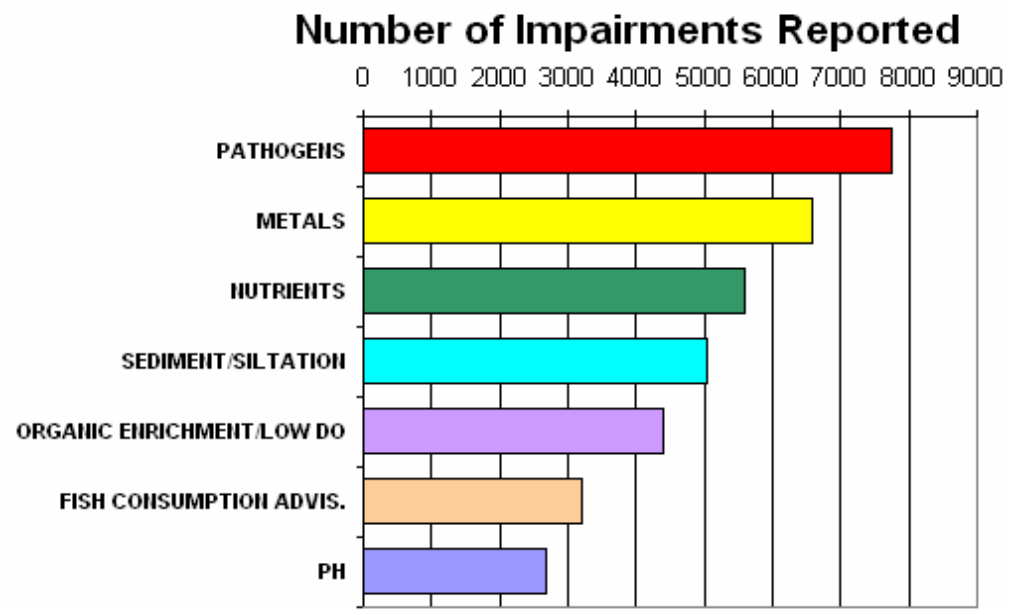


Figure 1-1. Number of reported surface water quality impairments (top 7) since January 1, 1996.

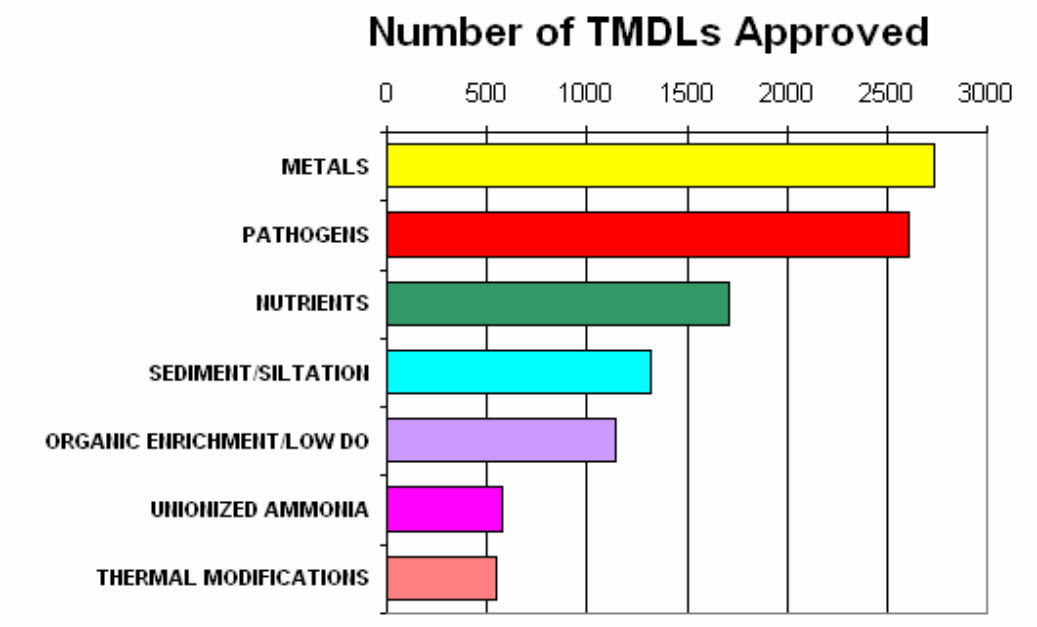


Figure 1-2. Number of approved TMDLs by pollutant (Top 7) since January 1, 1996.

Researchers have correlated aquatic microorganism densities with terrestrial watershed factors such as land use, density of housing, population, development, percent impervious area, and domestic animal density (Young and Thackston, 1999; Mallin, 1998; Glenne, 1984; Francy *et al.*, 2000; Selvakumar and Borst, 2006). Surface runoff samples from more densely populated, sewered areas generally showed higher bacterial counts than runoff from less developed areas serviced by septic tanks (Young and Thackston, 1999). Selvakumar and Borst (2006) found microorganism concentrations from high-density residential areas were higher than those associated with low-density residential and landscaped commercial areas.

Stormwater BMPs

A stormwater best management practice (BMP) is a technique, measure, or structural control that is used to manage the quantity and improve the quality of stormwater runoff in the most cost-effective manner. The USEPA (1999) defines BMPs as "schedules of activities, prohibitions of practices, maintenance procedures, and other management practices to prevent or reduce the pollution of waters of the United States." There are two general types of BMPs used to reduce the threat of stormwater runoff pollution from urbanizing areas: (i) nonstructural or source control BMPs; and (ii) structural or treatment BMPs (USEPA, 1993).

Nonstructural BMPs refer to those stormwater runoff management techniques that use natural measures to reduce pollution levels, do not require extensive construction efforts, and either limit the generation of stormwater runoff, or reduce the amounts of pollutants contained in the runoff. They do not involve fixed, permanent facilities and they usually work by changing behavior through government regulation (e.g., planning and environmental laws), persuasion, and economic instruments (Taylor and Wong, 2002). These BMPs include institutional, educational, or pollution prevention practices.

Structural BMPs are engineered systems and methods designed to provide temporary storage and treatment of stormwater runoff for the removal of pollutants (MWLAP, 1992; MDE, 2000; Clar *et al.*, 2003). Structural BMPs improve the quality; control the quantity of stormwater runoff or both. The

USEPA recommends installing stormwater BMPs within the landscape to influence runoff rates and reduce stressor levels in the stormwater runoff before it reaches the receiving water.

Stormwater runoff is not identical in each location. The character of the drainage area strongly influences not only the runoff volume and rate from a given rain event but also the stressor concentrations. Regardless of the landscape where the BMP is installed, the same potential processes occur within the structure to mitigate the stressors and flow. These processes control the effluent rates and stressor levels regardless of the designated use of the receiving water. Fundamentally, a constructed wetland or retention pond of given characteristics attenuates the stressor load runoff regardless of land use or the receiving waters. The BMP's capabilities are established by their design, construction, and maintenance, and not whether the device installed is part of the source water protection strategy or is a means to protect recreational water, e.g., as part of a TMDL strategy.

If it is determined that a BMP approach (including an iterative BMP approach or treatment train) is appropriate to meet the stormwater component of a TMDL, USEPA recommends that the regulatory language within the TMDL reflect this. Reductions in concentrations in effluents reaching the recreational waters depend on BMP performance. To estimate the reduction in stormwater pollutant concentrations passing through BMPs for developing TMDL allocations, the performance of each BMP must be well established. Much of the existing information on BMP performance comes from current literature and the American Society of Civil Engineers (ASCE) International BMP Database (www.bmpdatabase.org). This database, although one of the largest collections of data on BMP performance, has a paucity of information to adequately assess the performance of many stormwater BMPs (Andrews *et al*., 2004).

Pollutant Attenuation

BMPs are generally passive tools that use physical, chemical, and biological processes that promote natural microbiological inactivation to reduce this and other stressor concentrations in the effluent. These systems are not a means of chemical disinfection as in wastewater treatment. Determining the dominant mechanisms of stormwater bacterial and pathogen removal by these devices is an important step in predicting trends in effluent concentrations to meet state and federal WQSs and for developing TMDLs. Few quantitative studies have been carried out to determine the relative importance of various removal mechanisms by constructed wetlands and retention ponds for indicator bacteria, consequently the ability of these BMP treatments to reduce concentrations in stormwater runoff before reaching receiving waters where WQS must be met, are poorly understood.

Two commonly-used structural BMPs for controlling pollutants in stormwater are constructed wetlands and retention ponds. Treatment and therefore design within these two systems rely predominately on slowing water transport time that provides increased settling. Other environmental factors that contribute to the natural decay process (referred to here as inactivation) in these management practices include irradiance (sunlight), temperature, turbidity, salinity, toxic substances, and predation. A simple first-order decay model:

$$C_t = C_o e^{-K_o t} \qquad (3\text{-}1)$$

Where*:* C_t = concentration of organism at time t (CFU/100 mL); C_o = concentration of organism at time zero (CFU/100 mL); K_o = overall inactivation rate constant at the environmental conditions (h^{-1}); and t = elapsed time since time zero (h); is commonly used to predict the effluent concentrations in these systems. The literatures report a wide range of values for *K*, however. Those values are typically based on a single source making extrapolation to different conditions difficult.

The primary objectives of this study were to:

(1) determine, using bench-scale studies, the factors most important to evaluate commonly used inactivation models for indicator bacteria;
(2) document the effects of two types of structural best management practices (retention pond and constructed wetland) on the removal efficiencies of indicator organisms (total coliforms, fecal coliforms, *E. coli*, and enterococci) in stormwater;
(3) evaluate the applicability of first-order decay model;
(4) evaluate the effluent concentrations of indicator organisms in stormwater runoff as they flow from constructed wetland and retention pond BMPs to determine overall inactivation rates from these systems for various indicator organisms;
(5) record physical, chemical and biological parameters to determine any correlation with effluent indicator bacteria concentrations; and
(6) develop relationships to serve as predictors for concentrations of indicator bacteria in the effluent of selected BMPs.

Investigating the inactivation of indicator organisms from stormwater runoff that passes through retention ponds and constructed wetlands is a complex undertaking. This project involves the analysis of various types of environmental and biological factors and multiple laboratory methodologies. A combination of bench- and pilot-scale studies were selected to take advantage of controls and conditions each scale has to offer. Bench-scale work was done to identify timing of samples and gain an understanding of the magnitude select factors would have on bacteria inactivation rates. It was also recognized early that there was little chance of obtaining constant conditions in a pilot-scale study subject to many environmental influences. Likewise, the replication of all potential environmental factors and their combinations within the laboratory was not feasible. Studies of both scales proved to provide the necessary conditions and controls to complete the project. The following chapter provides, in detail, the primary factors and supporting literature considered when considering the affect environmental factors have on indicator organism inactivation.

Chapter 2 Factors Affecting Microbial Indicator Concentrations

Stormwater runoff often accounts for a large fraction of total microbial loading to many receiving waters (Jamieson *et al.*, 2004; Crabill *et al.*, 1998; Nix, 1994; Qureshi and Dutka, 1979; Olivieri *et al.*, 1977; Wanielista, 1977; Geldreich *et al.*, 1968; Weibel *et al.*, 1964) with the potential to adversely impact drinking water sources, contact recreation areas, and protection and propagation of aquatic life (Sunen and Sobsey, 1999; Haile *et al.*, 1999). Studies have also identified potential links between stormwater runoff and waterborne disease outbreaks in human populations (Currieo *et al.*, 2001; Rose *et al.*, 2001).

Once introduced into the environment, microorganisms are affected by various environmental factors. There are known effects from chemical, physical, and biological sources that influence indicator bacterial growth, die-off, and inactivation. This chapter reviews some of these factors. While not exhaustive in its coverage, it covers the factors believed to be the most pertinent to retention ponds and constructed wetlands.

Temperature

Temperature plays an important role in microorganism die-off and has often been cited as the most important environmental factor. In general, microorganisms survival is prolonged at lower temperatures (Ferguson *et al.*, 2003). Experiments conducted by Selvakumar *et al.* (2004) showed that growth rates of indicator organisms are greatly reduced at 4^{o}C. Geldreich *et al.* (1968) noted that organism persistence remained higher at 10^{o}C than similar samples at 20^{o}C.

In the natural environment, several studies reported different die-off rates for various microorganisms in surface water (Table 2-1). Medema *et al.* (1997) found that the die-off of *E. coli* and enterococci were approximately ten times faster than die-off of *Cryptosporidium parvum* oocysts, but die-off rates of *Clostridium perfringens* were slower than those of oocysts. They also noted that die-off of these indicators was faster at 15^{o}C than at 5^{o}C. Dutka and Kwan (1980) reported that *E. coli*, *Streptococcus faecalis*, and *Salmonella thompson* could survive in 17-18^{o}C waters for at least 28 days and *E. coli* was found in greater concentrations than *Streptococcus faecalis*. Baudisova (1997) reported that the die-off rate of *E. coli* is greater than that of total and fecal coliforms. Canteras *et al.* (1995) noted a clear negative relationship between die-off and temperature. At 10^{o}C, 36 h was necessary to reduce the population of *E. coli* to 10% of the initial concentration compared to 8.4 h at 42^{o}C. Greater reduction of the die-off rate was noticed in the range between 10 and 18^{o}C than between 18 and 42^{o}C.

Table 2-1. Some Reported Die-off Rates (*K*) for Indicator Organisms

Indicator Organism	*K* (h^{-1})	Condition	Reference
Total Coliforms	0.042-0.229	Freshwater (20°C)	Thomann and Mueller (1987)
	0.033	Average freshwater (20°C)	
	0.058 (0.029-0.125)	Seawater (20°C)	
	0.018	River water (12 d)	Baudisova (1997)
Total or Fecal Coliforms	0-0.1	New York Harbor, salinity 2-18 ppt, dark samples	Thomann and Mueller (1987)
	0.104-0.254	New York Harbor, salinity 15 ppt and sunlight	
Fecal Coliforms	1.542-4.583	Seawater and sunlight	Thomann and Mueller (1987)
	0.021	River water (12 d)	Baudisova (1997)
Enterococci	0.003	River water (5°C); 42 d	Thomann and Mueller (1987)
	0.009	River water (15°C); 0-14 d	
E. coli	0.003-0.083	Seawater, salinity 10-30 ppt	Thomann and Mueller (1987)
	0.022	River water (12 d)	Baudisova (1997)
	0.004	River water (5°C); 42 d	Medema *et al.* (1997)
	0.008	River water (15°C); 0-14 d	
Fecal Streptococci	0.75-2.292	Seawater and sunlight	Thomann and Mueller (1987)

Much of the earliest work on bacterial removal assumed that temperature was the most important factor controlling the removal mechanism, as described by the first-order equation developed by Marais and Shaw (1961). Studies, such as Klock (1971) and Ferrara and Harleman (1981) also emphasized on first order concentration reductions with temperature-dependent rate constants.

Recent investigations considered bacterial removal as a more complex mechanism involving interactions between the physical, chemical and biological systems present in wetlands and retention ponds, although temperature clearly remains an important parameter. For example, Polprasert *et al.* (1983), Pearson *et al.* (1987a, b), Barzily and Kott (1991), Mara *et al.* (1992a, b), and Mezrioui *et al.* (1995a, b) all found that removal rates of fecal coliforms increased with increasing temperature. No matter which indicator organism is tested, temperature clearly affects indicator bacteria.

Sunlight

Numerous studies have shown sunlight as an important factor in microorganism die-off though it is difficult to separate effects from other factors entirely. Sinton *et al.* (1994) studied inactivation in sunlight of fecal coliforms and enterococci from sewage and meat works effluent concluding that die-off rate of fecal coliforms was 2-4 times that of enterococci and inactivation is generally slower at lower light intensities. Alkan *et al.* (1995) found that variability of enteric bacteria (i.e., enterococci and *E. coli*) die-off due to the effect of sunlight depends on the variability of the intensity of light and other small scale environmental factors such as turbidity, sewage content, and degree of mixing. Importantly, they further

reported that the die-off rates of *E. coli* and enterococci from exposure to light were similar. Canteras *et al.* (1995) reported that sunlight was the most important factor affecting die-off of *E. coli* with 90% concentration reductions within about 1 h (i.e., inactivation rate of about 0.89 h^{-1}) at 18°C and 8.5% of salinity when light radiation was greater than 120 W/m^2 (12 mW/cm^2). Yukselen *et al.* (2003) studied the effects of solar radiation and temperature on bacterial die-off rates in Black Sea coastal waters and found that solar radiation was the most significant factor affecting the mortality of coliform bacteria. No significant effect of temperature was observed in the presence of solar radiation. However, the effect of temperature is significant in dark experiments with die-off taking approximately 20 times longer to reach 90% concentration reductions compared to values in the light. Davies-Colley *et al.* (1999) reported that sunlight is the main factor causing natural attenuation in waste stabilization ponds, although dissolved oxygen (DO) and pH can also influence the rate of disinfection. Die-off studies on *E. coli* and *Salmonella* were conducted in two different ecosystems: Morlaix estuary in English Channel and Bay of Toulon on Mediterranean Sea. In the Morlaix estuary, most of the bacteria were mixed with turbid waters and were able to survive a long time as light penetration was prevented by suspended matter, lowering the effect of sunlight. On the contrary, through lack of nutrients and very high sunlight intensity, die-off rates in Mediterranean waters were high with 90% mortality within 2 h near the water surfaces, and several hours in deep waters (Pommepuy *et al.*, 1992).

A close relationship was found between the light intensity and the decay rate. Gameson and Gould (1975) concluded that about half the lethal effect of light is attributable to wavelengths below 370 nm with an additional quarter of the lethal effect attributable to the 370-400 nm and 400-500 nm bands, respectively. The effect of longer wavelengths, greater than 500 nm, is negligible.

The exact mechanism whereby microorganisms become non-viable after sunlight exposure is not entirely clear. Photons can excite exogenous or endogenous sensitizers (e.g., humic acid) present in the water that damage DNA or other cellular components of the bacteria, directly. Photons can also cause damage indirectly by promoting the production of free radicals in the presence of dissolved oxygen and organics.

Chamberlin and Mitchell (1978) and Eisenstark (1971) noted that the mechanism of light-induced bacterial decay depends on the presence of endogeneous sensitizers or chromophores, which adsorb light energy and cause cell damage directly or by reaction with oxides to form superoxides, which in turn may cause damage to the cells.

Physical Processes (Sedimentation, Sorption, and Filtration)

Microbes in the water column may associate with particles or remain in the ‘‘free’’ or unassociated phase. This free phase includes organisms that exist individually or as agglomerated groups (aggregates) held together by organic and inorganic particles. Microbes associated with particles, particularly denser inorganic particles, will tend to settle from the water column more quickly than free organisms or those associated with less dense particles that remain more mobile in the environment. It has also been observed that microbes associated with particles tend to survive longer in natural waters than free microbes (Howell *et al*., 1996; Sherer *et al*., 1992; Burton *et al*., 1987; Gerba and Schaiberger, 1975). These particle associations can affect not only microbial fate and transport, but also the time these organisms remain a threat to human health.

Gannon *et al*. (1983) and Davies and Bavor (2000) assessed the performance of stormwater impoundments and constructed wetlands for microbial concentration reductions by measuring inflow and outflow, with results suggesting that sedimentation was a primary mechanism of removal. Gannon *et al.* (1983) also demonstrated that a significant fraction of fecal coliforms in the water column was retained by a 5 mm filter, indicating that some bacteria were attached to particles, but making no distinction regarding the nature of the particles. In subsequent work, Schillinger and Gannon (1985) analyzed the partitioning

of several bacterial indicators in samples taken from the water column of a stormwater drain under wet weather conditions. Similar filtration experiments revealed that at least half the bacteria in the water column passed through a 5 mm filter, but again the nature of the particles was not addressed.

Characklis *et al.* (2005) found that substantial fractions of five different microbial organisms, including bacterial, protozoan, and viral indicators, were associated with settleable particles in stormwater. The results also found partitioning behavior varied by organism and with conditions (e.g., storm vs. background). This study attempted to correlate the microbe–particle association with specific environmental factors (e.g., TSS, TOC, particle number) but did not yield strong evidence of a relationship. However, the results suggest that for some organisms (e.g., fecal coliforms) there may be a relationship between the fraction that is particle associated and particle concentration, microbial concentration, or both.

The processes of sedimentation, sorption, and filtration are difficult to separate. In the project discussed in this report, the physical processes are treated collectively and referred to as sedimentation. Some consider sedimentation as the main mechanism of pollutant removal in general in constructed wetlands and retention ponds. With the tendency for a significant portion of some stormwater pollutants to bind to particulates, sedimentation is often considered as the primary factor when designing treatment practices. Longer detention periods in retention ponds promote sedimentation of solids in the course and medium size fractions. Similarly, the presence of extensive vegetation in constructed wetlands can encourage sedimentation (Pundsack *et al*., 2001).

Salinity

Osmotic stress can also play a role in the concentrations of concentrations of indicator bacteria. The die-off rate is generally much faster in marine and estuarine waters than in freshwater (Thoman and Mueller, 1987). Yan *et al.* (2000) found that both light intensity and salinity have significant effects on the inactivation of *E. coli* in wastewater discharged into the ocean through submarine outfall system. Solic and Krstulovic (1992) found inactivation rate increased as salinity increased. They also noted that higher salinity and high levels of solar radiation combined produced a synergistic effect, resulting in higher mortality rates of fecal coliforms. Hanes and Fragala (1967) showed that *E. coli*, coliforms and enterococci had greater inactivation rates with increasing salinities, with 6.2, 4.6, and 1.6 times greater death rate, respectively, at 100% seawater compared to 0% seawater. Similarly, Anderson *et al.* (1979) found a decreased survival rate for *E. coli* with increasing salinity, ranging from 53.5% survival at 10 ppt after 8 days of exposure, to 2% survival at 30 ppt for the same period. Fuijoka *et al.* (1981) found that seawater caused rapid inactivation of fecal streptococci and fecal coliform, whereas the organisms remained stable for three days in freshwater. Mancini (1978) indicated that components in seawater in addition to salt may be responsible for inactivation in seawater.

Salinity concentration is an important factor in coastal receiving waters such as estuaries or the coastal ocean. Although many estuarine and coastal systems receive stormwater runoff, generally stormwater retention ponds and constructed wetlands are not constructed in areas that are tidally influenced or are in a continuously saline environment. The project discussed in this report only briefly address bacterial attenuation from this stressor.

Predation

Wetlands and retention ponds can support a diversity of aquatic animals including micro-crustaceans (copepods, ostracods, and cladocerans), shrimp, crayfish, insects (dragonfly larvae, water beetles, and water boatman), pond snails, tadpoles, frogs, and fish. These organisms are a crucial component of

wetland and shallow open-water ecosystems, providing food-web linkages between plants, microorganisms and other animals. Predator–prey relationships are important in the control of mosquitoes (Greenway *et al.*, 2003) and may contribute to the control of bacterial populations (Davies and Bavor, 2000) in these systems. Green *et al.* (1997) determined bacterivorous activity was an important factor in the removal of bacteria in constructed wetlands treating wastewater. Mandi *et al.* (1993) determined predation by nematodes and Decamp and Warren (1998) by ciliates and rotifers were significant factors in determining bacterial densities in constructed wetlands although in the former study the predation was not quantified. In the latter, Decamp and Warren (1998) determined that ciliates such as paramecium ingested as many as 13 fluorescently labeled *E. coli* per cell per minute. Fernandez *et al.* (1992a, b) also concluded that predation and competition were extremely important in the removal of fecal coliforms. As part of a large study to model the removal of fecal coliforms, Troussellier *et al.* (1986) investigated the effects of grazing by rotifers and of biological oxygen demand (BOD) loading. They found rotifers can significantly affect fecal coliform concentrations in aquatic systems.

Other Potential Factors

There are many other factors that can affect the densities of indicator organisms. BOD, pH, and DO have all previously been mentioned as potential contributing factors to inactivation rates of bacteria. Solic and Krstuvolic (1992) noted that fecal coliforms thrived at a pH range of 6-7, declining in numbers outside of this range, with greater rate of mortality in acidic environments. Chemical factors include oxidation, exposure to biocides excreted by plants, and sorption to organic matter. Additional biological removal mechanisms may include antimicrobial activity of root exudates (Kickuth and Kaitzis, 1975; Axelrood *et al.*, 1996), activity of lytic bacteria or viruses (Axelrood *et al.*, 1996), retention in biofilms (Brix, 1997), and natural die-off (Gersberg *et al.*, 1989a, b).

Few studies have thoroughly investigated the effect of nutrients (including dissolved organic carbon and trace metals) on the inactivation of microorganisms in stormwater in the environment. Thomas *et al.* (1999) found bacterium *Campylobacter* had low survival rates in nutrient-containing microcosms. In microcosm studies in fresh and salt waters, Noble *et al.* (2004) found that nutrient levels had an insignificant effect on the persistence of fecal indicator bacteria.

Summary

As can be seen, literature values on indicator bacteria inactivation in surface waters are quite variable. Much of the literature pertains to wastewater treatment and dairy waste studies. There have been few studies conducted investigating the viability of indicator bacteria using stormwater as a medium. Of the field studies on surface waters, conditions of the watershed and storm information such as intensity and duration are not well documented or are incomplete. Therefore, interpreting data and comparing results are often tenuous at best.

Which of these factors has the greatest influence on indicator bacteria inactivation? Are there combinations of factors that have the greatest affect? What conditions affect the contribution from each individual or group of factors? The next three chapters detail both bench- and pilot-scale experiments conducted for data collection and evaluation of the first-order decay model that uses environmental factors to determine the inactivation rate, K, to predict effluent concentrations in BMPs.

Chapter 3 Bench-Scale Research

Purpose

The bench-scale research tested the proposed descriptive relationships between concentration and identified presumptive controlling variables (time, temperature, light intensity, and salinity) outlined in the literature and listed below. The work used statistical analysis, specifically nonlinear regression, to quantify the organism-specific inactivation rate constants for traditional (fecal streptococci, and total and fecal coliforms) and alternate (enterococci and *E. coli*) microbial indicators in stormwater. The research separately assessed the influence of time, temperature, light intensity, and salinity on the inactivation rate constants to isolate the effects and maintain the analytical load within laboratory capacity. This approach simplifies the analysis but neglects the potential interactions among the independent variables.

The research exposed stormwater samples to controlled-conditions to measure the change in microorganism concentrations after known exposure periods. The experimental design selected the controlled independent variables and their ranges based on the broad, literature-reported influence and the likelihood of the condition existing in the structural BMPs (e.g., retention ponds and constructed wetlands). As widely reported in the literature, time, temperature, and light intensity are important environmental variables which determine the rate of change of indicator organism concentrations. Salinity was included in this study as it is reported as an environmental factor which influences the microbial decay and can be potentially important in some BMPs installed in coastal settings or when the stormwater runoff results from areas where communities apply road salt during winter.

Inactivation Rate Models

The literature reports indicator organism inactivation rates in various water types (Table 2-1). However, information on inactivation rates for indicator organisms in stormwater and effects of natural factors on survival rates is limited except for one study by Geldreich *et al.* (1968).

Most published studies use first-order decay known as Chick's Law to describe indicator organism inactivation with time. Under this premise, the concentration-time relationship is:

$$C_t = C_o e^{-K_o t} \qquad (3\text{-}1)$$

Where: C_t = concentration of organism at time (CFU/100 mL);
C_o = concentration of organism at time zero (CFU/100 mL);

K_o = overall inactivation rate constant at the environmental conditions (h^{-1}); and
t = elapsed time since time zero (h).

There are several approaches to estimate the effects of environmental variables on the overall rate constant. The simplest approach assumes additive effects:

$$K_0 = K_T + K_S + K_l + K_f \quad (3\text{-}2)$$

Where: K_T = inactivation rate constant due to temperature (h^{-1});
K_S = inactivation rate due to salinity (h^{-1});
K_l = inactivation rate constant due to light (h^{-1}); and
K_f = inactivation rate constant due to other factors such as sorption, filtration, and sedimentation (h^{-1}).

Temperature

The effect of temperature is often approximated by using the Arrhenius-van't Hoff equation (Khatiwada and Polprasert, 1999):

$$K_T = K_{20}\Phi_T^{(T-20)} \quad (3\text{-}3)$$

Where: K_T = inactivation rate constant due to temperature at T = T^0C (h^{-1});
K_{20} = inactivation rate constant due to temperature at T = 20^0C (h^{-1});
T = temperature in ^{0}C; and
Φ_T = temperature coefficient (dimensionless).

The selection of the reference temperature (20^0C) is somewhat arbitrary. Other temperatures can and have been used. As much of the literature uses 20^0C as the reference value, this research follows this convention.

Salinity

The simplest approach to estimating the effect of increasing salinity on the rate constant assumes a linear increase with salinity.

$$K_S = \Phi_S S \quad (3\text{-}4)$$

Canteras *et al.* (1995) proposed a variation on the additive effects described by equation (3-5). That work proposes a combined effect due to the combination of temperature and salinity.

$$K_{T,S} = K_{20}\Phi_T^{(T-20)}\Phi_S^{S} \quad (3\text{-}5)$$

Where: $K_{T,S}$ = inactivation rate constant due to temperature and salinity (h^{-1});
K_{20} = inactivation rate constant at dark, 20^0C and zero salinity (h^{-1});
Φ_T = temperature coefficient (dimensionless); and
Φ_S = salinity coefficient (dimensionless).

Light

The effect of light intensity on the inactivation rate constant is normally expressed as:

$$K_l = \Phi_l I_Z \quad (3\text{-}6)$$

Where: Φ_l = light proportionality coefficient (cm^2/mW-h); and
I_Z = light intensity at depth Z below the surface (mW/cm^2).

Unlike temperature and salinity which can be reasonably assumed to be uniform throughout the system, light intensity varies with depth below the water surface. The intensity at a given depth, I_Z,, decreases exponentially with distance (Gameson and Gould, 1975). The value is often estimated as:

$$I_Z = \frac{I_O}{\tau h}(1 - e^{\tau h}) \quad (3\text{-}7)$$

Where: I_o = light intensity at the earth surface (mW/cm^2);
τ = vertical light extinction coefficient (1/m); and
Z = depth of water (m).

The extinction coefficient varies with water proprieties including color and turbidity (Lee and Rast, 1997).

Combining equations, the overall equation using the Canteras *et al.* (1995) assumption for salinity is:

$$K_o = K_{20}\Phi_T^{(T-20)}\Phi_S^{S} + \Phi_l I_Z + K_f \quad (3\text{-}8)$$

Materials and Methods

Sample Collection

Stormwater was collected from an outfall after that drained a 10-acre portion of the Middlesex County College Campus near the USEPA facility in Edison, New Jersey (Figure 3-1). The drained area was predominantly campus maintenance buildings and student parking lots. Samples were only collected for this work when the rain event met the USEPA monitoring guidance (USEPA, 1992). Generally, the project required at least 3 mm (1/8th in.) total rainfall, preceded by at least 72 h without measurable precipitation. Automatic samplers (Hach, Loveland, CO) placed in the outfall collected a flow weighted composite sample when the flow water depth in the outfall initially reached 2.54 cm (1 in.). Area-velocity flow meters (Hach, Loveland, CO) connected to the automatic samplers triggered the internal peristaltic pump to add 1-L aliquots to a 20-L, pre-cleaned container when an incremental specified flow volume was measured. The incremental volume was set based on forecasted total rainfall.

After collection, the samples were transported to the on-site laboratory and allowed to quiescently settle for 10 to 20 min at room temperature to allow most settling solids to fall to the container bottom. The water from the settled collection container was transferred leaving about 1 in. in the composite container bottom to limit the potential effects of settleable particulates on the experiments and avoid interference with the enumeration process. While continuously stirring the container holding the decanted supernatant (Stir Pak Mixer), a peristaltic pump transferred aliquots to 250-mL pre-cleaned HDPE bottles. All subsample bottles were completely filled leaving no headspace.

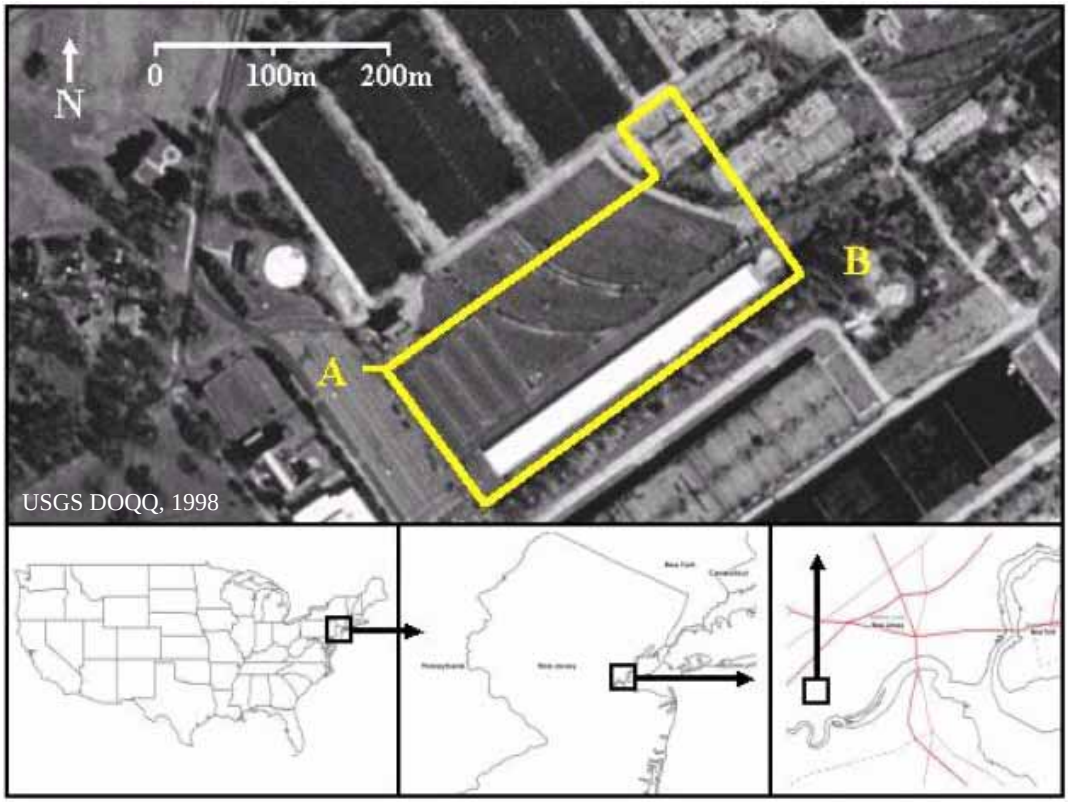


Figure 3-1. Drainage area (A) and outfall location (B) of the study in Edison, New Jersey.

Experimental Methods

The experiments were conducted by placing the 250-mL HDPE containers in water baths (Precision, A Division of Jouan Inc., Winchester, VA) to maintain constant temperature. The temperature of each water bath was established at least one day before inserting the bottles. Aluminum foil wrapping on the outside of the bottles prevented light exposure for experiments other than those investigating the effects of light exposure. The temperature of the water bath and the temperature of an equal volume of deionized water in separate containers were monitored using a NIST-traceable digital thermometer and recorded at 1-min intervals using logging thermisters (Onset Corp, Bourne, MA). The recorded temperatures confirmed that the stormwater in the 250-mL containers required from 30 to 340 min to reach the water bath temperature. The temperature varied less than 1^{o}C during the experiment.

The experiments defined time zero as the time when the sample reached the designated temperature as described below. Bottles were removed periodically during the experiment for sampling and analysis. The times when bottles were removed from the water bath were established based on the expected exponential concentration decline and to collect samples primarily during the normal workday as a cost control measure. Samples collected from the bottles were analyzed for five indicator organisms following membrane filtration procedure. A set of four samples was collected for the initial time. Subsequent sampling collected duplicate samples from the bottle. Time was monitored using commercially-available (La Crosse Technology, La Crosse, WI) clocks synchronized to the US Naval Observatory atomic clock. The reported elapsed time for removing the bottle from the temperature bath is believed to be accurate to within 1 min. DO and pH were monitored daily from independent sample bottles for the duration of the

experiment.

Temperature Study

The temperature-dependence die-off study targeted temperatures of 10°C, 20°C, and 30°C. Earlier USEPA research, measuring the diurnal temperature fluctuation in local BMPs showed that summer temperatures reach about 30°C and fall to less than 5°C in the winter. While extreme water temperatures in BMPs are likely to span a slightly wider temperature range; the temperature range used in this study should represent the most often encountered temperatures within the BMP. The mean temperatures recorded by the data loggers are used in the analysis (usually slightly lower than the targeted temperatures), however, for simplicity, the descriptive target temperatures were used. Temperature monitoring was included as part of the light and salinity studies.

Salinity Study

The salinity-dependence die-off study targeted four salinity levels (0, 10, 20, and 30 ppt). The target temperature was set at 25°C. The salinity range was selected to represent concentrations encountered in partially diluted seawater. The salinities were established by adding synthetic sea salt (Instant Ocean, Aquatic Systems, Mentor, OH) directly to stormwater while mixing. When the lowest salinity was achieved, stormwater was dispensed to the sample bottles. Then, more sea salt was added to achieve the next highest salinity level. The salinity of the resulting solution was measured using Hach CO 150 Conductivity Meter (Loveland, CO). The analytical results showed salinity concentrations of 0.45, 8.1, 16.1, and 23.5 ppt, while those concentrations for samples other than those in the salinity study were not measured.

Light Study

The light-dependence study established a target temperature of 25°C. Samples were exposed to light at four intensities including one dark sample. The respective light intensities for different light conditions were established using Reptisun 5.0 UVB fluorescent bulbs (Zoo Med Laboratories, Inc., San Luis Obispo, CA). The manufacturer reports that the bulbs produce light at UVA (320-400 nm) (30%) and UVB (290-320 nm) (6%) wave lengths. Adjusting the distance between the light source and the surface of the container controlled light intensities. The distance was maintained at 12.7, 22.9 or 35.6 cm above the water surface to have light intensities varying between 20 and 100 mW/cm^2 (200 to 1000 W/m^2). Average intensity of sunlight is about 120 mW/cm^2 (1,200 W/m^2). Light intensities at the sample surface were measured using a light meter (International Light IL 1400A with thermopile detector) daily throughout the experiments. The measured light intensity varied about 5% during the experiment. Table 3-1 lists the average light intensities measured throughout the experiment.

The container used to hold the sample in the light study was 250-mL thin, flat sided polystyrene flasks with canted neck and plug seal cap (BD Biosciences, Bedford, MA). This container was selected because it did not have any effect on the light attenuation and would reduce the depth of water assuring a more homogenous light dose.

Table 3-1. Light Intensities Corresponding to the Height of Light Source above the Water Surface

Distance (cm)	Light Intensity (mW/cm^2)	Average Light Intensity (mW/cm^2)
12.7	89.0 – 97.8	94.70
22.9	50.2 – 58.4	55.23
35.6	19.7 – 21.9	20.86

Analysis of Indicator Organisms

All samples were analyzed for five indicator organisms (total coliforms, fecal coliforms, fecal streptococci, enterococci, and *E. coli*) using membrane filtration methods following Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 1998) (Table 3-2) and described below.

Table 3-2. Summary Table of Analytical Procedures

Indicator Organism	Method Number	Method Title
Total Coliforms	SM 9222B	Membrane Filter Procedure
Fecal Coliforms	SM 9222D	Membrane Filter Procedure
E. coli	SM 9222G	Membrane Filter Procedure
Fecal Streptococci	SM 9230C	Membrane Filter Procedure
Enterococci	SM 9230C	Membrane Filter Procedure

Total coliforms were determined by incubation on M-Endo agar (24 h at 35°C) and confirmed by gas formation in lauryl tryptose broth and brilliant green lactose broth. Fecal coliforms were incubated on M-FC agar (24 h at 44.5°C) and were confirmed by gas formation in lauryl tryptose broth and EC broth. *E. coli* levels were measured by transferring the membrane from the Endo-type medium to a nutrient agar containing 4-methylumbelliferyl-β-D-glucuronide (NA-MUG) and incubating 4 h at 35°C. Production of blue fluorescence on the periphery of colonies under long wavelength UV indicated *E. coli*. Fecal streptococci were determined by incubation on m-Enterococcus agar (48 h at 35°C). Colonies were transferred to brain heart infusion (BHI) agar. Transfers were made to BHI broth and incubated at 35°C for 24 h, with confirmations made by retransfer to bile esculin agar, BHI broth incubated at 45°C, and BHI with 6.5% NaCl. Growth on bile esculin agar BHI broth verifies that the colony is of the fecal streptococci group. Growth at 45°C and in BHI with 6.5% NaCl indicates that the colony belongs to the enterococci group.

Samples were sequentially diluted with sterile buffered water using three dilution factors based on previous analyses of similar samples. Dilution factors were estimated to obtain the method-recommended colony count on at least one dilution set. Sequential dilutions usually used at least 10 mL aliquots and always used at least 5 mL. All results were volume normalized to give concentrations in colony forming units (CFU) per 100 mL.

Each analytical batch included laboratory blanks and positive controls. Blanks were run before and after each analytical set. Verification was performed on ten colonies for each organism according to the procedures listed in Standard Methods (APHA *et al.*, 1998). After incubation, the plates were manually

enumerated. Positive controls showed the growth of particular indicator organisms.

Data Analysis/Reduction

The data analysis used all incubated plates with colonies in the countable range. The count from each incubated plate was normalized to the source concentration using the dilution factor and volume filtered. The uncertainty in each sample was estimated as the propagated error using the methods outlined by Taylor (1997). The dilution is assumed to be error free. The uncertainty in the filtered volume is estimated as ±0.4 mL, the tolerance of the ASTM class A graduated cylinders used in this study. The uncertainty in the number of counted colonies is estimated as 10% of the count with a minimum of 1 colony.

The sample weighted-average concentration and associated uncertainty was calculated using the uncertainty in the individual estimates as the weighting factor. This approach reduces the multiple (5 to 12) results from the samples and dilutions associated with the original source (bottle) to a single concentration estimate for the organism representing the experimental result resulting from the environmental condition. Alternate pooling strategies for combining the multiple analyses can be developed. For example, the multiple dilutions from each sample could be pooled using the same weighting strategy to obtain either four or two estimates for each elapsed time.

This data reduction relies on regression analysis of the concentration time series developed under the established experimental conditions. The approach estimates the effect of the various conditions (treatments). The treatments identified were based on the breadth of earlier-published research. No attempt was made to establish why the environmental exposure reduces the measured concentration, e.g., cellular wall degradation or DNA damage. Because of the reliance on the statistical techniques, it is important to prevent artificially increasing the degrees of freedom associated with the analysis that would suggest greater confidence in the results. The pooling approach described accomplishes the objective of not artificially increasing the degrees of freedom and placing greater emphasis on results with less uncertainty. Generally, the concentration time series developed for the individual experiments showed undetectably low concentrations for the final analyses.

The weighted-average concentration for each organism was regressed on the independent variables using nonlinear least-squares regression techniques. The nonlinear regression method used the Levenberg-Marquardt technique (a modified algorithm of the Gauss-Newton least-squares technique) in Statistica software package (version 7.1, Statsoft, Inc.). All regressions are run at the 95% level of confidence (α=0.05). The reported uncertainty in the calculated coefficients is the confidence interval reported by the Statistica software package. After the regression was complete, an Analysis of Variance (ANOVA) was run to test the significance of the proposed model.

Results

Time and Temperature

The regression used the proposed time-dependent decay function, known as Chick's Law (equation (3-1). The concentrations at each elapsed time for a given temperature were used to estimate the inactivation rate constant for the indicator organism at that temperature. Table 3-3 lists the regression results from the temperature study with the 25^{o}C results from the light and salinity studies (discussed below). These experiments were also conducted in salt-free, dark, isothermal conditions and represent data that was included in the analysis.

Table 3-3. Inactivation Rate Constants for Each Indicator Organism at Tested Temperatures in the Isothermal Experiments

Temperature (°C)	Total Coliforms	Fecal Coliforms	*E. coli*	Fecal Streptococci	Enterococci
	Inactivation Rate Constants (h^{-1})				
9.07	0.007±0.010	0.03±0.025*$^{\Delta}$	0.027±0.015*	0.027±0.022*$^{\Delta}$	0.021±0.014
19.87	0.017±0.035	0.01±0.140	0.085±0.033*	0.076±0.077	0.095±0.038*
29.32	0.016±0.045	0.76±0.510*	0.136±0.072*	0.100±1.300$^{\Diamond}$	0.150±0.420
26.17 [1]	0.013±0.020	0.07±0.060*	0.019±0.072$^{\Diamond}$	0.039±0.055	0.027±0.015*
24.74 [2]	0.0131±0.37*	0.10±0.180	0.029±0.048	0.056±0.029*	0.044±0.038

* Indicates K_T value is statistically significant at $\alpha = 0.05$

$^{\Delta}$ The first concentration is omitted from the analysis as an apparent outlier

$^{\Diamond}$ ANOVA shows regression model is not significant

[1]Data from light experiment

[2]Data from salinity experiment

In all but two cases, the post-regression ANOVA confirmed the model significance; however, in about half the cases the temperature-specific inactivation rate constant was not significant. This is taken to mean that these results do not provide a reason to reject the first-order decay model but that the data set is often not numerically sufficient to obtain quantitative estimates of the temperature-specific inactivation rate constant that excludes zero at the established level of confidence. This result generally occurred when the time series included few data triplets (time, temperature, concentration) leading to high uncertainty in the numeric values calculated.

The results listed in Table 3-3 for the portion of the study emphasizing temperature generally demonstrate an increase in the calculated decay constant with increasing temperature. Completing the same analysis on the results from the subsequent investigations for the light and salinity studies at roughly 25°C do not produce rate constants expected by interpolating between the bounding temperatures of 10 and 20°C. The calculated values (Table 3-1) are not consistent for all organisms although the temperatures were within about 1.5°C. These later two experiments used stormwater collected at the same outfall but at a different time.

The experimental design planned to pool the results of the temperature study data across the experimental temperatures. The weighted-average concentration results were regressed on elapsed time using the same nonlinear procedures to test the model proposed by equation (3-3). As discussed above, the temperature study used a common stormwater source for the samples exposed to the selected temperature conditions. These samples had uniform starting concentrations allowing for the time delays in reaching time zero. The studies for light and salinity effects used different stormwater samples with dramatically different initial concentrations as would be expected from samples collected at different times of the year. To pool the data into a common set, the results were normalized to the estimated initial concentration using the calculated value of the initial concentration, $\hat{C}_o$ for the specific source calculated above. After transforming the data to $C_t/\hat{C}_o$ the above-described procedures were applied to estimate the reference temperature decay constant and the temperature coefficient using equation (3-9):

$$\frac{C_t}{\hat{C}_o} = e^{-K_{20}\Phi_T^{T-20}t} \qquad (3\text{-}9)$$

Table 3-4 lists the regression coefficients. In all cases the temperature coefficient (Φ_T) and the reference decay constant at 20°C (K_{20}) are statistically significant. The post-regression ANOVA confirms the

significance of the regressions and significantly there is consistency of the individual coefficients across organisms.

Table 3-4. Organism-Specific Reference Temperature Rate Constant and Temperature Coefficient Determined Using Salt-Free Dark Experimental Results

Indicator Organism	Reference Temperature Rate Constant (K_{20}) (h^{-1})	Temperature Coefficient (Φ_T)
Total Coliforms	0.016±0.009*	1.057±0.085*
Fecal Coliforms	0.042±0.030*	1.090±0.110*
E. coli	0.036±0.019*	1.023±0.072*
Fecal Streptococci	0.047±0.031*	1.044±0.040*
Enterococci	0.042±0.014*	1.057±0.045*

* Coefficient is statistically significant at α=0.05.

The Φ_T values for all the organisms range between 1.02 and 1.09, which is consistent with the values reported in the literature. Mancini (1978) and Khatiwada and Polprasert (1999) suggested a value of 1.07 for fecal coliforms which agrees with the calculated value in this work of 1.09±0.11.

The DO monitoring showed a steady decline with time. The rate of decrease increased with temperature. The DO declined at 0.4, 1.4, and 1.7 mg/L/day at 10, 20, and 30°C, respectively. At 30°C, the DO was nearly depleted after 60 h. At 20°C, the DO was depleted after 70 h. At 10°C, DO was at 2.75 mg/L after 72 h. Except for total coliforms, the plates produced non-quantitative counts within 23 h at 30°C. This suggests the die-off is not due to depleted DO, but due to the combined effects of time and temperature. The pH of the samples varied slightly, but remained within the near- neutral range (6.5 to 7.0) throughout the experiment. Solic and Krstuvolic (1992) noted that fecal coliforms survived within the pH range of 6 to 7, and declined outside of this range, with greater rate or mortality in acidic environments. The average TSS in the sample was 41 mg/L. These water quality indicators are within the range reported in the NSQD (Maestre and Pitt, 2005).

Light

The analysis assumes the light intensity measured at the sample surface is representative of the exposure throughout the container. The limited water depth in the selected container bottles supports this assumption. The analysis of the results of the light exposure experiments were examined in a two-step process. The weighted-average concentration was first used to calculate the overall coefficient under the established condition of light and temperature ($K_{T=26.17,l}$) for each indicator at each exposure level using the model in equation (3-10).

$$C_l = C_o e^{-K_{l,T} t} \qquad (3\text{-}10)$$

Table 3-5 lists the regression-estimated values of $K_{T=26.17,l}$ for each indicator organism at each light level. The statistical quality of the fecal coliform and *E. coli* results is generally poor. The increased light intensity affects the calculated decay constant confirming that light influences the decay process. The calculated decay constant values increase with increasing light intensity. These results support the presumptive additive effect of light on the overall rate constant, i.e., $K_{T,l} = K_T + \Phi_l l$.

Table 3-5. Regression-Estimated Values of Inactivation Rate Constants at 26.17°C for

Experimental Light Intensities

Indicator Organism	Light Intensity (mW/cm²)			
	0	20.86	55.23	94.7
Total Coliforms	0.131±0.037*	0.21±0.16*	0.23±0.053*	0.32±0.12*
Fecal Coliforms	0.10±0.18	0.07±0.17◊	0.08±0.25◊	0.20±0.24
E. coli	0.029±0.048	0.14±0.16	0.16±0.23	0.26±0.20*
Fecal Streptococci	0.056±0.029*	0.205±0.063*	0.254±0.022*	0.469±0.025*
Enterococci	0.044±0.038*	0.202±0.070*	0.19±0.15*	0.96±0.14*

* Coefficient is statistically significant

◊ Regression result is not statistically significant at α=0.05

The pooled data were then used to estimate the coefficients in the presumptive relationship:

$$C_t = C_0 e^{-(K_{T=26.17} + \Phi_L I)t} \quad (3\text{-}11)$$

Table 3-6 lists the estimated values of the constants for each organism. The effect of light on the decay coefficient varies by a factor of four across the organisms showing a difference in light sensitivity. As expected, the values of $K_{T=26.17,l}$ generally agree with the values listed in Table 3-5 for the dark experiments. The values also agree with the expected estimated values using $K_T = K_{20}\Phi^{(T-20)}$ evaluated at 26.17°C. The light-free exposure for this data set is included in the previous analysis. The added light has minimal effects on the decay rates for total and fecal coliforms. There is an order of magnitude smaller than the effects on the other indicators.

Table 3-6. Regression-Estimated Coefficients from Light Experiment

Indicator Organism	Inactivation Rate Constant ($K_{T=26.17}$) (h^{-1})	Light Proportionality Coefficient (Φ_L) (cm^2/mW-h)
Total Coliforms	0.155±0.047*	0.0016±0.0012*
Fecal Coliforms	0.070±0.090	0.0130±0.0027
E. coli	0.040±0.037*	0.0025±0.0019*
Fecal Streptococci	0.046±0.015*	0.0057±0.0018*
Enterococci	0.034±0.020*	0.0076±0.0038*

* Coefficient is statistically significant at α=0.05

The intensity of natural sunlight varies during the course of the day. The exposure levels are further variable when considering the clouds that produce the rainfall and resulting runoff. The clouds will filter or block incident radiation to differing degrees. This work used artificially generated light to maintain constant exposure levels. Other researchers reported that sunlight showed the greatest bactericidal effect on organisms. Most research identifies UVB (290-320 nm), UVA (320-400 nm) and blue green visible light (400-550 nm) as the portion of the solar spectrum responsible for inactivating microorganisms. The UVB portion of the solar spectrum is believed to be the dominant bactericidal agent causing direct DNA damage (Sinton *et al.*, 1999). For this reason, UV is used for disinfection in water and wastewater treatment processes (Ferguson *et al.*, 2003; Giese and Darby, 2000).

The pH of the samples varied between 6.5 and 7.5. Dissolved oxygen content of the samples varied between 8.2 and 12.3 mg/L. The difference in DO from the temperature study is noteworthy. The decline

observed in the temperature was not observed in this effort. The light exposure experiments lasted nearly 50 h. Total suspended solids concentration in the sample was measured at 85 mg/L. While about double the concentration recorded in the temperature study, this is still in the typically reported range.

Salinity

The data analysis to establish the effects of salinity parallels the approach used to evaluate light effects. The individual time series were analyzed for each organism at each salinity level to obtain a preliminary assessment of the salinity effect. Table 3-7 lists the values of $K_{T,S}$ determined for each indicator organism at each salinity level.

The effect of salinity is not consistent across indicator organisms. The rate constant for fecal streptococci is not significant for any of the salinity levels evaluated. The results for other organisms, e.g., fecal coliforms and enterococci, are generally significant.

The results listed in Table 3-7 do not show a clear pattern for the salinity effect on the calculated decay rate. Applying the technique proposed by Canteras *et al.* (1995) requires fitting the concentrations to the form:

$$C_t = C_0 e^{-K_T \Phi_s^S t} \qquad (3\text{-}12)$$

Table 3-7. Regression-Estimated Values of Inactivation Rate Constants Determined for Salinity Concentrations

Indicator Organism	Salinity (ppt)			
	0	8	16	24
Total Coliforms	0.013±0.020	0.039±0.022*	0.020±0.035	0.044±0.038*
Fecal Colifroms	0.065±0.061*	0.031±0.020*	0.058±0.099	0.417±0.094*
E. coli	0.020±0.072◊	0.079±0.079*	0.010±0.110◊	0.012±0.042
Fecal Streptococci	0.039±0.054	0.016±0.079◊	0.027±0.098◊	0.027±0.089◊
Enterococci	0.027±0.015*	0.035±0.019*	0.005±0.011	0.019±0.008*

***** Coefficient is statistically significant at α =0.05*****

◊ Regression result is not statistically significant at α=0.05

Table 3-8 lists the regression results using the proposed relationship. Overall, the effect of increased salinity at the tested concentrations is small. The calculated value of Φ_S is not generally distinguishable from unity for organisms other than total and fecal coliforms. This suggests that, for the span of salinity values studied, the added salt had little effect on the decay rate constant. This supports the results reported by Canteras *et al.* (1995) who found largest salinity effect occurs only when the salinity values were over 35 ppt. Thoman and Mueller (1987) reported that the inactivation of fecal coliforms is generally much faster in marine and estuarine waters than in freshwater. Mancini (1978) also indicated that components in seawater in addition to salt may be responsible for greater inactivation effect in seawater.

The pH of the samples varied between 7.0 and 8.0. Dissolved oxygen content of the samples varied between 8.3 and 12.2 mg/L. Total suspended solids in the sample were measured at 44 mg/L.

Table 3-8. Regression-Estimated Coefficients from Salinity Experiment

Indicator Organism	Calculated Coefficients	
	K_S (h^{-1})	Φ_S
Total Coliforms	0.011±0.011	1.073±0.061*
Fecal Coliforms	0.035±0.023*	1.095±0.060*
E. coli	0.024±0.041	0.990±0.100*
Fecal Streptococci	0.025±0.032	0.998±0.086*
Enterococci	0.032±0.016*	0.957±0.036*

* Coefficient is statistically significant at $\alpha = 0.05$

Summary

The results of this experiment demonstrated that the concentration of the tested indicator organisms decrease exponentially with time. The first-order decay process reasonably models the concentration time series for the durations tested. The analysis of the weighted-average concentrations enabled developing organism-specific inactivation rate constants in stormwater assuming time, temperature, light intensity and salinity are the most significant parameters.

The temperature study indicated that the indicator organisms persisted at higher concentrations at lower temperatures. The inactivation rates increased with increasing light intensity. The added light has minimal effects on the inactivation rates for total and fecal coliforms. Different indicator organisms exhibited different trends with salinity. Taken as a whole, the results indicate that salinity had little or no effect on inactivation rates for these indicator organisms for the salinities tested.

The difference in temperature results from the temperature study suggests that differences in the stormwater influence the reference decay rate. The major measured difference in the characterization of the stormwater was the initial concentration of indicator organisms. This further suggests that the constants measured in the bench-scale experiments must be viewed as the rate for the specific stormwater sample evaluated and cannot be extrapolated to all stormwater sources. The variability of the constants between sources, if any, cannot be estimated from these data.

Chapter 4 Pilot-Scale Research

This project was designed to determine the factors that influence the rate of microbial inactivation as urban stormwater passes through retention ponds and constructed wetlands. Research on constructed wetlands inactivation of fecal indicators in wastewater is well documented (Bavor *et al.*, 1987; Gersberg *et al.*, 1987; Ottová *et al*., 1997). Removals of fecal streptococci and coliforms generally exceeded 80% and 90%, respectively (Kadlec and Knight, 1996). Gersberg *et al.* (1987) and Garcia and Bécares (1997) concluded that extensively vegetated systems remove indicator bacteria at significantly higher rates from wastewater than unvegetated systems. However, because of the potentially high indicator bacteria concentrations in stormwater runoff, the remaining 10 - 20% (assuming a performance of 80-90% is achieved) in the effluent may increase receiving water concentrations beyond WQS. This is in contrast to sanitary and combined stormwater and sanitary systems which, other than during sewer overflow, chemically treat the wastewater routed to treatment plants.

This pilot-scale project intended to build on the bench-scale studies to evaluate the variation of rates of inactivation using first-order decay with changes in environmental conditions. The mesocosms designed and constructed for this project offer a unique environment where many parameters concerning stormwater characterization and flow can be held constant (i.e., characteristics of influent, residence time, and pollutant loading). By varying testing dates with typical climatic conditions experienced throughout the year, an assessment of the impact of environmental change on bacterial inactivation rates can be assessed. A comparison of rates of inactivation with seasonal wet weather events can indicate whether water quality managers can rely on model predictions to be accurate during periods when loading may be greatest. More information is needed to determine whether models that use first-order decay functions when predicting bacteria effluent concentrations from field BMPs (usually as a point source) are accurately providing effluent concentration predictions and concomitant loads. This can directly impact the loading allowed when determining TMDLs for meeting WQS.

Study Site and Experimental Design

A pair of rectangular mesocosms of the same size with two different stormwater BMP treatments (constructed wetland and retention pond) were constructed at the Urban Watershed Research Facility (UWRF) in Edison, New Jersey (see Figure 4-1). Mesocosm housings were purchased fiberglass aquaculture tanks. Mesocosms were constructed by placing a perforated PVC under drain with valve, about 4 cm of pea gravel, 2.5 cm of sand, and 25 cm of topsoil. Local cattail plants were transplanted into constructed wetland mesocosms. The retention ponds were constructed similarly with like layers and depths of gravel, sand, and topsoil, but with no plantings. Tanks had a length, width, and depth of 1.78

meters, 0.74 m, and 0.65 m, respectively with a stormwater volume of approximately 227 L. Both systems were constructed in August of 2002.

Creating Bacterially Loaded Stormwater

Feed concentrations in the stormwater runoff collected on site were low (10^1-10^3 CFU/100 mL) compared to many urban watersheds. To achieve a higher loading concentration (10^4-10^6 CFU/100 mL) for this study, a 500 mL aliquot of stormwater runoff was collected from on-site runoff as described in Chapter 3 and placed in growth media to encourage growth of the desired bacteria, consequently producing higher densities of bacteria in the stormwater. Complete method development is described in Appendix A.

(A) **(B)**

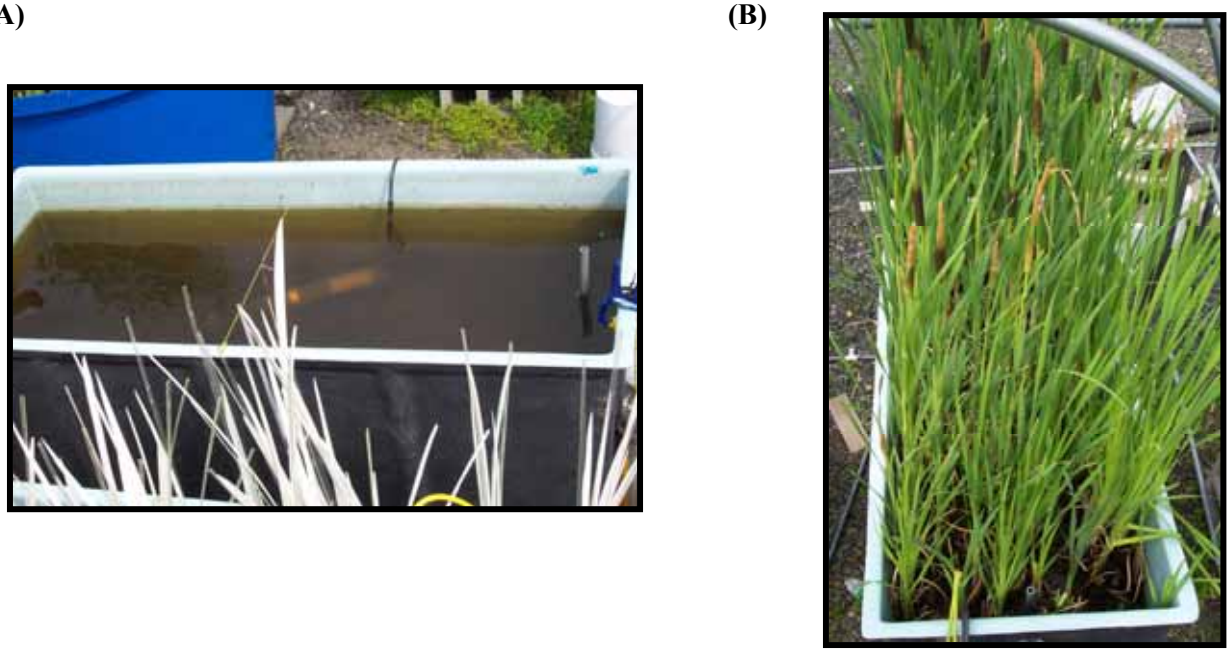


Figure 4-1. Pictures of the retention pond (A) constructed wetland (B) treatment systems.

Target indicator bacteria concentrations in mesocosms following addition of the enriched stormwater are found in Table 4-1. The cultured stormwater was introduced over a 30-45 min time period (in an attempt to limit thermal shock) to a common supply tank that contained approximately 1000 L of recently captured stormwater and mixed for 30 min. Constructed wetland and retention pond mesocosms were filled from this same supply source.

Table 4-1. Target Indicator Organism Densities in Mesocosms after Addition of Enriched Stormwater

Indicator Organism	Target Concentration (CFU/100 mL)
Total Coliforms	$10^6 – 10^7$
Fecal Coliforms	$10^6 – 10^7$
E. coli	$10^4 – 10^5$
Enterococci	$10^4 – 10^5$

It is recognized that the relative proportion of indicator bacteria in the bacterially enriched stormwater will change from the original stormwater and may be less representative of the true indicator bacteria community. Another recognized difference in the bacterially enriched stormwater is the particle association of the indicator bacteria is suspected to be different than the source with less bacteria associated with larger particles and more bacteria associated with finer particles or unattached. Although this was not measured, it is believed that this property results in a more conservative estimate for the indicator organisms when using bacterially enriched stormwater for indicator bacterial loading experiments. The thought is fewer colonies are expected to settle out of the water column through attachment to larger particles resulting in increased effluent concentrations compared to the stormwater source.

Storm Event Simulation

Runoff events were simulated by distributing 220 L of collected stormwater runoff from a common supply tank that was mixed for 30 min using four vortex mixing eductors to each mesocosm. Additions were made sequentially at an average rate of 0.75 L/s measured using a Seametrics WT-P turbine meter (SeaMetrics, Kent, WA). At this stormwater discharge rate, it took approximately 5 min to load the BMP mesocosms. Using the same method and equal volume as in the mesocosms, cultured stormwater was distributed to a 227 L container placed near the mesocosms serving as a control tank. This tank, based on proximity, was expected to have the same ambient environmental conditions as the pilot-scale tanks.

Effluent flow rates and hence detention times in the mesocosms were regulated by the effluent pipe orifice (diameter 2 mm) simulating a riser flow control structure as in field BMPs. Mean effluent flow rates ranged from 57 – 76 L/hr during the loading event until levels return to their pre-event (static) levels. Generally, it took between 20-22 h for water levels to return to their original pre-event level. Between simulated storm events, and after drawdown of stormwater to a static pre-event level during simulated storm events, mesocosm water levels were maintained through semi-continuous flow from a nearby water supply regulated by a float valve positioned opposite and slightly above the effluent orifice.

Residence times of the mesocosms were previously determined by Struck *et al.* (2004) using conservative dye tracer tests to determine the residence time distribution with Rhodamine WT dye distributed to each mesocosm. Tracer concentration in effluents were measured with a YSI Rhodamine WT and verified with a Turner Designs 10 AU field flourometer (Sunnyvale, CA). Exponential curves were fit to tracer concentration data to calculate the measured mean residence time (Levenspiel, 1999).

Storm events were planned over a two-year period with three simulated storm events each year. No event was to occur within the same month. The dates selected represented typical climatic conditions during that time to incorporate changing environmental conditions as a factor in bacterial inactivation.

Water Quality Monitoring, Solids, Light, and Bacterial Indicator Sampling

The constructed wetland, retention pond, and control tank each had a YSI water quality sonde placed on the sediment surface at a depth of 4 cm in the constructed wetland and 25 cm in the retention pond near the overflow orifice. These sondes recorded *in-situ* temperature, DO, pH, conductivity, and turbidity averaged over 10-minute intervals.

Light intensity was measured using an on-site weather station (Onset Corporation, Bourne, MA). Grab samples of light were also recorded on six separate occasions between 12:00pm and 3:00pm at the water surface in the retention pond and constructed wetland using a hand held light photometer (IL1400, International Light Inc., Newburyport, MA).

Effluent from the riser pipe of the retention pond and constructed wetland was collected in pre-washed 1-L HDPE bottles placed at the effluent drainpipe (to collect enough volume for the sample but subject to continuous replacement). Microbiological and TSS samples were collected from these 1-L containers using automatic samplers (Hach Company, Loveland, CO) and placed into cooled pre-washed 1-L HDPE bottles within the sampling device at elapsed times found in Table 4-2. Timed samples were also collected at a depth of 5 cm below the water surface in the control tank and labeled as "light controls". If grab samples were necessary due to autosampler failure discrete samples using pre-cleaned PVC bottles were collected to obtain the desired sample.

Six 500-mL bottles were wrapped in aluminum foil filled with 400 mL of inoculated stormwater, sealed, and placed in the control container to duplicate environmental conditions (other than sunlight) in the mesocosms and control tank. These samples were collected daily and with the extended times (beyond 90 h) as noted in Table 4-2, as "dark controls" along with the "light" control samples collected from the control container.

These samples separate light and dark affects on the microbial population in the control groups. It should be noted that sample #1 in Table 4-2 (time 0) was collected 30 min before stormwater addition for the constructed wetland and retention pond recording antecedent baseline conditions. Stormwater controls were then loaded and sampled with sample #1 (time 0) representing the influent concentrations and conditions of the supply tank and the dark and light controls.

Table 4-2. Effluent Time of Hand Collected and Programmed Autosample Collection

Sample #	h	Sample Collected
1	0	influent, light control, in-situ (background)
2	5	timed effluent
3	10	timed effluent
4	15	timed effluent
5	21	timed effluent
6	27	timed effluent, light control, dark control
7	33	timed effluent
8	39	timed effluent
9	45	timed effluent, light control, dark control
10	51	timed effluent
11	60	timed effluent, light control, dark control
12	69	timed effluent
13	78	timed effluent
14	90	timed effluent, light control, dark control
15	114	timed effluent, light control, dark control
16	150	timed effluent, light control, dark control

All samples were transferred to appropriate sized plastic pre-cleaned containers for analyses depending on the type of analyses and the source of the sample. Samples were transported to the laboratory for splitting, filtering, and preservation as necessary within specified holding times. If storage was necessary, samples were stored in a refrigerator at 4ºC.

Bacteria indicator samples were analyzed for four indicator organisms (total and fecal coliforms, enterococci, and *E. coli*) following the standard procedures listed in Chapter 3 (Table 3-2). Fecal streptococcus analyses were not done because the increased quantity of samples this indicator added was beyond laboratory capabilities.

The expected indicator concentration in the effluent for total and fecal coliform, enterococci, and *E. coli* was 10^4 to 10^6 CFU/100 mL during the first sampling period. Each timed sample was mixed and split into three equal volumes of sample for each organism. Three dilutions of each split sample were analyzed to assure at least one dilution provided concentrations between 5 and 200 colonies per plate. These ranges were expected to shift as the concentrations of indicator bacteria decrease with time (Table 4-3).

Automatic samplers were programmed to collect two additional 1-L samples 15-20 min following the first timed sample, allowing enough time for refilling of the effluent collection bottle. These samples served as additional samples for TSS analysis and secondary indicator bacteria samples in the event of an error in the first programmed sample collection.

Table 4-3. Expected Beginning Densities After Loading and Expected Dilution Factors

Indicator Organism	Expected Density (CFU/100 mL)	Dilution
Retention Pond Mesocosm		
Total Coliforms	$10^3 - 10^5$	10^2,10^3,10^4
Fecal Coliforms	$10^3 - 10^5$	10^2,10^3,10^4
E. coli	$10^2 - 10^4$	10^1,10^2,10^3
Enterococci	$10^2 - 10^4$	10^1,10^2,10^3
Constructed Wetland Mesocosm		
Total Coliforms	$10^4 - 10^6$	10^3,10^4,10^5
Fecal Coliforms	$10^4 - 10^6$	10^3,10^4,10^5
E. coli	$10^4 - 10^6$	10^3,10^4,10^5
Enterococci	$10^3 - 10^5$	10^2,10^3,10^4

Sediment Sampling Procedures

Mesocosms were divided into four spatial areas to randomize sampling location and avoid spatial biases. Four samples were collected from each section in the retention pond and constructed wetland systems. Sediments were collected using a clear cylinder (modified syringe) inserted about 5 cm into the undisturbed sediments and capped at one end. After removing the sediment core from the mesocosm, the core was transferred to a centrifuge tube. Sediment samples were mixed using a sterile spatula. Samples were then analyzed using multiple-well fermentation tests for enterococci and *E. coli* (and total coliforms) using Enterolert® and Colilert®, respectively, manufactured by IDEXX Laboratories (Westbrook, ME). This method was selected instead of membrane filtration because of the difficulty in counting colonies with obstructing sediment and due to the potential of growth inhibition by accumulated sediments on the filter paper.

Predation Sampling Procedures

Laboratory experiments have suggested that ingestion rates should increase with food concentration and

that feeding preference should be shown for the most abundant of available foods. Analysis of larger macroinvertebrates was the most practical method to determine the potential of predation on bacteria populations, due to the time and cost of other methods. Identification and enumeration of macroinvertebrates as small as 0.5 mm were done on 5 samples each from the retention pond and constructed wetland to determine the populations of either bacteriovores (bacteria eating predators) or the presence of macroinvertebrates. The guides Freshwater Macroinvertebrates of Northeastern North America (Peckarsky *et al.*, 1990) and An Image-Based Key to the Zooplankton of the Northeast (University if New Hampshire, 2005) were used for identification of invertebrates. A top-down approach, looking at the densities of macroinvertebrates including those from a higher trophic level (especially bacteriovores), was used to qualitatively assess the bacteria concentrations based on the presence and quantity of bacteria-eating organisms. The assumption is that an increase in the abundance of bacteria would also lead to an increase in the predators that use bacteria as a major food source. Ideally, when using this monitoring approach, one would take samples before, during, and after each storm event. However, because of the enormous analyses requirements to identify and enumerate the macroinvertebrates, as well as notice of recruitment of macroinvertebrates midway through the study, macroinvertebrate data are limited to a single sampling event.

Data Management

The volume of data for each simulated storm event was large. Much of the continuous data was placed into spreadsheets for geometric means and statistical calculations based on time and storm event. For the purposes of comparing the physical and environmental characteristics data was averaged over the time between each sample collection as noted in Table 4-2. Bacteria indicator organism concentration data required some synthesis to calculate sample time averages and inactivation rates as noted in Chapter 3. Since indicator bacteria samples were split into three samples and then three separate dilutions, it was possible to have up to 12 indicator concentrations with each organism for each timed sample. This was usually not the case as often one or more of the three dilutions would result in either values of zero or TNTC (plates containing over 200 colonies) as designed to bracket the actual culturable colony-forming units. All plates were enumerated, with the exception of those designated as TNTC. For each split sample, dilutions with plates containing 1-200 organisms were used for data analysis. Each split sample was averaged across dilutions. The three split samples for each sample collection time (total of 16 times) were log transformed and regressed with time. Uncertainty was calculated as in Chapter 3. Each simulated storm event produced more than 192 data points for indicator bacteria indicators.

Statistical Analyses

Sample sizes were large enough to perform statistical comparisons (ANOVA and correlation analyses) between constructed wetland, retention pond treatment practices, dark and light control inactivation rates, and physical and chemical characteristics. Likewise, sample size allowed for regression analyses of the bacterial indicator concentrations on elapsed time between the constructed wetland, retention pond, and dark and light controls for the each stormwater event. Slopes of the regressions gave inactivation rate constants, K, for each event. Chemical parameters were compared between the treatments and controls using correlation matrices. All statistical analyses that have significant p-values and $\alpha = 0.05$ were noted.

Results

Physical and Chemical Properties of the Pilot-scale Systems

Physical and chemical parameters measured in the study are listed in Table 4-4. Water temperatures averaged 2.15ºC less in the constructed wetland compared to the retention pond. This difference was

likely due to shading from the macrophytic vegetation (*Typha latifolia*, average stem density = 39.3 stems/m^2). This temperature difference was more notable in the warmer sampling events (difference of 3.08ºC and 1.82ºC, respectively) compared to the colder sampling events (difference of 0.46ºC).

DO was higher in the retention pond compared to the constructed wetland; the May, July, and September events had the highest values. The process of decomposition of organic matter in the constructed wetland can consume some of the DO, causing low concentrations (sometimes near 0 mg/L) during periods of increased decomposition. Increases in decomposition are temperature-related with a positive correlation between the two. Also, diurnal fluctuations in DO and temperature were reduced during initial storm event loading. Values for these parameters did not generally reach pre-event diurnal fluctuations until after 48 h of detention for most events. Conductivity was nearly the same in the two systems while pH was circumneutral to alkaline in the retention pond but tended to be acidic in the constructed wetland. This pattern was noted by Mitch and Gosselink (2000) in constructed wetlands with mineral soils and lake sediments by Stumm and Morgan (1996). These differences were attributed to the organic matter build-up in sediments and decomposition which tends to make the pH less than 7. The ORP was much less (and often negative) in the retention pond compared to the constructed wetland system. The depth of inundation of the free water in the retention pond was generally three times that of the constructed wetland. This would substantially increase the potential for more reducing conditions through both reduced oxygen diffusion with water depth and the lack of photosynthetic oxygen production with absence of macrophytic vegetation, resulting in lower ORP values in the retention pond. Light intensity in the constructed wetland was consistently 9-10% of that measured in the retention pond. The difference in recorded hand-held light intensity for each event was used to adjust the measured irradiance for the light control and retention pond to calculate a corrected irradiance expected at the surface of the constructed wetland to make irradiance values between pilot-scale systems comparable.

Most storm events had maximum initial TSS and turbidity values of less than 100 mg/L and 150 NTUs, respectively, after stormwater loading to the retention pond and constructed wetland. As expected, turbidity and TSS values decreased with time in each system. Geometric mean turbidity values for sampling events before October 2005 are shown in Figure 4-2. Turbidity values were averaged for each time step and then over each sampling event.

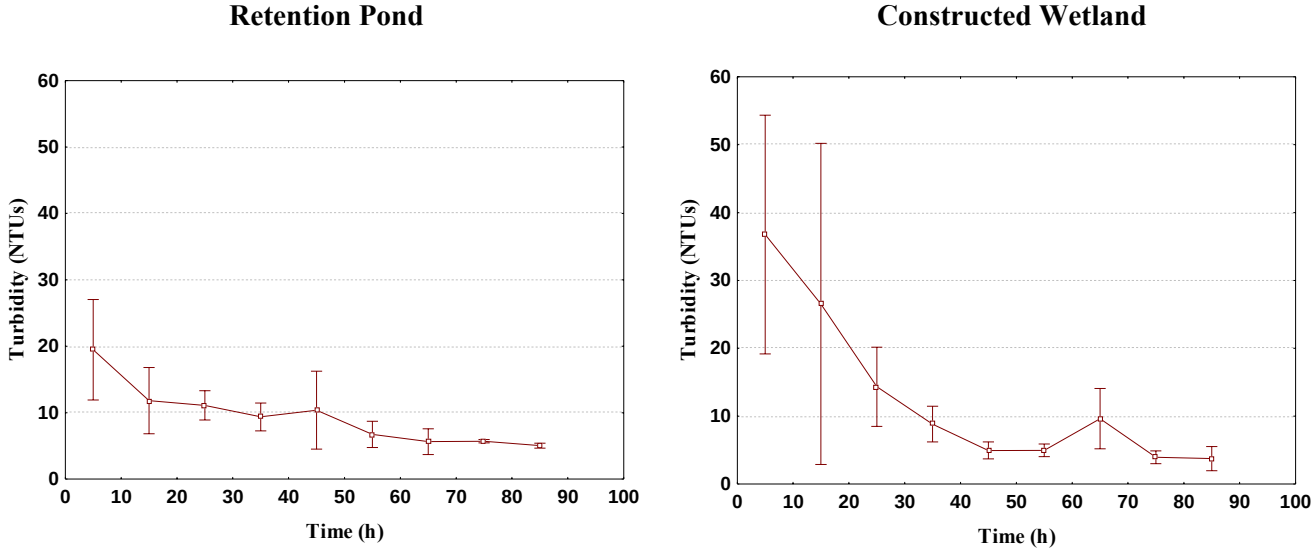


Figure 4-2. Mean turbidity in the retention pond and constructed wetland in all storm events except October 2005. Whiskers indicate 95% confidence intervals.

Table 4-4. Average Event *in-situ* Physical and Chemical Results

		Retention Pond						Constructed Wetland					
Date	Parameter	Valid N	Mean	Minimum	Maximum	Standard Deviation	Standard Error	Valid N	Mean	Minimum	Maximum	Standard Deviation	Standard Error
	Temp (ºC)	36	27.0	21.9	32.2	2.9	0.5	36	25.5	22.2	29.4	2.2	0.4
	Cond (mS/cm)	36	0.316	0.296	0.345	0.016	0.00261	36	0.284	0.265	0.316	0.014	0.002
	D0 (mg/L)	36	5.8	1.9	11.4	2.6	0.4	36	3.6	0.8	9.2	2.3	0.4
Jun-04	pH	36	8.0	7.3	9.1	0.6	<0.1	36	6.7	6.6	7.0	0.1	<0.1
	ORP (mV)	36	364	200	465	93	15	36	465	317	529	67	11
	Turbidity (NTU)	36	11.6	5.1	31.1	5.7	0.9	36	10.3	6.5	23.8	4.8	0.8
	Irradiance (kJ/m^2)	12	43.9	<0.1	139.1	48.9	14.1	12	4.1	<0.1	12.8	4.5	1.3
	Temp (ºC)	36	22.8	18.7	27.3	2.8	0.5	42	19.4	16.1	22.1	1.8	0.3
	Cond (mS/cm)	36	0.217	0.196	0.237	0.011	0.002	42	0.204	0.190	0.237	0.013	0.002
	D0 (mg/L)	36	8.5	5.8	11.5	1.9	0.3	42	4.1	0.7	13.4	3.6	0.5
Sep-04	pH	36	7.9	7.5	8.3	0.2	<0.1	42	6.4	6.3	6.7	0.1	<0.1
	ORP (mV)	36	-288	-354	-15	95	16	42	555	524	591	19	3
	Turbidity (NTU)	36	11.0	7.9	20.7	3.2	0.5	42	6.6	3.7	18.5	3.8	0.6
	Irradiance (kJ/m^2)	12	31.6	<0.1	125.0	45.1	12.0	14	3.4	<0.1	11.5	4.7	1.3
	Temp (ºC)	42	11.1	5.6	16.1	3.3	0.5	42	10.1	4.6	15.9	3.2	0.5
	Cond (mS/cm)	42	0.189	0.159	0.216	0.018	0.003	42	0.179	0.130	0.208	0.021	0.003
	D0 (mg/L)	42	9.5	6.2	13.9	2.0	0.3	42	9.0	2.3	14.3	4.1	0.6
Nov-04	pH	42	7.2	7.0	7.3	0.1	<0.1	42	6.3	6.1	6.8	0.2	<0.1
	ORP (mV)	42	-285	-322	-211	29	4	42	403	376	452	19	3
	Turbidity (NTU)	42	7.8	4.0	11.5	1.9	0.3	42	8.4	0.4	27.6	6.5	1.0
	Irradiance (kJ/m^2)	14	19.0	<0.1	84.4	26.5	7.1	14	1.9	0.0	7.8	2.4	0.7
	Temp (ºC)	33	19.9	16.6	27.0	3.2	0.6	39	17.1	15.3	22.3	1.9	0.3
	Cond (mS/cm)	33	0.447	0.394	0.555	0.047	0.008	39	0.680	0.585	0.771	0.052	0.008
	D0 (mg/L)	33	10.5	8.5	15.6	1.8	0.3	39	1.4	0.1	4.4	1.2	0.2
May-05	pH	33	8.3	7.3	9.3	0.5	0.1	39	6.8	6.8	7.0	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	33	1.8	<0.1	11.2	2.8	0.5	39	4.4	2.5	12.0	2.4	0.4
	Irradiance (kJ/m^2)	12	26.9	<0.1	119.0	40.2	11.1	13	2.6	<0.1	17.4	5.6	1.6
	Temp (ºC)	24	26.7	24.0	31.2	2.2	0.4	24	24.5	23.1	27.1	1.3	0.3
	Cond (mS/cm)	24	0.253	0.235	0.297	0.018	0.004	24	0.259	0.197	0.371	0.054	0.011
	D0 (mg/L)	24	5.5	3.0	9.5	1.9	0.4	24	1.3	0.1	3.7	1.3	0.3
Jul-05	pH	24	7.5	7.2	8.2	0.3	0.1	24	6.4	6.2	6.6	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	24	26.0	6.5	92.0	20.2	4.1	24	61.0	13.5	167.2	50.9	10.4
	Irradiance (kJ/m^2)	8	29.4	<0.1	119.0	40.3	14.3	8	2.7	<0.1	10.9	3.7	1.3
	Temp (ºC)	48	14.6	12.6	17.6	1.6	0.2	48	14.7	11.6	18.4	1.7	0.3
	Cond (mS/cm)	48	0.156	0.066	0.240	0.048	0.007	48	0.184	0.092	0.273	0.046	0.007
	D0 (mg/L)	48	3.2	<0.1	6.3	1.6	0.2	48	2.8	<0.1	9.1	3.0	0.4
Oct-05	pH	48	7.0	6.1	7.5	0.4	0.1	48	6.0	5.8	6.5	0.1	<0.1
	ORP (mV)	Not Recorded											
	Turbidity (NTU)	48	849.1	<0.1	1236.5	396.9	57.3	48	937.4	11.2	2141.4	782.5	112.9
	Irradiance (kJ/m^2)	16	18.7	<0.1	135.3	39.8	10.0	16	1.9	<0.1	12.4	3.7	0.9

The October 2005 experimental run had starting TSS values averaging 2,999 mg/L, and turbidity values averaging 2,173 NTUs, which is near the maximum of the range expected for most stormwater runoff. Active construction in the watershed was evident in the stormwater runoff during this sampling event. Inclusion of this stormwater runoff greatly increases the variability in solids concentration, overwhelming the smaller concentrations found in the previous runoff events. Thus some analyses occurred with the exclusion of this event as noted.

Bacteria Indicator Organisms

Samples were not analyzed for enterococci in the first experiment (June 2004) but resumed for all subsequent events. Graphs showing the relationship of bacterial indicator organism concentrations for the constructed wetland and the retention pond with event physical and chemical parameters can be found in Figures 4-3 through 4-5. Conductivity and DO did not appear to significantly effect bacterial concentrations over the ranges observed (Figure 4-3). ORP may have moderately affected fecal coliforms and *E. coli* concentrations around 200 mV in the retention pond while densities of fecal coliforms, *E. coli*, and enterococci decreased between 500 and 600 mV in the constructed wetland, but only three events were monitored for this parameter. Densities of fecal coliforms and *E. coli* decreased above a pH of 8.5 in the retention pond but remained unaffected over the range of observed pH values in the constructed wetland (Figure 4-4). Temperature appears to affect bacterial indicator organism concentrations in the retention pond. The optimal temperature range that resulted in the greatest number of observed bacteria colony forming units was between 11ºC and 26ºC. A similar trend was noticed in the constructed wetland for a temperature range between 11º C and 23ºC (Figure 4-5).

There was a distinct relationship with concentration of all indicator organisms with turbidity in this experiment. While there was much scatter in concentrations at turbidities less than 20 NTU, at turbidities greater than 100 NTUs there was a predictable increase in bacteria organism concentrations with increasing turbidity in both the retention pond and constructed wetland (Figure 4-6). Similar results have been shown by the United States Geological Survey (USGS) in larger rivers in northern and central Virginia as well as by the USEPA in smaller streams in northern Virginia (Hyer and Moyer, 2003; Struck *et al.,* 2006). These solids can potentially affect rates of bacteria attenuation as discussed in Chapter 5.

Overall inactivation rates for all simulated storm events are shown in Table 4-5. Significant differences were observed between the constructed wetland and retention pond in eight of the bacteria indicators for the six runoff events. The retention pond had six of these with inactivation rates for: total coliforms in June and July; *E. coli* in May; fecal coliforms in July and enterococci in May and November significantly larger than in the constructed wetland. However, the constructed wetland had a significantly larger inactivation rate compared to the retention pond for fecal coliforms in June and enterococci in July. Both treatments had greater inactivation rates compared to light and dark controls in September and November for total coliforms, *E. coli*, and fecal coliforms. Retention pond inactivation rates were also greater than controls for total coliforms in June and July, for *E. coli* in May and July, and fecal coliforms in May, June, and July while constructed wetlands rates were greater for *E. coli* in July, fecal coliforms in May and June, and enterococci in July. Light controls were greater than dark controls in 9 instances, including May and June for total coliforms and *E. coli*, July for fecal coliforms, and May, July, September, and November for enterococci. This indicates that light does have an impact on bacteria indicator organisms.

Bacteria indicator concentrations decreased with time for the retention pond and constructed wetland (Figure 4-7). The exponential regression coefficients indicate the inactivation rate. A two step process of generating an overall inactivation value for each bacterial indicator organism from 0-50 h and from 50-100 h was used to generate a best fit relationship. This timeframe was determined by maintaining R^2 values of regressions greater than 0.70 while varying the time interval between 0 and 100 until the difference in slope (inactivation rate) was maximized for the majority of the bacteria indicator organisms.

This method used R^2 values (Figure 4-7) of the whole timeframe compared to 0-50 and 50-150 h partial timeframes. In all instances, the R^2 values improved when dividing the duration of the experiment into the two timeframes, suggesting that inactivation rates vary as a function of time with greater rates of inactivation during the first 50 h timeframe compared to the second 100 h timeframe.

Table 4-5. Inactivation Rates for the Constructed Wetland, Retention Pond, and Dark and Light Controls for all Indicator Bacteria Organisms for each Sampling Event

		Retention Pond				Constructed Wetland			
Month	Year	Total Coliforms	*E. coli*	Fecal Coliforms	Enterococci	Total Coliforms	*E. coli*	Fecal Coliforms	Enterococci
		(h^{-1})				(h^{-1})			
June	2004	0.2419*+	0.1484	0.1814+		0.1529	0.1651	0.3277*+	
September	2004	0.144+	0.1164+	0.1192+	0.2030	0.1204+	0.1204+	0.1515+	0.1786
November	2004	0.1653+	0.1164+	0.1485+	0.1730*	0.1235+	0.1157+	0.1137+	0.1245
May	2005	0.0949	0.3350*+	0.1417+	0.1717*	0.1090	0.0919	0.1233+	0.0852
July	2005	0.1811*+	0.1957+	0.2610*+	0.1240	0.0733	0.1894+	0.1025	0.2112*+
October	2005	0.0437	0.0524	0.0566	0.0512	0.0427	0.0597	0.0536	0.0594

		Dark Control				Light Control			
Month	Year	Total Coliforms	*E. coli*	Fecal Coliforms	Enterococci	Total Coliforms	*E. coli*	Fecal Coliforms	Enterococci
		(h^{-1})				(h^{-1})			
June	2004	0.0247	0.0276	0.0249		0.1390♦	0.1502♦	0.0242	
September	2004	0.0700	0.0563	0.0527	0.0773	0.0588	0.0789	0.0760	0.2027♦
November	2004	0.0815	0.0480	0.0445	0.0711	0.0658	0.0724	0.0692	0.1787♦
May	2005	0.0258	0.0725	0.0514	0.0351	0.0679♦	0.1158♦	0.0828	0.0884♦
July	2005	0.0637	0.0509	0.0619	0.0944	0.0720	0.0712	0.1136♦	0.1681♦
October	2005	0.0538	0.0605	0.0514	0.0194	0.0676	0.0862	0.0822	0.0316

* Indicates a significantly higher value between retention pond and constructed wetland

+ Indicates a significantly higher value between retention pond or constructed wetland values and control values

♦ Indicates a significantly higher value between light and dark control values

Fecal coliforms and enterococci in the retention pond were an exception to this generalization. Several of the inactivation rates during the 50-150 h timeframe had values nearing zero suggesting that these organisms may have reached or nearly reached background concentrations after 50 h. This is supported by the average pre-event background concentrations in the retention pond and constructed wetland found in Table 4-6.

Bacteria Concentrations in Sediment

Results from the sediment bacteria indicator organism concentrations collected and analyzed one day before and two days after the November 2004 storm event are shown in Table 4-7. Sediment bacteria increased substantially for total coliforms and *E. coli* after the storm event. However, concentrations of enterococci decreased somewhat over the experiment. The bacteria indicator organisms measured before the experimental run are considered as background concentrations as the previous input of indicator organisms through stormwater runoff was two months prior to this event.

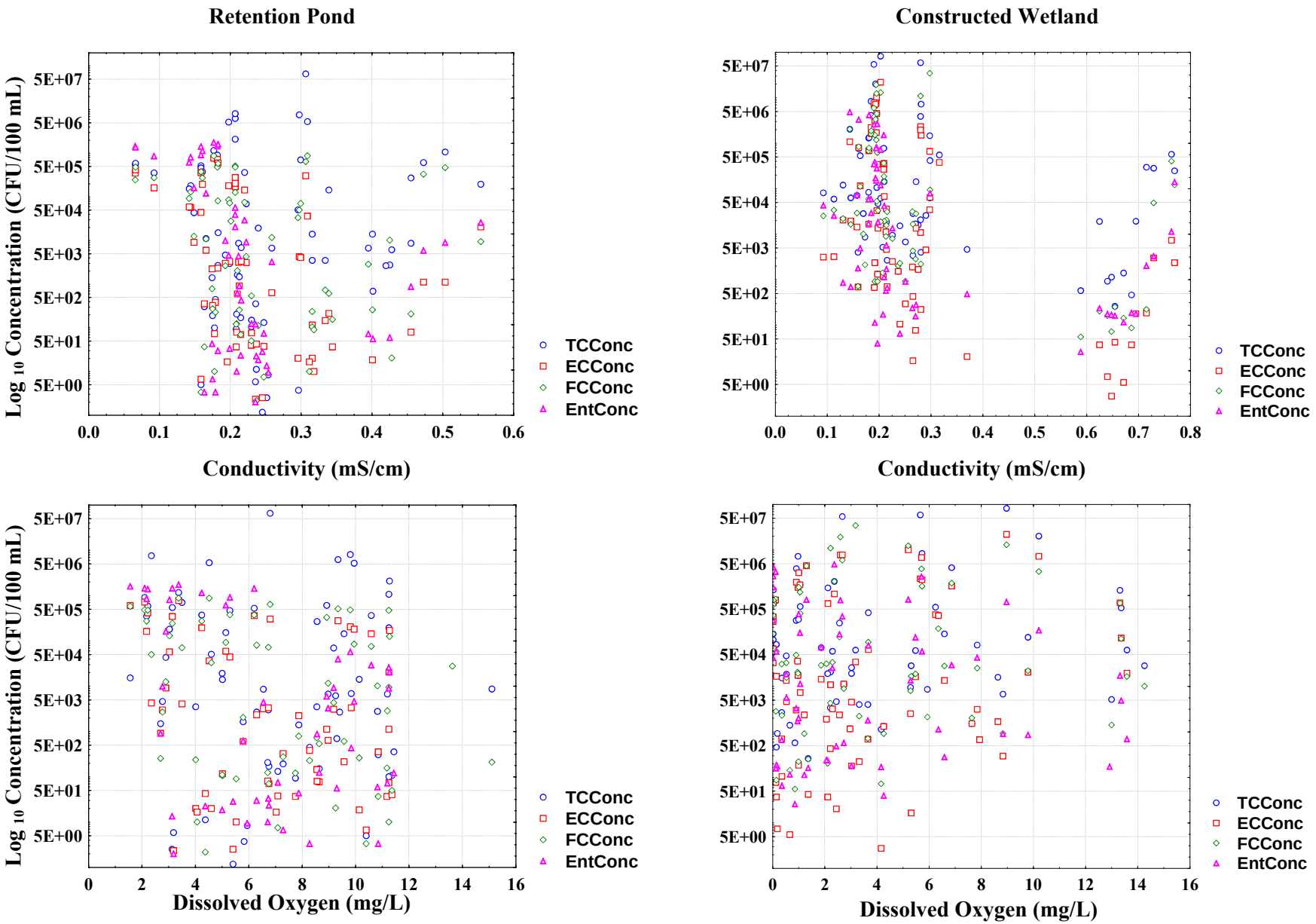


Figure 4-3. Effluent concentration of indicator organisms with conductivity and dissolved oxygen.

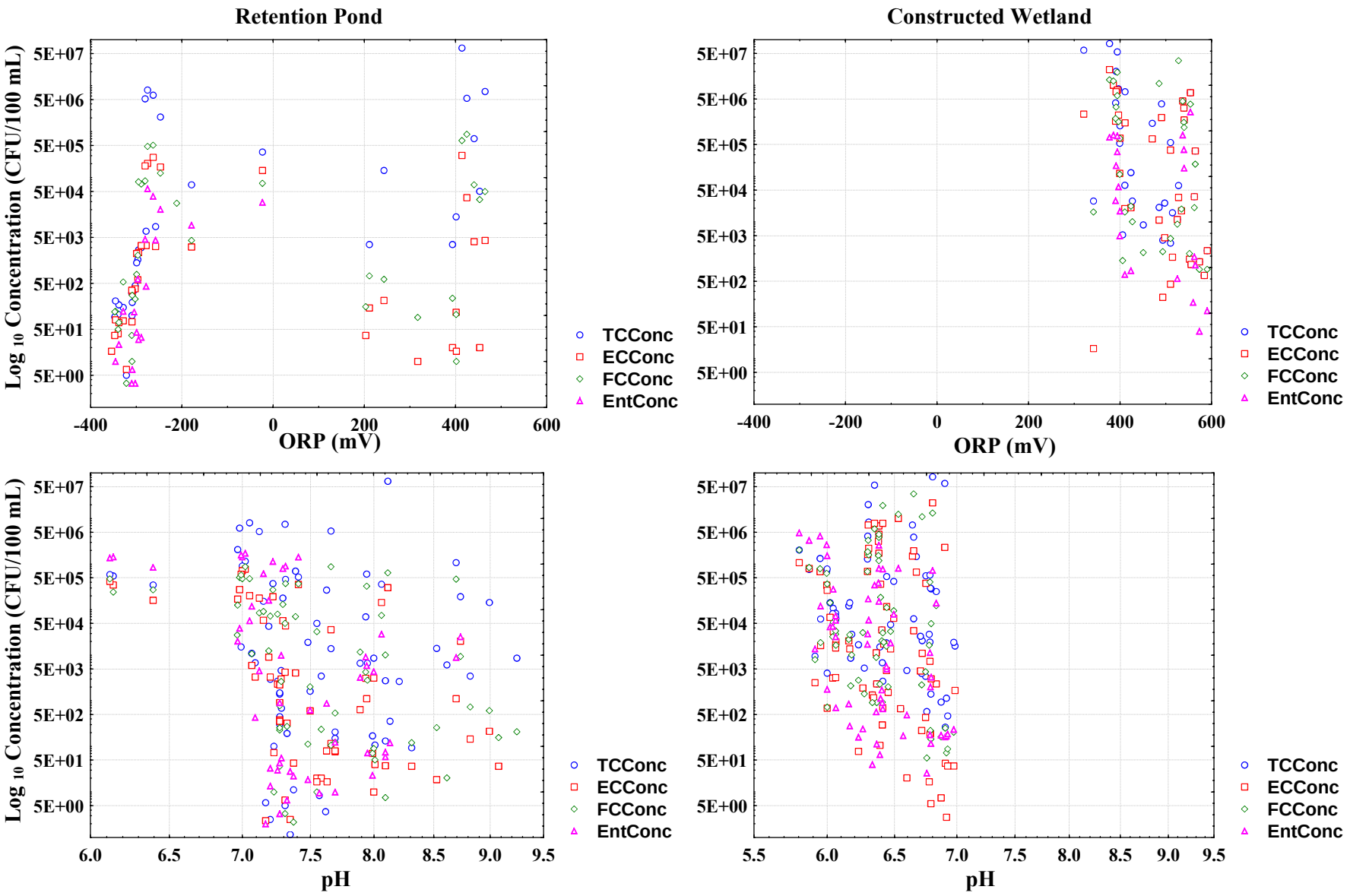


Figure 4-4. Effluent concentrations of indicator organisms with ORP and pH.

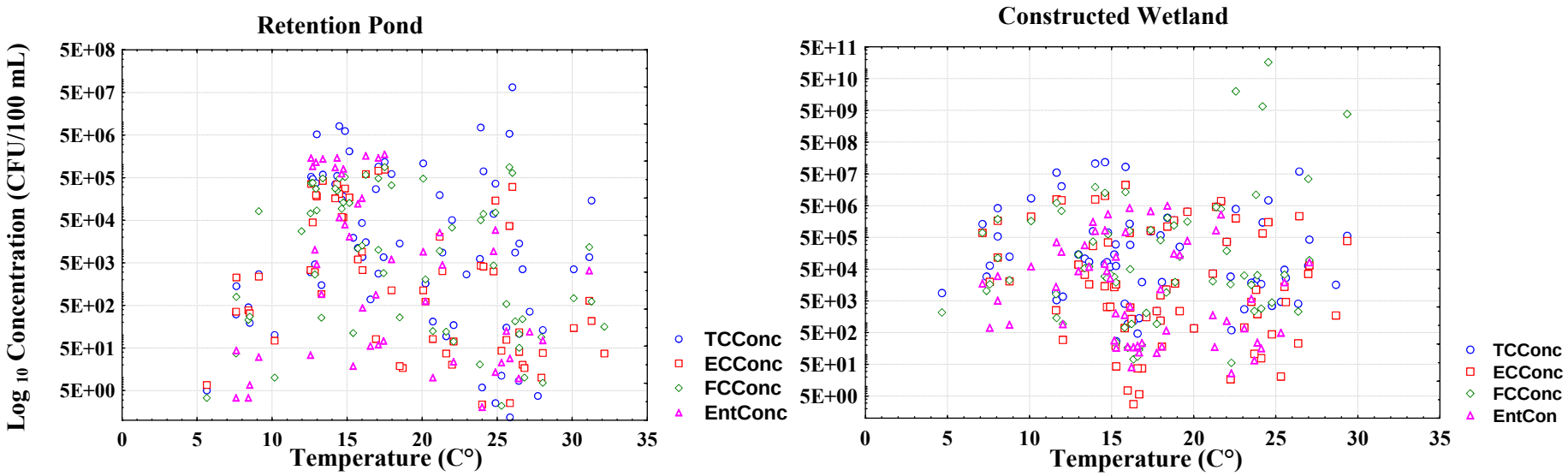


Figure 4-5. Effluent concentrations of indicator organisms with temperature

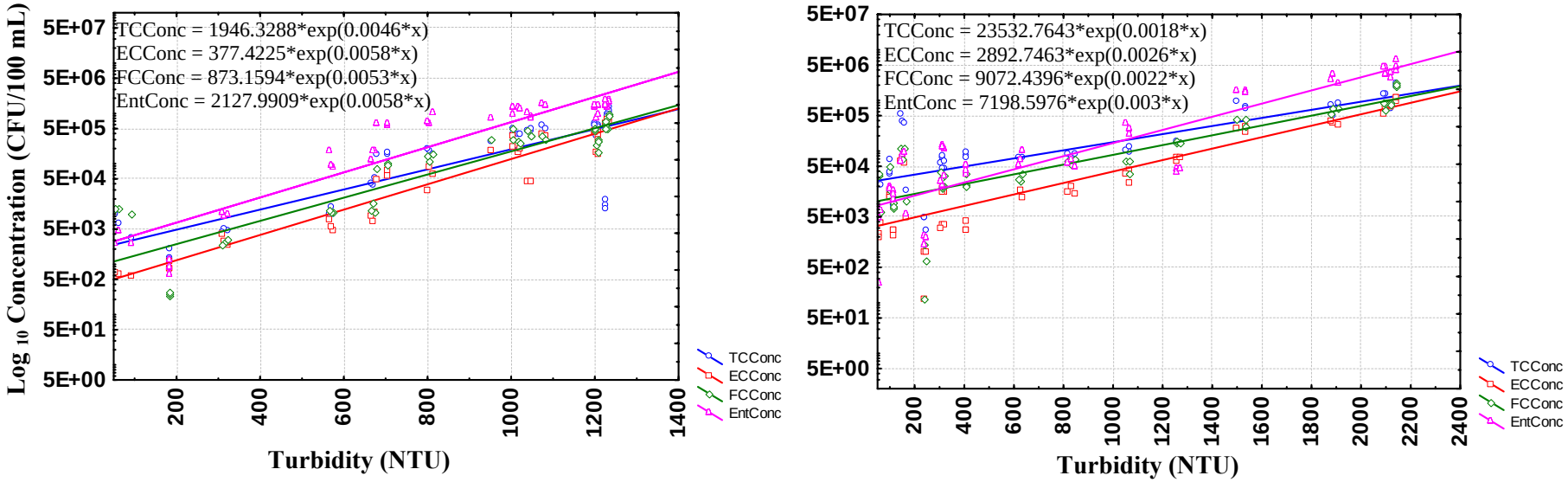
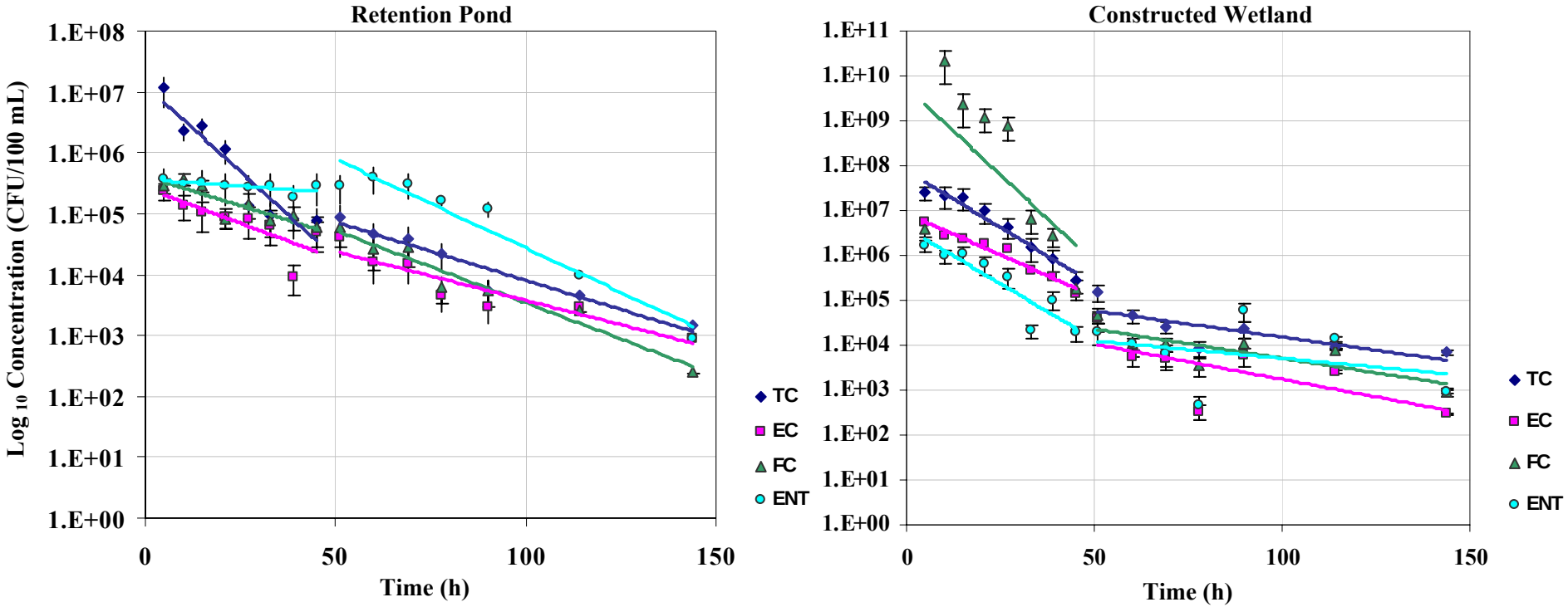


Figure 4-6. Effluent indicator bacteria concentrations with *in-situ* turbidity.



Organism	$K_{T\ 0\text{-}50hr}$ (h^{-1})	R^2	$K_{T\ 50\text{-}150hr}$ (h^{-1})	R^2
TC	-0.1308	0.895	-0.0044	0.937
EC	-0.0534	0.628	-0.0369	0.876
FC	-0.0440	0.464	0.0547	0.956
ENT	-0.0044	0.765	-0.0672	0.937

Organism	$K_{T\ 0\text{-}50hr}$ (h^{-1})	R^2	$K_{T\ 50\text{-}150hr}$ (h^{-1})	R^2
TC	-0.1164	0.959	-0.0270	0.636
EC	-0.0853	0.960	-0.0361	0.475
FC	-0.1797	0.383	-0.0298	0.664
ENT	-0.1142	0.824	-0.0180	0.116

Figure 4-7. Indicator organism concentrations with time. Regressions fits are for time = 0-50 h and 50-150 h. Regression coefficients (k-values) of the exponent (slope) are shown in the tables.

Table 4-6. In-situ Indicator Organisms Average Background Concentrations

Indicator Organism	Background Concentration (CFU/100 mL ± Standard Error)	
	Retention Pond	Constructed Wetland
Total Coliforms	$1.39x10^4 \pm 3.85x10^3$	$3.37x10^4 \pm 4.04x10^3$
E. coli	$6.42x10^0 \pm 7.22x10^0$	$2.55x10^1 \pm 6.21x10^0$
Fecal Coliforms	$1.02x10^4 \pm 2.55x10^3$	$8.09x10^3 \pm 1.12x10^3$
Enterococci	$3.70x10^1 \pm 8.29x10^0$	$2.01x10^1 \pm 5.46x10^0$

Bacteria indicator organisms present in the sediments have the potential to be resuspended in the water column with turbulent flow or disturbance, and may contribute to maintained or increased effluent bacteria indicator organism concentrations in the future.

Predation

Table 4-8 shows the groups of organisms identified in the samples. The average number of organisms enumerated (invertebrate density) in the constructed wetland was 777 and in the retention pond was 442 organisms. However, due to the high variance in the samples, this difference was not significant.

The difference in the number of organisms present (invertebrate taxa richness) was significant between the constructed wetland and retention pond, averaging 15.75 organisms and 7.5 organisms, respectively. These groups often had more than one species in a taxonomic group represented in each system as shown in parenthesis. Ostrocods were not speciated below this subclass.

Table 4-7. Sediment Bacteria Indicator Organisms Sampled in November of 2004

Indicator Organism	Indicator Organism Concentrations BEFORE Stormwater Loading (MPN)	Indicator Organism Concentrations AFTER Stormwater Loading (MPN)
	Retention Pond	
Total Coliforms	$2.25x10^4$	$1.94x10^5$
E. coli	<MDL	$7.41x10^4$
Enterococci	$1.62x10^4$	$1.88x10^3$
	Constructed Wetland	
Total Coliforms	$3.70x10^4$	$>2.41x10^5$
E. coli	$2.37x10^4$	$>2.41x10^5$
Enterococci	$2.02x10^5$	$8.67x10^3$

Table 4-7. Macroinvertebrate Groups Identified in the Retention Pond and Constructed Wetland

Taxa	Retention Pond	Constructed Wetland
Oligochaetae	1	231 (3)
Chironomidae	0	12
Cladocerans	1479 (3)	190 (2)
Coleoptera	0	9
Collembola	0	1591 (2)
Copepoda	22 (2)	269 (2)
Ephemeroptera	42	0
Hemiptera	0	8 (2)
Hydracnidia	0	9 (2)
Ostrocoda	186	782
Rotifera	39	1

*Parentheses indicate the number of taxa identified in that group

Cladocerans were the dominant species present both in richness (3 species) and in density (83%) in the retention pond. Ostrocods (11%), ephemeroptera (2%), rotifers (2%), copepods (1%), and oligochaetes (<1%) made up the rest of the composition within the retention pond. The dominant invertebrate species in the constructed wetlands were collembola, ostrocods, copepods, and oligochaetes composing 51%, 25%, 9%, and 7%, respectively.

Scaling Consideration

Mesocosms have a history of use as a research tool for ecological studies of aquatic and terrestrial ecosystems (Grice and Reeve, 1982; Odum, 1984; Lalli, 1990; Adey and Loveland, 1991; Beyers and Odum, 1993; Kangas and Adey, 1996). They have been used in commercial scale applications, such as in wastewater treatment, food production (Kangas and Adey, 1996), and in ecosystem restoration (Callaway *et al*., 1997). Use of mesocosms, particularly in wetland science, has become more common as a research tool for use in studies of the fate and effect of pollutants, biogeochemical cycles, and the effects of nutrients on ecosystem dynamics.

A given condition when considering comparing mesocosms natural ecosystems is that ecological complexity is to some degree reduced or lost in microcosm or mesocosm studies depending on the size of the mesocosms being used relative to large ecosystem-scale research. Scale can change nutrient cycling, the number of trophic levels, number of species within trophic levels, habitat types, and connectivity between habitats (Beyers and Odum, 1993). Because of this, some caution needs to be used when extrapolating mesocosm results to larger systems. Once models created using mesocosms are validated in the field, application of model results at a larger scale can be made.

Summary

The results highlight the varying influence environmental factors have on the inactivation of indicator bacteria routed into constructed wetland and retention pond systems. The differences between these treatment systems and light and dark controls are statistically measurable implying some variables may have a greater influence than others. A detailed discussion of these results and their relation to the bench-scale study are considered in Chapter 5.

Chapter 5 Discussion of Results and Conclusions

Microbial contamination from fecal origins in stormwater runoff poses a risk to human health through the consumption of drinking water and recreational and bathing contact with surface waters. Indicator bacteria serve as the regulatory meter by which water quality is measured and standards must be met. This chapter discusses the results of the bench and pilot-scale studies to evaluate the use of the first-order decay function for predicting indicator bacteria concentrations in BMP effluent. It compares the two studies to determine similarities and differences in inactivation rate constants, coefficients, and affects of environmental conditions on bacterial indicators.

A primary factor affecting indicator bacteria, often understated in microorganism studies, is time. Time is incorporated into every inactivation rate. By definition the inactivation rate is the change in concentrations of indicator bacteria (presumably decrease) for a designated period. In both the bench- and pilot-scale experiments, time was always a significant variable when evaluating the parameters affecting indicator bacteria concentrations. Many types of BMPs increase the storage time and decrease flow velocities as a primary mechanism of operation.

Results from the bench-scale (Chapter 3) and pilot-scale (Chapter 4) studies show environmental conditions affect indicator bacteria concentrations in retention ponds and constructed wetlands. Temperature, sunlight, and salinity, were investigated in these studies. Other environmental factors typical of constructed wetlands and retention ponds were also considered.

Effects of Temperature

Generally, the results from the bench-scale and pilot-scale experiments agree with the literature (e.g., Easton *et al.*, 2005; Ferguson *et al.*, 2003; Geldreich *et al.*, 1968; Medema *et al.*, 1997; and Canteras *et al.*, 1995) emphasizing temperature generally demonstrates an increase in the calculated inactivation rate constant with increasing temperature. Similarly, inactivation rates are lower at lower temperatures. This trend was most notable in the pilot-study during the October 2005 sampling event. Selvakumar *et al.* (2004) noted that concentrations of organisms did not change significantly when the samples were stored at 4^{o}C beyond the standard holding time of 24 h. Geldreich *et al.* (1968) noted that organism persistence remained at higher levels at 10^{o}C compared to 20^{o}C. In the pilot-scale experiment the optimal temperature range for growth (as indicated by overall indicator bacteria concentrations) was similar to values reported in the literature with indicator concentrations increasing with temperature, reaching a maximum concentration from 20^{o}-25^{o}C in both the retention pond and constructed wetland. Medema *et al.* (1997) found that inactivation was faster at 15^{o}C than at 5^{o}C. Canteras *et al.* (1995) noted a clear positive correlation between inactivation and temperature. In their study, when test conditions were at 10^{o}C, 36 h was necessary to reduce the population of *E. coli* to 10% of the original as opposed to 8.4 h at

42°C. Greater inactivation was also noticed in the range between 10 and 18°C than between 18 and 42°C.

The pilot-scale study found indicator organism concentrations were much greater over the first 50 h as compared to the following 100 h. Although over a longer period of time, Easton *et al.* (2005) reported that the 0-7 day inactivation rates were much larger than the 7-21 day rates. It may be possible that during the 0-7 days studied by Easton *et al.* (2005) there have been varying rates of die-off. In the pilot-scale study, by increasing the temporal resolution of the first 150 h compared to seven days (168 h), the changes in bacteria inactivation could be more easily observed.

Effect of Sunlight/Light Intensity

Many studies have shown that sunlight is an important factor in bacteria indicator inactivation (Sinton *et al.*, 1994; Canteras *et al.*, 1995). The bench-scale study supported this by showing that the effect of light on the overall decay coefficient was substantial, especially for non-coliform bacteria. If, for example, ambient light levels are 100 mW/cm², then the light increases the *E. coli* inactivation rate constant by 0.25 $h^{-1,}$ which is a six-fold increase over the dark value. Similarly, the pilot-scale study shows statistically lower inactivation rate constants in the dark control compared to the light control for total coliforms and *E. coli* for the months of May and June (Table 4-5). According to Table 4-4, June had the greatest irradiance of any of the dates sampled while May ranked fourth in light intensity (because of cloudy conditions during the experiment). Enterococci showed the greatest difference in inactivation rate constants between light and dark controls followed by total coliforms and *E .coli*. The primary difference between these controls was the exposure to sunlight. These differences in rate constants, up to 0.12 h^{-1} for enterococci and *E. coli*, are substantial and are also supported by the inactivation rates observed in the bench-scale study for total coliforms, fecal coliforms, fecal streptococci, enterococci, and *E. coli* indicator bacteria exposed to the highest light intensity.

Effects of Sedimentation, Sorption, and Filtration

Sedimentation, sorption, and filtration processes are generally accepted as the dominant mechanism for the removal of solids and other sediment-related stressors such as heavy metals. The settling velocity has been used as an approximation of the overall rate constant due to these factors in stormwater treatment systems (Wong and Geiger, 1997). Because the particle settling velocities are related to the grading, shape, and density of the particles entering the system, settling velocities measured in the laboratory can only serve as an indicator of the rate constant for sedimentation. Other environmental factors such as non-ideal flow conditions would be expected to increase solids in the water column through resuspension, while some posit that higher density vegetation can increase the rate of the settling constant (Wong and Geiger, 1997).

The pilot-scale experiment compared the effluent concentration of a constructed wetland and a retention pond. While treatment volume was quite different between the two systems, the major difference between these systems was the presence of vegetation. Results comparing the TSS and turbidity in the two systems indicate that the constructed wetland and retention pond showed little difference in turbidity and effluent TSS. However, settling velocity appears to be greater in the constructed wetland under the higher (>100 NTUs) sediment loading observed in October 2005.

The difference in indicator bacteria concentrations and the inactivation rate constants between the constructed wetland and retention pond in our study support settling as a contributing but not primary factor in bacterial inactivation. With the overall differences in turbidity and TSS between the constructed wetland and retention pond, relatively small for most of the simulated storm events (Figure 4-2), it is probable a large portion of the influent may have been unassociated (free) or associated with very fine particles. Similarly, the effects of settling may be artificially small as an artifact of the manner in which

the enriched stormwater is created (described in Chapter 4). The smaller particles would result in a longer time necessary to settle in these systems and was likely to occur within the duration of the experiment. Another possibility is that the increased solids characterized in the influent (especially for October 2005) may reduce the effect of other environmental variables. Increased particulates may occlude light penetration or prevent predation by bacteriovores through limiting access and harboring the indicator bacteria that are agglomerated to these solids.

Wong and Geiger (1997) suggest, when selecting an appropriate *K* value for sedimentation, filtration, and sorption using the settling velocity of the fiftieth percentile sediment grade with adjustments for increased effectiveness for wetlands having higher vegetation density. However, as experienced in this study, this may not adequately predict the effluent concentrations of stormwater runoff passing through passive treatment systems if bacteria are either unassociated with settleable particles or if they are associated with the fine particle fraction, i.e., less than 2 um in size (Davies and Bavor, 2000).

Effect of Salinity

Salinity was only assessed in the bench-scale study and was not included in the pilot-scale study. The bench-scale study results indicate that different organisms exhibited different trends at varying salinity concentrations. Overall, the effect of increased salinity at the tested concentrations was small. The calculated value of Φ_S was not generally significant from non-saline controls. This suggests that, for the span of salinity values studied, the added salt has little effect on the inactivation rate constant and supports the results reported by Canteras *et al.* (1995) who found the largest salinity effect occurs when the salinity values were over 35 ppt. Thoman and Mueller (1987) reported that the inactivation of fecal coliforms is generally much faster in marine and estuarine waters than in freshwater. Mancini (1978) indicated that components in seawater in addition to salt may be responsible for inactivation in seawater.

Salinity is less often a factor in most BMPs but it is a consideration when stormwater controls are to be placed in the coastal and estuarine environments, or when a BMP receives runoff from areas treated with road salts.

Effect of Predation

Previous research has suggested bacterivory can significantly reduce indicator bacteria organism concentrations (Green *et al.*, 1997; Mandi *et al.*, 1993; Decamp and Warren, 1998; Pretorius, 1962; Fernandez *et al.,* 1992a; and Troussellier *et al.,* 1986). There are a variety of invertebrates present in the constructed wetland and retention ponds in this study. While the retention pond and constructed wetland had different taxa represented in each of these systems, dominant invertebrates in both systems have been shown to consume large quantities of indicator bacteria depending on the population size of both predators and prey. The major difference in species richness between the systems was the retention pond was dominated by cladocerans while the constructed wetland had populations of oligochaete, collembola, copepod, and ostrocod invertebrates. The difference in predatory effects of the dominant species on indicator bacteria concentrations in each system is not known. Characteristics of the constructed wetland and retention pond do suggest why there may be different invertebrate communities between the different systems. Constructed wetlands generally had taxa that are associated with greater organic matter (derived from the macrophytic vegetation) (i.e., oligochaetes, collembola, copepods, and ostrocods) (Peckarsky *et al.*, 1990). Collembola and ostrocods are reported to feed on detritus algae, fungi, and dead animal matter with collembolan having special mouthparts for consuming the surface film or underlying bacterial populations (Peckarsky *et al.,* 1990). Therefore, their numerical importance in the constructed wetlands was not surprising. Cladocerans and copepods can affect bacterial populations in both wetlands and open water systems. Both taxa have been shown to consume greater than 25% of the bacterial populations in near shore areas of lakes (Heath *et al.*, 1999). The association of collembolans with detrital organic

matter and cladocerans with more open water habitat may explain the relative densities of these invertebrates in both the retention pond and constructed wetland environments.

The identification and enumeration of the taxa did not provide quantifiable results to determine the predatory contribution of invertebrates on indicator bacteria concentrations. However it does provide anecdotal evidence that invertebrates may contribute to the reduction of indicator bacteria in natural systems. The pilot-scale study, while not directly measuring bacteria indicator inactivation rates due to predation, included the overall effects of predation by incorporating this effect into the cumulative inactivation rate as discussed in the section addressing collective environmental factors below.

Effect of Other Potential Factors

The many other factors (i.e., DO, pH, conductivity, oxidation reduction potential) that can contribute to inactivation rates of indicator bacteria were not directly assessed in this report. The bench-scale study addressed DO in a peripheral manner while the pilot-scale study did not address other potential factors individually but grouped their effects into the overall inactivation rates discussed as collective environmental factors in the next section.

Inactivation Rates Due to Collective Environmental Factors

The data on inactivation rates for microorganisms in stormwater and effects of natural factors on survival rates are limited except for one study by Geldreich *et al.* (1968). They reported a decay rate of 0.061 h^{-1} at 20^{o}C for fecal coliforms. The inactivation rate constant of fecal coliforms at 20^{o}C obtained in the bench-scale study (0.047±0.031 h^{-1}) was similar to Geldreich *et al.* (1968) and the 0-50 h and 50-150 h rates for fecal coliforms observed in the retention pond in the pilot-scale study.

The bench-scale results observed showed a good relationship with the first-order equation. In this portion of the study, total coliforms had a much slower inactivation rate than other indicator organisms. Traditional indicators (total coliforms and fecal coliforms) had lower inactivation rates than the alternate indicators (*E. coli* and enterococci) suggesting that use of traditional indicators may tend to predict higher concentrations compared to the alternate indicators. Depending on the stressor(s) for which the BMP is designed, this could affect the necessary retention time calculated when designing BMPs.

When correlation analysis was done for chemical and physical parameters with overall inactivation rates in the pilot-scale study, conductivity with *E. coli* and enterococci with DO were significantly correlated in the retention pond. The constructed wetland had no significant physical or chemical correlations with inactivation rates. Conductivity of the *in situ* water may be a surrogate for total dissolved solids. However, standard methods suggest the relationship is not constant (APHA *et al.*, 1998). The relationship between total dissolved solids and conductivity is a function of the type and nature of the dissolved cations and anions in the water (i.e., the ability of the water to carry a charge). Some total dissolved solids measuring devices measure the conductivity of the water with the assumption that the primary dissolved minerals are either a combination of NaCl or KCl. Other anions and cations, such as sodium sulfate, sodium bicarbonate, or possibly some organic molecules with ionic and cationic charges can contribute to the conductivity in water samples suggesting total dissolved solids, while not directly measured in the experiment, may be correlated with *E. coli* concentrations in the retention pond if other mineral or organic compounds are present.

The pilot-scale study generally followed the first-order rate equation. A jackknife relationship showing a different rate constant for the first 50 h compared to longer periods, as in Figure 4-7, was appropriate for some indicator bacteria. This relationship was also observed by Thomann and Mueller (1987) for bacteria

distributions in rivers with resistant strains. In addition, with indicator species concentrations (Figure 4-7) having an average predicted background concentration of 10^1–10^4 organisms/100 mL and *in situ* background concentrations ranging from $6.42x10^0$-$3.37x10^4$ organisms/100 mL, there is reasonable support for changes to the first-order rate equation in wetland and retention pond BMPs. Kadlec and Knight (1996) suggest that because of residual indicator bacteria populations present in wetlands, bacteria removal efficiency is a function of the inflow bacteria concentrations. Removal efficiency typically is higher at high inflow concentrations, but declines to low or negative values when inflow concentrations are lower than the *in situ* bacteria production rates. However, during periods when influent flow rates are turbulent, causing resuspension of the previously settled solids, removal efficiency may not depend on influent concentrations alone. Because these settled sediments are associated with *in situ* bacteria populations, there may be an increase in effluent concentration of indicator bacteria with turbulent or high flow or when sediments are disturbed by other means (i.e., waterfowl, muskrats, etc.) compared to the influent concentration. Similarly, sediment resuspension may be more likely to occur in wetland and retention pond BMPs that are poorly designed, have reached the design life, or are not maintained and may contribute to lower or negative indicator bacteria inactivation rates (and removal efficiencies).

Evaluation of the First-Order Decay Equation

Recalling that one of the primary objectives of this research was to evaluate the first-order decay equation for predicting bacteria indicator concentrations affected by environmental conditions, a bench-scale study to look at selected variables identified in the literature as important to inactivation rates was developed. With the bench-scale information as the primer, the pilot-scale experiments utilized controlled mesocosms to further develop the possible effects of typical environmental conditions (similar to expected field BMP conditions) have on indicator bacteria concentrations in BMP effluent. Both the pilot-scale study and the bench-scale study demonstrated the first-first order decay function adequately models indicator bacteria concentrations in the short term. However, during longer periods, the first-order decay equation may not apply to effluent from these types of BMPs. Literature has reported that the assumptions for a first-order decay function (i.e., steady flow conditions) may seldom be met in studies concerning stormwater runoff in constructed wetlands and retention ponds (Wong and Geiger, 1997). Other researchers have suggested using surface area based models for wetlands constructed for the treatment of wastewater (Kadlec and Knight, 1996). One of these models is known as the *K*-C* model which incorporates a concentration term, C*, that represents the background concentration often present in natural systems. The formula is:

$$\frac{(C_{out} - C^*)}{(C_{in} - C^*)} = e^{-K} \qquad (5\text{-}1)$$

Where: C_{out} = effluent concentration; C_{in} = influent concentration; C^* = background concentration; K = rate constant for the water quality parameter being treated based on time of detention.

However, Wong and Geiger (1997) point out that the stochastic nature of stormwater-related systems introduces significantly different system functions compared to wastewater treatment. These authors formulate a procedure that incorporates the use of the *K*-*C** model and the interaction between the requirements for wetland storage for inflow stochasticity and stormwater treatment.

They recommend an adaptation of the Kadlec and Knight's *K*-*C** model with the formula:

$$C_{out} = C^* + (C_{in} - C^*)e^{-KA/Q} \qquad (5\text{-}2)$$

Where: C_{out} = effluent water quality target; C_{in} = influent event mean concentration; C^* = background concentration; K = rate constant for the water quality parameter being treated; A = constructed wetland or retention pond area; and Q = steady state flow.

It should be noted that equations 5-1 and 5-2 have attempted to incorporate conditions that meet the assumptions for the first-order decay equation or include environmental realities such as background concentrations of indicator organisms (C*) to improve prediction of this stressor. The rate constant *K*, which governs inactivation rate determinations in the first-order decay equation, is the only means of incorporating environmental variables to better predict effluent concentrations in surface water models. The bench and pilot studies discussed in this report have estimated inactivation rate constants for indicator bacteria. Further, constant coefficients have also been estimated to predict the effect that environmental factors have on overall indicator bacteria inactivation rates.

Returning to Khatiwada and Polprasert (1999) and Canteras *et al.* (1995) equations from Chapter 3, the following formula for overall inactivation rate constant is proposed:

$$K_{overall} = K_{20}\Phi_{T}^{\,T-20} + \Phi_l I + K_f + K_p \qquad (5\text{-}3)$$

Where: $K_{overall}$ = overall inactivation rate constant; K_{20} = inactivation rate constant due to temperature at 20°C; Φ_T = temperature coefficient; Φ_l = light proportionality coefficient; l = light intensity (mW/cm^2); K_f = inactivation rate constant due to other factors such as sorption, filtration, and sedimentation; and K_p = inactivation rate constant due to predation.

Temperature and light could be quantified through the combination of both studies in this report. Salinity was found to have little effect on bacteria indicators in the constructed wetland and retention pond systems used in this study. Due to the inability to separate sorption, sedimentation, predation, and other environmental factors in the study, substituting the variable K_{other} instead of K_f and K_p to include these (and other) processes in one variable is proposed. As a result, the inactivation rate formula from above can be written as:

$$K_{overall} = K_{20}\Phi_{T}^{\,T-20} + \Phi_l I + K_{other} \qquad (5\text{-}4)$$

Where definitions are as above and K_{other} = inactivation rate constant due to other factors such as sorption, filtration, sedimentation, predation, pH, DO, conductivity, oxidation reduction potential, etc.

Table 5-1 lists coefficients for light and temperature from the bench-scale study and the light and dark controls of the field-scale study. Inactivation rates at 20°C (K_{20}) obtained in the field study are larger than the values obtained from bench-scale experiments except for *E. coli*. Temperature coefficients (Φ_T) obtained from both bench- and pilot-scale studies are not statistically significant different. Light proportionality coefficients (Φ_l) obtained from pilot-scale study are much larger than the values obtained from bench-scale study supporting the conclusion that natural sunlight has a much larger effect on inactivation rates compared to artificially induced light.

Using the light and dark control inactivation rates, the inactivation rate due to other parameters was calculated as the measured $K_{light\ +temperature}$ value. Subtracting the $K_{temperature}$ value from the $K_{light\ +temperature}$, results in a calculated K_{light} value. The K_{other} value was then calculated by subtracting K_{light} and $K_{temperature}$ from the $K_{overall}$ value that was measured for the retention pond and constructed wetland. All K values for the retention pond and constructed wetlands can be found in Tables 5-2 and 5-3, respectively.

Table 5-1. Inactivation Rate Coefficients from Batch and Field Studies

Indicator Organism	Batch Study			Field Study		
	K_{20} (h^{-1})	Φ_T	Φ_L (cm^2/ mW-h)	K_{20} (h^{-1})	Φ_T	Φ_L (cm^2/ mW-h)
TC	0.016±0.009*	1.057±0.085*	0.0016	0.066±0.007*	1.005±0.011	0.0092
FC	0.042±0.030*	1.090±0.110*	0.0130	0.053±0.006*	1.017±0.004*	0.0047
EC	0.036±0.018*	1.023±0.072*	0.0025	0.057±0.008*	1.013±0.002	0.0022
ENT	0.042±0.014*	1.057±0.045*	0.0076	0.054±0.006*	1.050±0.014	0.0070

***** Coefficient is statistically significant at α=0.05.

Table 5-2. Retention Pond Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

Indicator Organism	Month/Year	$K_{overall}$ (measured)	K_{temp} (measured)	K_{light} (calculated)	K_{others} (calculated)
		(h^{-1})			
TC	June 2004	0.242	0.025	0.114	0.103
	September 2004	0.144	0.070*	-0.011*	0.085
	November 2004	0.165*	0.082*	-0.016*	0.010
	May 2005	0.095	0.026	0.042*	0.027
	July 2005	0.181	0.064*	0.008*	0.109
	October 2005	0.044*	0.054*	0.014*	-0.024
	Annual Average	0.145	0.054	0.025	0.052
FC	June 2004	0.181	0.025	-0.001	0.157
	September 2004	0.119	0.053*	0.023*	0.043
	November 2004	0.149*	0.045*	0.024*	0.079
	May 2005	0.142	0.051*	0.033	0.059
	July 2005	0.261	0.062*	0.052*	0.147
	October 2005	0.057*	0.051*	0.031*	-0.025
	Annual Average	0.152	0.048	0.027	0.077
EC	June 2004	0.148	0.028	0.122	-0.002
	September 2004	0.116	0.056*	0.023*	0.038
	November 2004	0.116*	0.048*	0.024*	0.044
	May 2005	0.335*	0.073	0.043	0.219
	July 2005	0.196	0.051*	0.020*	0.125
	October 2005	0.052*	0.061*	0.025*	-0.034
	Annual Average	0.161	0.053	0.043	0.065
ENT	September 2004	0.203	0.077*	0.126*	0.001
	November 2004	0.173*	0.071*	0.108*	-0.006
	May 2005	0.172	0.035*	0.053*	0.083
	July 2005	0.124	0.094*	0.074*	-0.044
	October 2005	0.051	0.019*	0.013	0.019
	Annual Average	0.145	0.059	0.075	0.011

***** Coefficient is statistically significant at α=0.05

The $K_{light\ +temperature}$ value for the constructed wetland was not directly measured but could be calculated. To calculate a the $K_{light\ +temperature}$ value for the constructed wetland the K_{light} value from the light control was multiplied by the weighted average of light intensity expected at the surface of the constructed

wetland. The weighted average was calculated as 10% of the light that reached the retention pond surface (based on light meter measurements) for six hours out of 24 hours of effective light exposure, multiplied by 18 hours out of 24 hours in which the exposure was relatively the same as in the retention pond. This resulted in a multiplication factor of 0.775 •K_{light} of the retention pond. All negative calculated values were assumed to be a propagation of error and are therefore expected to be within the range of error for the respective inactivation rate constant.

Table 5-3. Constructed Wetland Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

Indicator Organism	Month/Year	$K_{overall}$ (measured)	K_{temp} (measured)	K_{light} (calculated)	K_{others} (calculated)
				(h^{-1})	
TC	June 2004	0.153	0.025	0.088	0.040
	September 2004	0.120	0.070*	-0.009*	0.059
	November 2004	0.124*	0.083*	-0.013*	0.054
	May 2005	0.109	0.026	0.033*	0.050
	July 2005	0.073	0.064*	0.006*	0.003
	October 2005	0.043*	0.054*	0.011*	-0.022
	Annual Average	0.104	0.054	0.019	0.031
FC	June 2004	0.328	0.025	-0.001	0.304
	September 2004	0.152	0.053*	0.018*	0.081
	November 2004	0.114*	0.045*	0.019*	0.050
	May 2005	0.123	0.051*	0.025	0.047
	July 2005	0.103	0.062*	0.040*	0.001
	October 2005	0.054*	0.051*	0.024*	-0.021
	Annual Average	0.146	0.048	0.021	0.077
EC	June 2004	0.165	0.028	0.095	0.042
	September 2004	0.120*	0.056*	0.018*	0.046
	November 2004	0.116*	0.048*	0.019*	0.049
	May 2005	0.092*	0.073	0.033	-0.014
	July 2005	0.189*	0.051*	0.016*	0.123
	October 2005	0.060*	0.061*	0.019*	-0.020
	Annual Average	0.124	0.053	0.033	0.038
ENT	September 2004	0.179*	0.077*	0.098*	0.004
	November 2004	0.125*	0.071*	0.084*	-0.030
	May 2005	0.085*	0.035*	0.041*	0.009
	July 2005	0.211*	0.094*	0.057*	0.060
	October 2005	0.059*	0.019*	0.010	0.030
	Annual Average	0.132	0.059	0.058	0.015

***** Coefficient is statistically significant at α=0.05

Inactivation rate constants vary throughout the year based on different affects of the environmental factors. In general, the combination of other factors had the greatest effect on inactivation rates in the retention pond for the indicator bacteria evaluated in this study. Enterococci were an exception to this. Temperature was the found to be more important than light, however light is still a significant factor and should be considered when using the first-order equation. In the constructed wetland, temperature had the greatest effect on inactivation rates for the selected indicator bacteria. Other factors had a greater influence on inactivation rates for all organisms except for enterococci, where light appeared to be as

important as temperature. Inactivation rates for fecal coliforms were most affected by other factors followed by temperature and then light.

Application of the inactivation rate constants found in Tables 5-2 and 5-3 can provide an overall inactivation rate constant incorporating temperature, light intensity, and a lumped factor for other environmental variables. The overall inactivation rate constant can be applied to equation 5-2 to determine the required area necessary to achieve WQSs when designing constructed wetland or retention ponds. If a background concentration is determined, the overall rate constant can also be applied to equations 5-1 and 5-2 to predict effluent concentrations. The first-order decay equation is most accurate when used with the inactivation rates and uncertainties in short-term models to predict stormwater runoff effluent quality from constructed wetland and retention pond BMPs to improve or prevent further degradation of water quality. Longer-term modeling would benefit from applying separate inactivation rates for periods immediately following stormwater runoff and periods unaffected by stormwater runoff.

Conclusions

The studies in this report demonstrated that the concentration of the tested indicator organisms decrease exponentially with time. The first-order decay process reasonably models the concentration time series for shorter durations. The first-order decay model is a simple and efficient means of predicting indicator bacteria concentrations in stormwater runoff effluent from BMPs such as retention ponds and constructed wetlands. Results from the studies discussed in this report provide new data on inactivation rate constants coefficients, and uncertainties used in this equation. The factors of light, time, and temperature influence processes in all retention ponds and wetlands constructed to mitigate the effects of stormwater runoff on the receiving waters. A combination of other factors (e.g., predation, sedimentation, sorption, filtration, pH, BOD, pH, and DO) can also contribute to the inactivation of indicator bacteria in constructed wetland and retention pond BMPs. Reliable rates, coefficients, and the uncertainties expected in the reported values will add to the accuracy of surface water quality models. Water quality models are a primary tool for evaluating permit applications (e.g., NPDES) and have an important regulatory role in developing TMDL allocations. These models should incorporate the affects of BMPs to better model their potential for improving water quality in the watershed. The incorporation of simple reliable models is an important step in assuring that the models used in determining bacterial TMDL loading and allocations meet the state of the science.

BMPs were originally designed to control runoff volumes and rates by attenuating the flow. The attenuation increases the time between the rainfall-generated runoff and the water reaching the receiving water. The time lag serves to reduce the concentration of these indicator organisms. Structural BMPs then can be effective in reducing indicator bacteria concentrations contained in stormwater runoff. Low inactivation rates may occur in BMPs where inflow bacterial concentrations are lower than the *in situ* bacteria productions rates, or turbulent flow through the BMPs causes resuspension of sediments.

Quantitative microbial partitioning estimates can represent critical inputs in areas where sedimentation is a primary mode of indicator organism inactivation when modeling the location and severity of impaired waters. The lack of reliable partitioning information currently leads most surface water modeling efforts to assume that microbes exist in the free phase. The presumption of only free-phase organisms biases model results to greater dispersion and shorter microbial longevity. However, from the results obtained from this project, factors such as temperature and light intensity have been shown to be as, or more important to, indicator bacteria inactivation rates. This would suggest that when attempting to mitigate bacteria in runoff, watershed managers should construct BMPs to maximize the temperature increase from solar exposure. Similarly, the added effects of light, even at constant temperature, can increase inactivation rates, improving BMP performance. The extent to which shading in constructed wetlands, due to vegetation or the deeper water of retention ponds, attenuates the effect of incident light will vary

with runoff and *in situ* water properties (e.g., turbidity, color) in the BMP. It is also important to recognize that bacteria loading seldom acts as a single environmental stressor of concern. The watershed manager must consider the effects of the increased effluent temperature on the receiving waters, particularly when the receiving water is a low-order cold water stream. Also, the results from this study suggest that the regulatory indicator selected can influence BMP design. The apparent insensitivity of coliforms to light levels suggests that the shading effects may be reduced when this is selected as the water quality indicator. When the monitored indicator organism is *E. coli* or enterococci, the effect of light would be expected to be greater than for coliforms.

It is accepted that placement of appropriate BMPs in watersheds can lead to improvements in receiving water quality by reducing the overall load of pollutants to receiving waters. If watershed managers can reduce microbial loads in waterbodies using the range of possible BMPs, verification of these stormwater management tools will help MS4 Phase I and Phase II communities reduce microbial loadings and meet requirements set out by the TMDL process. Long-term microbial load reductions will improve the overall water quality and could potentially lead to increased consumption of fish and shellfish, increased use of recreational waters, reduced beach closures, and increased protection of source water used as drinking water sources.

The limitations of BMP effectiveness in reducing bacterial loading to WQS must be recognized. In most natural treatment systems there will be an irreducible concentration that is often maintained in system sediments. Dilution of BMP effluent likely plays a significant role in attaining WQS in receiving water. However, elimination of bacteria indicators may require chemical treatment. In addition, overall effectiveness and efficiencies of BMPs hinge on proper design and maintenance of these systems.

Chapter 6 References

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Appendix A Growth of Indicator Bacteria for Pilot-Scale Research

Introduction

To overcome the stochastic nature in stormwater runoff, researchers often employ the use of "synthetic stormwater", a laboratory-produced stormwater matrix of known pollutant concentrations, in their research. Literature indicates synthetic stormwater has been used most often for evaluating BMP performance for the removal of heavy metals and total organic carbon (TOC) (Driscoll *et al.*, 1990; Liu *et al.* 2001; Liu *et al.* 2001) but has also been used in nutrient (nitrogen and phosphorus) (Davis et al., 1998; Kim *et al*, 2003), chemical oxygen demand (potassium salt), and total organic carbon (motor oil) (Fassman and Yu, 2001; Hong *et al.* 2006) studies, as well. However, the authors could not find any wet weather flow studies that used bacterial indicator-based synthetic stormwater to evaluate the performance of BMPs. Consequently, the researchers developed a method for creating a stormwater source that has a known concentration of common indicator organisms (i.e., total coliforms, fecal coliforms, *E. coli*, and Enterococci) and contains genotypic representation (assumed, not measured) typical of bacterial indicators found in urban stormwater for use in evaluating pilot-scale stormwater BMPs.

Methods

Stormwater runoff from the Middlesex County College (9.75 acres) campus was collected in a 11,300 L tank from an outfall near the Urban Watershed Research Facility (UWRF) in Edison, NJ and stored on site (Figure 3-1). After thorough mixing, a 60 mL aliquot of stormwater was collected for each sampling event with 30 mL each placed in a flask containing 1L of tryptic soy (TS) broth (for coliforms and *E. coli*) and 1L of brain heart infusion (BHI) broth (for enterococci). A stir bar was added to each flask. The TS broth flask was then placed on a stir plate in a 37ºC incubator while the BHI flask was placed on a stir plate in a 41ºC incubator. Concentrations of bacteria in the inoculated stormwater (broth-stormwater mixture) were measured daily via membrane filtration using Standard Methods 9222B, 9222D, 9222G, and 9230C as described in Chapter 3. Methods for positive controls followed manufacturer's specifications (Becton Dickinson and Company, Sparks, MD).

Trend analysis of the concentration results from five- and seven-day sampling periods determined the growth curves of indicator bacteria populations. Regression analyses of the growth phases for each organism were used to determine the rate of growth. Rate of growth, in general, was grouped by days of positive growth versus days of no or negative growth. Results allowed for future prediction of bacterial concentrations so that experimental use of indicator bacteria could occur at the time of greatest bacterial concentration. A measured volume of inoculated stormwater could then be combined with the desired

volume of original stormwater runoff for use in BMP performance studies. The inoculated aliquot and stormwater was mixed, forming the "synthetic stormwater", and distributed to pilot scale BMPs for performance evaluation.

Results and Discussion

Average starting concentrations on day zero of total coliforms, fecal coliforms, *E. coli*, and enterococci were 2.3 x 10^5, 1.7 x 10^4, 5.8 x 10^3, 3.1 x 10^3 CFU/100 mL, respectively. All indicator organism concentrations reached a maximum on day two and then declined (Figure A-2). Enterococci concentrations declined at a much slower rate with day two and day five concentrations very similar (Figure A-2; Table A-1). Starting concentrations of fecal coliform were one order of magnitude lower and *E. coli* and enterococci starting concentrations were two orders of magnitude lower than the concentrations of total coliforms. After the second day all indicator bacteria concentrations except for enterococci were within the same order of magnitude (~10^9 CFU/100 mL). Enterococci concentrations remained greater than 10^9 CFU/100 mL until the seventh (last) day of the experiment.

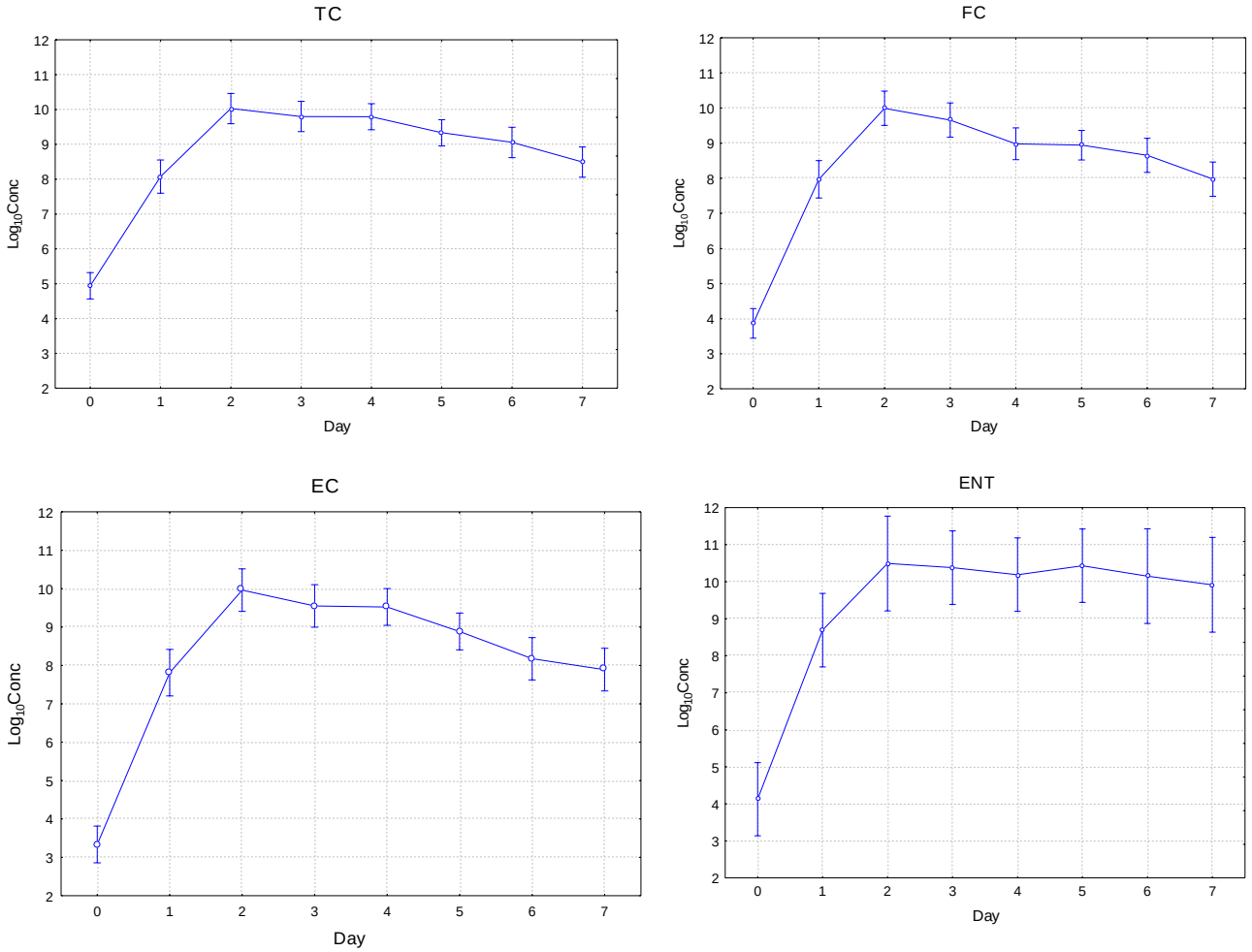

Figure A-1. Mean indicator bacteria concentrations by day. Vertical bars denote 95% confidence intervals.

Table A-1. Growth Rates of Bacterial Indicators Based on Regression Analyses

Indicator Organism	Growth Rate: Days 0-2 (R^2)	Growth Rate: Days 2-7 (R^2)
Total Coliforms	2.565 log (0.922)	-0.297 log (0.903)
Fecal Coliforms	3.106 log (0.916)	-0.373 log (0.877)
E. coli	3.356 log (0.913)	-0.434 log (0.920)
Enterococci	3.321 log (0.704)	-0.087 log (0.823)

Conclusion

The methods for developing indicator bacteria-rich synthetic stormwater in this laboratory experiment can be useful for applications that specifically require stormwater that is characterized by abundant bacterial indicator concentrations. Bacterial loading can be closely predicted using growth rate curves. A wide range of bacterial loading capabilities can be achieved by using this approach to stormwater research. One must recognize that the relative proportion of indicator bacteria will change from the original stormwater and may be less representative of the true microbial community structure. Likewise, the particle association of the indicator bacteria is suspected to be different with less bacteria associated with larger particles and more bacteria associated with finer particles or unattached as opposed to the initial stormwater runoff. However, it is believed that this property results in a more conservative estimate for the indicator organisms when using synthetic stormwater for indicator bacterial loading experiments as fewer colonies settle out of the water column.

Based on indicator bacteria concentrations from the first sample collection after spiking, there is some bacterial die-off that occurs when diluting original stormwater with the inoculated stormwater. A potential cause of this die-off may be thermal shock, although equilibration procedures attempt to minimize the temperature changes between the collected stormwater and the laboratory grown inoculated stormwater.

The synthetic stormwater runoff produced in this project has been effectively used in pilot studies for assessing the performance of small scale controlled stormwater wetland and retention pond BMPs.

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