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EVALUATING PATHOGEN REMOVAL IN STORMWATER BMPS

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Executive Summary

Microbial quality in surface waters is a concern across the United States due to human reliance on surface waters for food, recreation, and other life sustaining activities. Although pathogens are of utmost concern, indicator bacteria are typically used for regulatory purposes to indicate the presence of fecal matter, and thus the possible existence of pathogens. Total Maximum Daily Loads (TMDLs) are established for surface waters impacted by excessive indicator bacteria. Analyses are required to categorize sources of indicator bacteria, and a plan is developed to restore water quality in the impacted water by way of various management/control practices. Stormwater runoff has been shown to have high indicator bacteria concentrations, contributing to microbial degradation in surface waters.

Stormwater runoff is typically managed by implementation of SCMs. Although SCMs have been shown to sequester numerous pollutants, relatively little is known regarding their ability to sequester indicator bacteria. The effectiveness of SCMs in Wilmington, NC, was examined. Differences in performance were noted between SCMs studied in Wilmington, NC, and those studied in Charlotte, NC, by Hathaway et al. (2009). Differences are potentially due to variations in particle association of indicator bacteria between the relatively clayey soils in Charlotte, NC, and the sandy soils in Wilmington, NC. High water tables in Wilmington, NC, likely also influenced results, particularly for wet ponds, where dilution of stormwater runoff due to groundwater intrusion was likely. Although some SCMs showed statistically significant reductions of indicator bacteria ($p < 0.05$), some SCMs appeared to export indicator bacteria. These data suggest SCMs do possess treatment mechanisms which are effective at sequestering indicator bacteria; however, an environment may be present in some SCMs which allows indicator bacteria to persist and/or regrow. In general, bioretention has shown fairly consistent sequestration of indicator bacteria in locations throughout North Carolina. Further, infiltration-based SCMs offer some advantage, as mass removal of indicator bacteria can be realized through infiltration of runoff into subsoils.

Further study is needed to determine the impact of infiltrated stormwater on groundwater systems. Although infiltration of stormwater runoff may lead to reduced export to surface waters, little is known regarding the fate of microbes in groundwater being fed by SCMs. SCMs

constructed similarly and employing similar mechanisms of pollutant removal (wetland, wet ponds, etc.) exhibited varied performance for indicator bacteria in both this study and others in literature. More research is needed to determine why such variability is present. For instance, stormwater wetlands were shown to perform well in Charlotte, NC, but not Wilmington, NC. Although inferences can be made as to the cause behind these differences in performance, a refined understanding of microbial processes in these systems would allow a greater understanding of potential design modifications to SCMs which may lead to improved performance. If no such design modifications are possible, SCMs equipped with different or new treatment mechanisms may be required to treat microbes in stormwater runoff.

For the Wilmington, NC, SCMs, effluent indicator bacteria concentrations were observed to vary seasonally, although no significant relationships could be found ($p < 0.05$). Trends were apparent for both *E. coli* and enterococci for all but one SCM. The geometric mean effluent enterococci concentration was slightly higher during the non-swimming season for one wetland. These data suggest the potential for variations in indicator bacteria export from SCMs during warmer seasons. Such seasonal differences, if present, must also be considered in microbial TMDLs. However, due to the lack of statistically significant relationships identified in these data, additional study is needed to verify the trends identified herein. Additional research should also be focused toward determining if these apparent differences in effluent indicator bacteria concentrations are due to elevated influent concentrations or poorer sequestration of indicator bacteria during warmer months.

A paired watershed study in Wilmington, NC, showed differing performance between two bioretention cells constructed with varied media depth. Differences in function were potentially attributable to numerous factors, including differences in soil temperature, soil moisture, soil chemistry, and soil physical properties. These factors were evaluated, with the only notable differences between the cells being variedmedia depth and a slightly warmer and moister environment in the shallow bioretention area. The differences in temperature and moisture were not considered substantial enough to result in such dramatic differences in performance. Thus, soil media depth was identified as the most likely difference between the two cells leading to differences in indicator bacteria sequestration. This is due to the influence of media depth on hydrology within the system, with a shallow depth leading to higher soil water flux. This leads to

reduced hydraulic contact time, and possibly stripping of bacteria from the soil matrix. For bioretention cells, a minimum soil media depth appears to exist, below which poor sequestration of indicator bacteria may occur due to high soil water flux and low contact time.

Soil water flux appears to be an important consideration during bioretention design. Although an increase in soil media depth is one option for decreasing soil water flux, other design options may provide similar results. A reduction in ponding depth, and subsequent increase in surface area, will also lead to decreased soil water flux. Stormwater may also be slowed in bioretention areas by decreasing infiltration rate. This may be accomplished by increasing the content of fines within bioretention media. Increasing the percentage of fines may also lead to increased sorption of indicator bacteria. Thus, more shallow media depths may be possible provided the soil media utilized has a higher fraction of fine soils (clay and silt). However, such modification to soil media will also result in decreased hydrologic efficiency. Thus, the trade-off between indicator bacteria sequestration and hydrologic efficiency should be carefully considered. These design options should be explored in future research.

Related Documents

This report is a compilation of two manuscripts and a doctoral dissertation which were generated using data collected during the process of this study. Portions of this report may be published in the following documents:

- Hathaway, J.M., and W.F. Hunt. (in review). “Indicator bacteria performance of stormwater control measures in Wilmington, NC” *Journal of Irrigation and Drainage Engineering.*

- Hathaway, J.M., W.F. Hunt, A.K. Graves, and J.D. Wright. (in preparation). “Field evaluation of bioretention sequestration of indicator bacteria in Wilmington, NC” *Journal of Environmental Engineering.*

- Hathaway, J.M. (2010). “An Evaluation of Indicator Bacteria Transport in Stormwater Runoff and Removal in Stormwater Control Measures.” doctoral dissertation, North Carolina State University, Raleigh, NC.

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1 Indicator Bacteria Performance of Stormwater Control Measures in Wilmington, NC

Abstract

Indicator bacteria are a common source of impairment in surface waters in the United States. Urban stormwater runoff has been identified as a contributor to elevated indicator bacteria concentrations. Six Stormwater Control Measures (SCMs) were monitored in Wilmington, NC, for *Escherichia coli* (*E. coli*) and enterococci. Monitored SCMs included two stormwater wet ponds, two bioretention cells, and two stormwater wetlands. Sandier watersheds in Wilmington potentially lead to differences in SCM performance for indicator bacteria compared to SCMs implemented in clayey watersheds. Results showed *E. coli* and enterococci concentration reductions between 70 and 98% for the two wet ponds and a bioretention cell with a 60 cm deep fill media. Other SCMs showed poor removal of indicator bacteria, in some cases negative, with stormwater wetlands performing the poorest overall for the three SCM types. Further analysis showed that SCMs with high concentration reductions tended to have geometric mean effluent concentrations lower than the United States Environmental Protection Agency's (USEPA) target surface water concentration for *E. coli*. Conversely, no SCM had a geometric mean effluent enterococci concentration lower than the USEPA target value. SCM geometric mean effluent concentrations were typically higher during North Carolina's swimming season between the beginning of April and the end of October, although no statistically significant relationship could be found ($p < 0.05$). Despite a lack of statistically significant relationships, the potential for higher effluent indicator bacteria concentrations from SCMs during the peak recreational season may have implications for both public health and watershed management and should be further evaluated by the scientific community.

Introduction

Surface waters in the United States are commonly placed on the Environmental Protection Agency's (USEPA) 303(d) list due to impairment by pathogens (indicator bacteria) (USEPA, 2008). Subsequently, indicator bacteria Total Maximum Daily Loads (TMDLs) have been established for numerous surface waters. Stormwater runoff has been identified as a contributor to indicator bacteria pollution, with indicator bacteria concentrations in urban runoff commonly exceeding USEPA standards for surface waters (Hathaway et al. 2009, Krometis et al. 2009).

Typically, stormwater runoff mitigation involves the use of Stormwater Control Measures (SCMs – also known as Best Management Practices or "BMPs"). SCMs have been shown to effectively reduce numerous types of pollutants, yet their ability to remove indicator bacteria and pathogens is still under evaluation. Studies have indicated variable performance of SCMs for indicator bacteria from storm to storm and based on SCM type (Hathaway et al. 2009, Krometis et al. 2009, Passeport et al. 2009, Li and Davis 2009, Birch et al. 2004, Davies and Bavor 2000, Mallin et al. 2002). Evaluations of indicator bacteria removal in SCMs have typically been performed on data sets with less than 10 samples. Other than Hathaway et al. (2009), studies with more than 10 data points have collected samples at a predetermined time interval (monthly, biweekly, etc), and thus did not isolate SCM performance during storm flow.

Indicator bacteria are of particular concern in coastal areas, where human exposure can occur during recreational activities or consumption of shellfish (USEPA 2001). Such human health concerns have economic implications for the tourism and commercial fishing industries. Despite the need for microbial controls in coastal areas, few evaluations have been performed for stormwater wetlands, wet ponds, and bioretention areas in watersheds with similar characteristics to those of watersheds in the coastal Southeastern United States. In particular, limited data are present with regard to SCM removal and sequestration of enterococci, which is recommended for use as an indicator species in coastal areas and potentially has different survival characteristics than other indicator bacteria species in the environment (USEPA 2001). Only two field studies could be found in scientific literature where either a stormwater wetland or bioretention area was monitored for enterococci sequestration and removal (Davies and Bavor 2000, Jones et al. 2008)

Coastal areas in the Southeastern United States are characterized by sandy soils. This may lead to differences in SCM microbe removal efficiency. For instance, the percentage of incoming microbes attached to sediment may vary from that in clayey watersheds, as microbes predominately attach to smaller particles (Davies and Bavor 2000). Krometis et al. (2009) proposed that particle-microbe association occurs in upland areas, further suggesting that sediment type within the watershed may influence microbial characteristics at the SCM inlet. Also, microbes may persist for longer periods in the environment when associated with particles

(Sherer et al. 1992). Thus, SCMs receiving runoff with a small amount of particle associated bacteria may perform differently than those which receive high amounts of particle associated bacteria. Resuspension of captured microbe-particle colloids may also be possible in SCMs. Microbes have been shown to persist in stream and estuary sediments, where a similar environment to that found in SCMs may be present (Sherer et al. 1992, Jeng et al. 2005).Thus, scour or resuspension of sediments in SCMs during storm events may also resuspend microbes. This is specifically a concern in wet ponds and stormwater wetlands. Larger particles, such as sands, have a greater resistance to resuspension, potentially leading to reduced loss of particle associated bacteria from SCMs in sandy watersheds.

Bioretention is increasingly being used as part of Low Impact Development strategies in coastal areas. Bioretention performance for indicator bacteria has been evaluated primarily for systems constructed with media consisting of some combination of organic matter, fine particles, and sand or expanded slate fines. However, design specifications for bioretention fill media are typically focused on hydraulic efficiency (i.e., infiltration rate). Thus, it is possible that in-situ soils would be used as bioretention fill media in watersheds containing sandy soils. These potential fill soils have not been tested for indicator bacteria removal when used in bioretention designs. Although there has been some field evaluation performed on bioretention areas for indicator species removal by Hathaway et al. (2009), Li and Davis (2009), Dietz and Clausen (2005), and Passeport et al. (2009), no field evaluation has been performed on bioretention for enterococci other than a study in New England by Jones et al. (2008).

Another concern for management of surface waters is the variation observed in stormwater indicator bacteria concentrations based on season and temperature. Hathaway and Hunt (in review), Selvakumar and Borst (2006), and McCarthy et al. (2007) all showed higher indicator bacteria concentrations in stormwater runoff during warm seasons/temperatures. Such conditions coincide with peak recreational use of surface waters. In TMDL guidance provided by the USEPA, seasonal variations must be taken into account for microbial TMDLs (USEPA 2001).

Despite the understanding that indicator bacteria concentrations in stormwater runoff increase during warm periods of the year, little is known about how SCM efficiency or effluent indicator bacteria concentrations vary based on temperature and/or season. A field-monitoring study on

two bioretention areas by Li and Davis (2009) observed the highest influent *Escherichia coli* (*E. coli)* and fecal coliform concentrations during the summer; however, removal efficiency could not be correlated to temperature. There are public health implications for such information, as SCM efficiency may change throughout the year. Thus, watershed plans which apply one indicator bacteria removal percentage to a given SCM (i.e., not adjusted seasonally) may misrepresent the benefit of implementing such SCMs.

The objectives of this study were to build upon the current understanding of indicator removal in SCMs by: (1) evaluating the performance of SCMs implemented in sandy, coastal watersheds for both *E. coli* and enterococci, and (2) evaluating the influence of seasonality on SCM effluent concentrations and removal of indicator bacteria.

Materials and Methods

1.1.1 Site Descriptions

The experimental sites were located in Wilmington, North Carolina (Figure 5.1). Six SCMs were evaluated, including two wet ponds, two bioretention areas, and two stormwater wetlands. Samples were collected between January 2008 and February 2010. General SCM characteristics are given in Table 5.1. Soils in the watersheds contributing to the SCMs were typically either hydrologic group A or B (sands and fine sands).

Wet Pond 1 was located in a residential medium density neighborhood with a watershed area of approximately 7 ha (Figure 5.2a). The wet pond had poor vegetative growth around its perimeter and had a sinuous pathway between the inlet and outlet. Wildlife was not readily observed around the pond, and fencing around the pond likely restricted access from domestic animals. Wet pond 2 serviced a cinema parking lot and the surrounding area (Figure 5.2b). The watershed was approximately 15 ha. The wet pond had minimal vegetative growth along its perimeter and grass was manicured to the pond edge. Water fowl were noted at the site on occasion, but never in large quantity. Wet Pond 2 exhibited submergence at the outlet, leading to increased normal pool depth, but decreased storage depth relative to the design specifications in Table 5.1.

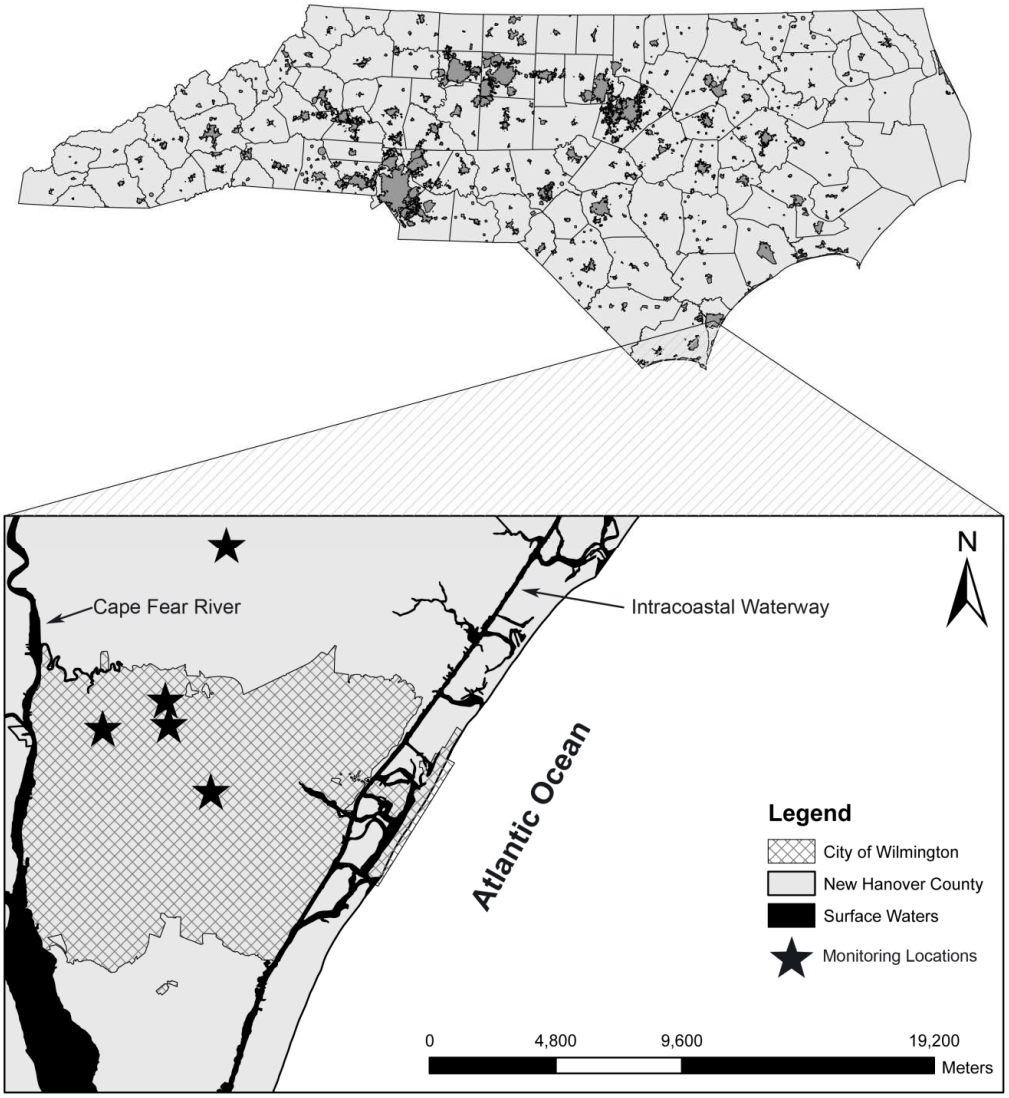


Figure 1.1: Monitoring Locations in Wilmington, NC

Table 1.1: General characteristics of Wilmington SCMs

Characteristic	Wet Pond 1	Wet Pond 2	Bioretention-D	Bioretention-S	Wetland 1	Wetland 2
Approximate Year Constructed	1999	1996	2006	2006	2005	2006
Drainage Area (ha)	7.6	14.8	0.10	0.05	12.7	2
Watershed Composition	Multi Family Residential (primarily duplex lots)	Commercial	Commercial (parking lot)	Commercial (parking lot)	Municipal (school)	Multi-family Residential
Estimated Imperviousness	45%	81%	100%	100%	20 %	42%
Primary Surrounding Soil Type (hydrologic group)[1]	Lynn Haven fine sand (B/D) and Seagate fine sand (B)	Seagate fine sand (B)	Baymeade fine sand (A)	Baymeade fine sand (A)	Leon Sand (B/D)	Baymeade fine sand (A)
Surface Area (ha)	0.18	0.59	0.006	0.006	0.1	0.09
Surface Area: Drainage Area Ratio	0.02	0.04	0.06	0.12	0.01	0.05
Storage Depth (cm)	46	52 (actual lower due backwater in effluent pipe)	28	28	31	> 15 (due to well infiltrating soils)
Estimated Average Depth (cm)	198	168	60[2]	25[2]	7	17 (typically less due to well infiltrating soils)

1. NRCS 2010 – Soil Data Mart (http://soildatamart.nrcs.usda.gov/)
2. Average depth represents soil depth for bioretention cells

(a) **(b)**

(c) **(d)**

(e)

Figure 1.2: Illustrations of SCMs: (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D and Bioretention-S, (d) Wetland 1, and (e) Wetland 2

The two bioretention areas were located within the same parking lot which serviced a coffee shop (Figure 5.2c). A paired watershed design was sought, with each bioretention having a similar footprint, but watershed area differed due to microtopography within the parking lot. One bioretention was constructed with a soil depth of approximately 60 cm (Bioretention-D),

one with a soil depth of 25 cm (Bioretention-S). All fill soil for the bioretention areas came from on site sandy soils. Each cell was constructed with a 10-cm underdrain to facilitate sample collection. It should be noted that underdrains are not typically required for bioretention areas in the sandy soils of coastal areas, thus this design differs from standard practice in the region. Runoff entered each bioretention cell as sheet flow. A small flume was installed at the pavement edge in a location presumed to be representative of the entire watershed. This allowed some pooling of runoff as it entered the bioretention cell, facilitating sampling of the inlet. The bioretention areas were covered with turf grass and had a small number of shrubs.

Wetland 1 serviced a 2-ha watershed consisting of a multi-family residential housing complex (Figure 5.2d). The wetland was constructed in sandy soils and typically had standing water only present in the deep pools. Therefore, the average depth of the system and ponding depth varied from design specifications. Wetland 2 serviced a 13-ha watershed containing a school and associated athletic fields (Figure 5.2e). Both wetlands were designed consistent with guidance by Hunt et al. (2008), including varied internal topography, emergent vegetation, and a design surface area based on capturing the water quality event for Wilmington, NC (3.8 cm). Large storms overflowed both wetlands by large weirs installed at each outlet.

1.1.2 Monitoring Methods

Short hold times and the increased man-hours and technical difficulty of using automatic samplers for microbial analyses led to the use of grab samples for SCM evaluations. This is a common methodology for sampling surface waters for indicator bacteria (USEPA 2002, Burton and Pitt 2002). All wet ponds and wetlands had one defined inlet and outlet. One sample set was collected from the inlet and outlet of each SCM for each storm event. Each sample set consisted of two sterile bottles to facilitate two bacteria analyses (*E. coli* and enterococci). Inlet samples were collected for both bioretention areas from the inlet flume mentioned previously. Outlet samples were collected from each respective bioretention cell's underdrain. There are valid concerns over the use of grab samples, as concentrations of a given pollutant may vary during the course of the storm. However, use of grab samples was necessary in this study, and studies such as McCarthy et al. (2008) have illustrated the uncertainties present in indicator bacteria field monitoring, which potentially overshadow the negative impacts of using single grab samples to some degree.

Samples were transported to Tritest, Inc for analysis. Hold times were generally less than 6 hours. Samples were analyzed for both *E. coli* and enterococci. *E. coli* were enumerated using Colilert® and enterococci were enumerated using Enterolert®. Each methodology is based on the use of a defined substrate media (IDEXX Laboratories Inc., Westbrook, Maine). Sample dilutions were performed as needed to adequately characterize bacteria concentrations. The Limit of Detection (LOD) was typically either 2 or 10 MPN / 100 ml depending on the dilution used. The Maximum Reporting Limit (MRL) was typically 24,196 MPN / 100 ml. The MRL for *E. coli* was typically higher than that of Hathaway et al. (2009), allowing a better overall estimation of functionality. Data are analyzed herein using the values at the reporting limit without adjustment.

1.1.3 Statistical Evaluations

Statistical analyses were made to evaluate the performance of each SCM. Removal percentages (Concentration Reduction "CR") were calculated for each SCM using a similar methodology to that used to generate efficiency ratios (USEPA, 2002); however, event mean concentrations are necessary to generate efficiency ratios. This was not possible due to the use of single grab samples in this study, leading to the use of Equation 1.

$$CR = \left(1 - \frac{Geometric\ Mean\ Outlet\ Concentration}{Geometric\ Mean\ Inlet\ Concentration}\right) \times 100\% \qquad (1)$$

Microbial water quality standards are concentration based. Thus, geometric mean effluent concentrations from each SCM were compared to water quality standards for *E. coli* and enterococci. Based on USEPA recommendations, geometric mean *E. coli* concentrations should not exceed 126 organisms / 100 ml over a 30-day period for fresh water designated as full body recreational waters (USEPA 1986). Similar recommendations exist for enterococci, whereby geometric mean concentrations should not exceed 33 organisms / 100 ml for fresh waters or 35 organisms / 100 ml for marine waters over a 30-day period (USEPA 1986).

A non-parametric Wilcoxon Signed Rank test was used to determine differences among influent and effluent concentrations. Non-parametric analyses also lessen the influence of high and low

concentrations, which is important when data sets contain values below the MDL or above the MRL. These analyses were supplemented with probability plots to evaluate the performance of each SCM over the entire range of influent concentrations. Probability was calculated using Equation 2 (Burton and Pitt 2002).

$$P = \frac{(i - 0.5)}{n} \qquad (2)$$

Where: P = probability of a given observation
i = rank of observation within group n
n = number of observations within a given data set

Additional statistical analyses were performed to evaluate differences in influent and effluent concentrations based on season. Samples were categorized based on the dates considered by the North Carolina Division of Environmental Health (NCDEH) to be the "swimming season" and "non-swimming season" (NCDEH 2010). Swimming season is defined as the period between the beginning of April and the end of October. SCM functionality may hold more importance during swimming season, as water-related recreation increases. Wilcoxon Rank Sum tests were used to statistically evaluate differences in effluent concentrations between swimming and non-swimming seasons. Also, the difference in geometric mean effluent concentrations between the two seasons was calculated using Equation 3.

$$Seasonal\ Difference = \left(1 - \frac{Geometric\ Mean\ Outlet\ Concentration\ (swimming)}{Geometric\ Mean\ Outlet\ Concentration\ (non-swimming)}\right) \times 100\% \qquad (3)$$

Results and Discussion

1.1.4 Summary Statistics

Between 15 and 20 storms were sampled for each SCM between January 2008 and February 2010. Summary statistics for these data are presented in Table 5.2. Samples were fairly well distributed throughout the seasons, with storm sizes ranging from 0.8 to 12.8 cm. The median storm size was 2.7 cm, suggesting the data set was somewhat shifted toward larger storm events. An analysis of historical rainfall data from Wilmington, NC, by Bean (2005) showed 70% of runoff was generated by storm events less than 2.5 cm. Because larger events may create

more scour and decrease detention time in SCMs, these data are likely conservative estimates of SCM function. There is some concern that large storms may dilute indicator species, resulting in decreased influent concentrations; however, McCarthy et al. (2007) and Hathaway and Hunt (in review) showed no significant correlation between indicator bacteria concentrations in urban stormwater runoff and storm size.

Table 1.2: Summary statistics for monitored storm events

Location	Number of Samples	Statistic	*E. coli*		enterococci	
			MPN / 100 ml		MPN / 100 ml	
			inlet	outlet	inlet	outlet
Wet Pond 1	15	geometric mean	2483	62	2356	237
		median	2851	40	2599	168
		maximum	24196	19863	24196	24196
		minimum	255	2	278	2
		standard deviation	6555	5082	8111	6491
Wet Pond 2	18	geometric mean	1273	60	274	37
		median	2489	34	179	20
		maximum	81640	3466	24196	1633
		minimum	10	2	2	2
		standard deviation	19639	843	6218	563
Bioretention-D	20	geometric mean	130	39	375	39
		median	122	10	440	42
		maximum	7701	8164	4839	1454
		minimum	2	2	30	2
		standard deviation	2106	1959	1355	323
Bioretention-S	20	geometric mean	130	284	375	378
		median	122	714	440	358
		maximum	7701	19863	4839	4839
		minimum	2	2	30	20
		standard deviation	2106	5632	1355	1536
Wetland 1	18	geometric mean	834	826	1018	316
		median	741	1167	1097	309
		maximum	14136	36540	24196	29090
		minimum	75	17	61	2
		standard deviation	3833	9870	6135	8437
Wetland 2	18*	geometric mean	425	503	866	510
		median	554	386	842	690
		maximum	9804	24196	24196	24196
		minimum	10	6	140	10
		standard deviation	2516	6335	5428	5827

* 19 enterococci samples collected for Wetland 2

The geometric mean of the influent *E. coli* samples was between 130 and 2483 MPN / 100 ml. This range was similar to that reported for *E. coli* concentrations entering SCMs in Charlotte, NC,

by Hathaway et al. (2009) and influent concentrations for one of two wet ponds studied in Durham, NC, by Krometis et al. (2009). Geometric mean enterococci concentrations ranged from 274 to 2356, slightly higher than influent concentrations for SCMs studied by Jones et al. (2008), but lower than values reported for influent concentrations to wet ponds studied by Krometis et al. (2009).

1.1.5 Concentration Reduction

Concentration reductions for each SCM are documented in Tables 5.3 and 5.4. Highest *E. coli* reductions were observed for the wet ponds, which also had the highest influent concentrations. Bioretention-D also performed well with a concentration reduction of 70%. Poor performance was noted for Bioretention-S and the two wetlands for *E. coli*, although both wetlands had fair removal of enterococci. For each SCM, removal performance was variable from storm to storm. Individual event concentration reductions varied from greater than 90% to an *addition* of both *E. coli* and enterococci for most SCMs. Similar inter-event variations in SCM performance for indicator bacteria were showed for bioretention areas by Li and Davis (2009) and for a stormwater wetland by Birch et al. (2004).

Table 1.3: *E. coli* concentration reductions for Wilmington SCMs

SCM Type	*E. coli* Concentrations (MPN/100ml)		
	Geometric Mean Influent	Geometric Mean Effluent	Concentration Reduction (%)
Wet Pond 1	2483	62	98
Wet Pond 2	1273	60	95
Bioretention-D	130	39	70
Bioretention-S	130	284	-119
Wetland 1	834	826	1
Wetland 2	425	503	-18

Table 1.4: Enterococci concentration reductions for Wilmington SCMs

SCM Type	Enterococci Concentrations (MPN/100ml)		
	Geometric Mean Influent	Geometric Mean Effluent	Concentration Reduction
Wet Pond 1	2356	237	90
Wet Pond 2	274	37	87
Bioretention-D	375	39	89
Bioretention-S	375	378	-1
Wetland 1	1018	316	69
Wetland 2	866	510	41

Results of a Wilcoxon Signed Rank analysis are shown in Table 5.5. Only the two wet ponds significantly reduced *E. coli* ($p < 0.05$), while both wet ponds and Bioretention-D significantly reduced enterococci. Significant relationships can be difficult to find in microbial data sets given the inter-storm performance variability noted in these data and in other studies. Statistical analyses generally support the concentration reductions in Tables 5.3 and 5.4, as wet ponds and Bioretention-D were found to perform well.

Table 1.5: Results of Wilcoxon Signed Rank Analysis

Location	*E.coli*		enterococci	
	p - value	significant difference?	p - value	significant difference?
Wet Pond 1	0.0002	**yes**	0.0134	**yes**
Wet Pond 2	0.001	**yes**	0.001	**yes**
Bioretention-D	0.1926	no	0.0001	**yes**
Bioretention-S	0.0808	no	0.5459	no
Wetland 1	0.6095	no	0.1187	no
Wetland 2	0.6322	no	1	no

Wet ponds have shown varied levels of treatment for indicator bacteria. A study of two wet ponds in Durham, NC, by Krometis et al. (2009) yielded different results. One pond showed poor performance with geometric mean concentration reductions of -41%, 0%, and -108% for fecal coliform, *E. coli,* and enterococci, respectively. The second pond showed modest removal, reducing geometric mean concentrations by 31%, 48%, and 36% for fecal coliform, *E.coli*, and enterococci, respectively. A study by Davies and Bavor (2000) on a wet pond near Sydney, Australia, showed similarly poor performance with fecal coliform and enterococci removal efficiencies of -2.5% and 23%, respectively. In a study by Mallin et al. (2002), 2 of 3 wet ponds in Wilmington, NC, removed fecal coliform with an efficiency higher than 50%, with the third wetland showing negative removal. Positive removal of indicator bacteria was also reported by Hathaway et al. (2009) for a wet pond in Charlotte, NC, with concentration reductions of 70% and 46% for fecal coliform and *E. coli*, respectively. Thus, from a removal efficiency metric, large variations in performance have been noted for wet ponds. Generally, performance for the wet ponds in Wilmington, NC, studied herein was good compared to studies in literature, and compared similarly to 1 of 3 wet ponds studied by Mallin et al. (2002) in Wilmington, NC, where a fecal coliform concentration reduction of over 85% was found. It should be noted that the other two ponds studied by Mallin et al. (2002) had low influent fecal coliform concentrations,

97 organisms / 100 ml and 74 organisms / 100 ml, potentially influencing microbial removal efficiency. Relatively poor performing wet ponds studied by Krometis et al. (2009) and Davies and Bavor (2002) potentially had different influent microbial particle association characteristics than those in Wilmington, NC, as finer soil types were likely present in their contributing watersheds. The soil types in the watersheds supplying runoff to the Wilmington, NC, SCMs were predominately fine sand. This may have implications for the amount of bacteria attached to particles at the inlet and the likelihood of resuspension of captured sediments (and associated bacteria) during subsequent events. Further, particle associated microbes have been shown to exhibit higher resistance to environmental conditions that otherwise cause their die-off (Sherer et al. 2002). Also, a high water table, characteristic of coastal areas in the Southeastern United States, may have resulted in dilution due to groundwater intrusion into the ponds. One of the two wetlands (Wetland 1) also intersected the groundwater table, but an improved performance was not evident.

Stormwater wetland indicator bacteria sequestration and removal has not been studied at length in peer-reviewed literature. A study by Birch et al. (2004) showed mean fecal coliform removal of 76%. Davies and Bavor (2002) reported removal efficiencies of 79% and 85% for fecal coliform and *E. coli*, respectively. However, results for two wetlands in Charlotte, NC, studied by Hathaway et al. (2009) were variable. One wetland exhibited fecal coliform and *E. coli* removal of 98% and 96%, respectively, while the other showed fecal coliform and *E. coli* removal of 56% and 33%, respectively. It should be noted that Hathaway et al. (2009) attributed high removal of indicator bacteria to a lack of vegetation in one of the wetlands, but vegetation deficiency is not a desirable attribute for stormwater wetlands. The results of studies in scientific literature generally indicate fair performance of stormwater wetlands for indicator bacteria. However, data from this research suggests poor performance of stormwater wetlands for *E. coli* removal, and modest performance for enterococci.

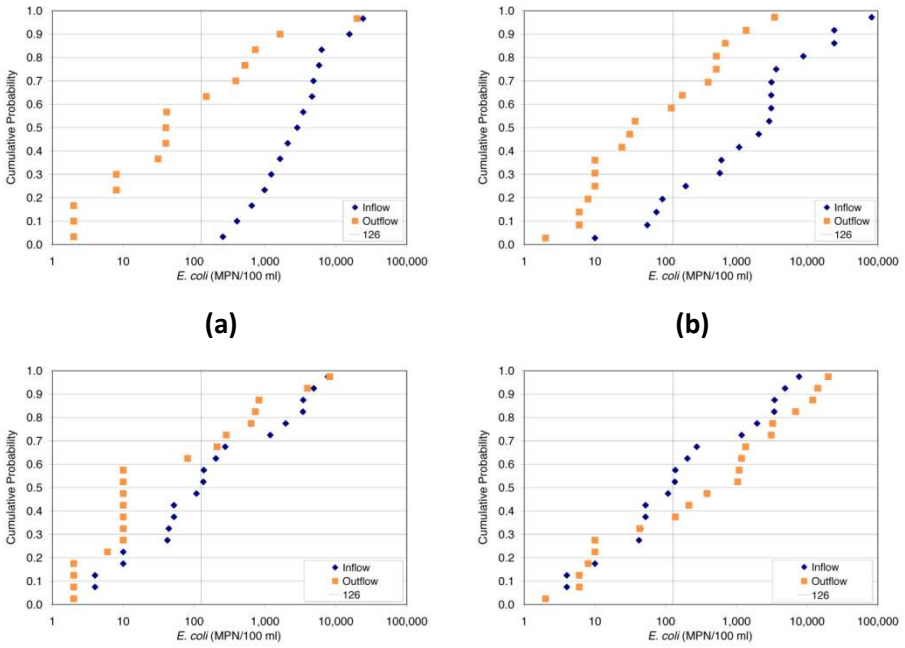
Differences in microbial removal efficiency between stormwater wetlands and wet ponds are not well established. A comparison of stormwater wetland and wet pond performance by Davies and Bavor (2002) indicated that wetlands may be more adept at indicator bacteria removal. However, a review of data from the International Stormwater BMP Database by USEPA (2003) suggested that wet ponds performed better for fecal coliform than wetlands and that data are

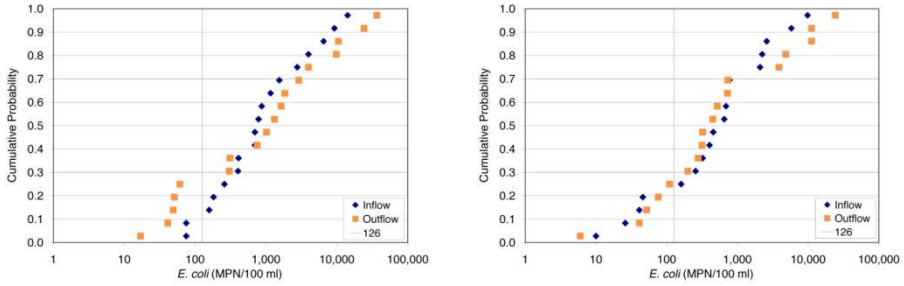
less variable from site to site. For the SCMs studied in Wilmington, NC, as part of this study, wet ponds appeared superior for removal of indicator bacteria. Numerous factors are likely associated with removal of indicator bacteria in stormwater wetlands and wet ponds, including predation, settling of particle associated microbes, and potential resuspension of captured particle associated microbes due to internal SCM hydrodynamics. Thus, numerous variables are present in stormwater wetlands and wet ponds which may explain variations in performance. Further research is needed to determine factors which contribute to the performance of these SCMs regarding microbe die-off.

Few field evaluations of indicator bacteria removal have been performed for bioretention, particularly for enterococci. Studies by Hathaway et al. (2009) on a bioretention area in Charlotte, NC, and Passeport et al. (2009) on two bioretention cells in Graham, NC, indicated high fecal coliform concentration reductions, with all three cells having concentration reductions above 85%. Hathaway et al. (2009) also reported a 92% *E. coli* concentration reduction for the bioretention area in Charlotte, NC. Jones et al. (2008) examined enterococci removal from a bioretention area in New Hampshire showing a concentration reduction of over 90%. Conversely, evaluations by Li and Davis (2009) on two bioretention areas in Silver Spring and College Park, MD, showed relatively poor performance for *E. coli* (median removal of 0% and 57%, respectively) and fecal coliform (median removal of 50% and 0%, respectively). Likewise, there was a substantial difference in functionality between the two bioretention areas studied in Wilmington, NC. The differing depth of media, nominally 60 cm for Bioretention-D and 25 cm for Bioretention-S, appeared to result in varied performance. Further investigation is planned to explore possible explanations for the difference in performance between cells. Potential causes are differences in organic content of the soils in the two cells, differences in soil moisture, differences in soil temperature, and differences in hydraulic function of the two systems (leading to differences in microbial sorption). It should be noted that both bioretention areas were constructed using native soils as fill media. Native soils were generally fine sands (NRCS 2010), which may lead to reduced effectiveness. Small clay particles have a greater ability than sand to facilitate sorption of microbes (Mankin et al. 2007).

1.1.6 Influent and Effluent Probability Plots

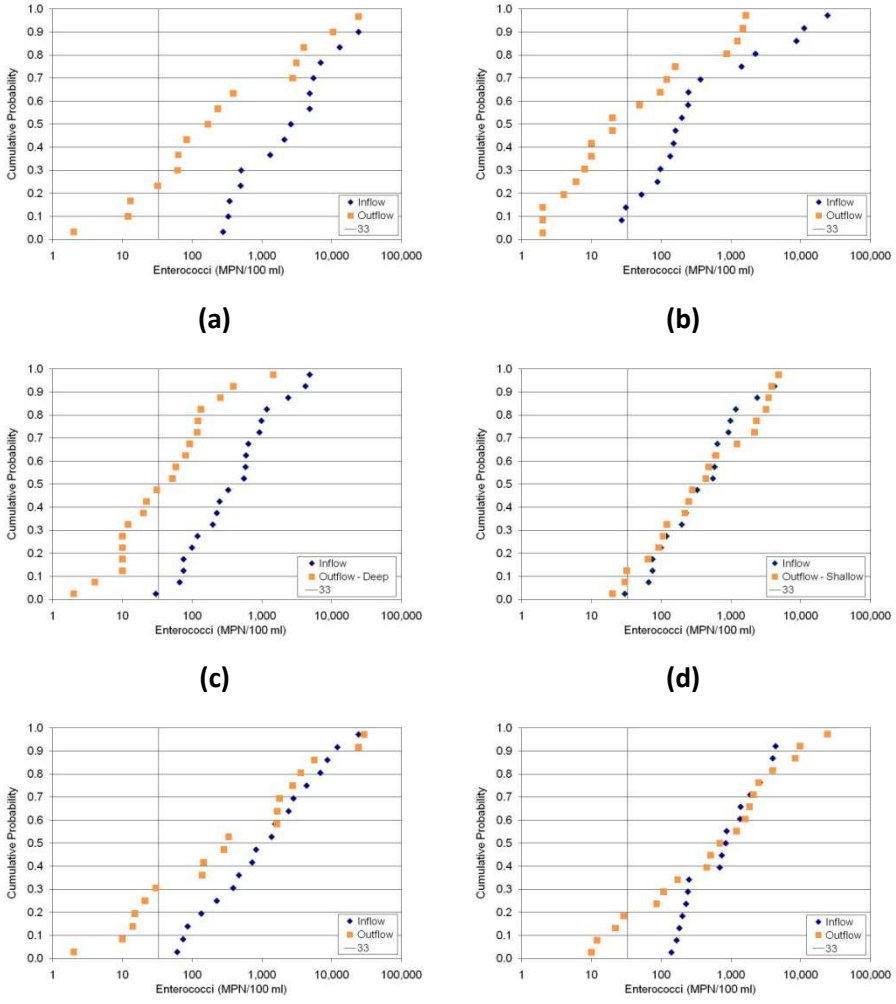
Probability plots allowed greater examination of influent and effluent indicator bacteria relationships for each SCM. Probability plots for *E. coli* are presented in Figures 5.3a-5.3e. Probability plots for enterococci are presented in Figures 5.4a-5.4e. These plots generally support performance observations made previously. Separation between influent and effluent probability curves are particularly noted for both wet ponds for *E. coli* and enterococci and Bioretention-D for enterococci. Some consistent separation between influent and effluent *E. coli* probability curves is noted for Bioretention-D; however, the separation is moderate in comparison to that exhibited in its enterococci probability plot. Wetland 1 also appears to function fairly well for enterococci based on the probability plots, supporting the moderate removal efficiency noted in Table 5.4. Probability plots for Bioretention-S for both indicator bacteria and the stormwater wetlands for *E. coli* show a lack of distinction between influent and effluent probability curves, indicating inconsistent, poor performance over the course of the study.





(e) (f)

Figure 1.3: *E. coli* probability plots for (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D, (d) Bioretention-S, (e) Wetland 1, and (f) Wetland 2



(a) (b)

(c) (d)

(e) (f)

Figure 1.4: Enterococci probability plots for (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D, (d) Bioretention-S, (e) Wetland 1, and (f) Wetland 2

1.1.7 Analysis of Effluent Concentrations

Microbial contamination is regulated by target concentrations established by the USEPA (1986). For fresh waters regulated for full body contact, the geometric mean over a 30-day period cannot exceed 126 organisms / 100 ml for *E. coli* or 33 organisms / 100 ml for enterococci. For marine waters regulated for full body contact, the geometric mean over a 30-day period cannot exceed 35 organisms / 100 ml for enterococci. Thus, effluent SCM concentrations can be compared to these values to determine how they will affect concentrations in receiving waters. Obviously, mass balances would be required to evaluate the full impact of these practices on targeted watersheds. Median effluent indicator bacteria concentrations are shown in Tables 5.6 and 5.7.

Table 1.6: Median effluent *E. coli* concentrations

SCM Type	*E. coli* Concentrations (MPN/100ml)			
	Geometric Mean Influent	Geometric Mean Effluent	Number of effluent samples less than 126 MPN / 100 ml	number of effluent samples less than 126 MPN / 100 ml (percentage)
Wet Pond 1	2483	62	9	9 of 15 (60%)
Wet Pond 2	1273	60	11	11 of 18 (61%)
Bioretention-D	130	39	13	13 of 20 (65%)
Bioretention-S	130	284	7	7 of 20 (35%)
Wetland 1	834	826	5	5 of 18 (28%)
Wetland 2	425	503	5	5 of 18 (28%)

Table 1.7: Median effluent enterococci concentrations

SCM Type	Enterococci Concentrations (MPN/100ml)			
	Geometric Mean Influent	Geometric Mean Effluent	Number of effluent samples less than 33 MPN / 100 ml	Number of effluent samples less than 33 MPN / 100 ml (percentage)
Wet Pond 1	2356	237	4	4 of 15 (27%)
Wet Pond 2	274	37	10	10 of 18 (56%)
Bioretention-D	375	39	10	10 of 20 (50%)
Bioretention-S	375	378	3	3 of 20 (15%)
Wetland 1	1018	316	6	6 of 18 (33%)
Wetland 2	866	510	4	4 of 18 (22%)

SCMs that provided good removal of indicator bacteria (Tables 5.3 and 5.4) also had low geometric mean effluent concentrations. Median effluent *E. coli* concentrations were below USEPA target concentrations for Wet Pond 1, Wet Pond 2, and Bioretention-D. For enterococci, no SCM had median effluent concentrations below USEPA targeted values, although Wet Pond 2 and Bioretention-D approached targeted values.

No SCM consistently provided *E. coli* or enterococci concentrations lower than USEPA targeted values. Wet Pond 2 and Bioretention-D provided the highest percentage of effluent *E. coli* and enterococci samples below the target value, while Wet Pond 1 had a high percentage of storms below only the *E. coli* target value. Bioretention-S and the two stormwater wetlands did not typically have effluent concentrations below the USEPA target values.

These results suggest that although positive reductions of indicator bacteria can be observed in SCMs, even those which perform well may not consistently produce concentrations below USEPA target values for surface waters. Similar observations were made by Hathaway et al. (2009). This is important in evaluating the effectiveness of watershed restoration activities. To reliably reduce indicator bacteria loadings to surface waters, SCMs must reduce runoff volume. SCMs may not consistently contribute to watershed restoration simply due to concentration reductions. To this end, a SCM like bioretention that has been repeatedly shown to reduce outflow volumes (Hunt et al. 2006, Li et al. 2009) holds the most promise.

Estimations of non-exceedance probabilities were generated using probability plots. A regression line was fit to the outlet data and the non-exceedance probability was estimated as the probability where the regression line crossed the USEPA targeted surface water concentration for *E. coli* and enterococci, respectively. This allowed some estimation of the probability a given SCM's effluent concentration will not exceed USEPA targeted surface water concentrations. Approximate non-exceedance probabilities are presented in Table 5.8. Generally, there is a higher probability of exceeding the enterococci target concentration, with non-exceedance probabilities being lower than 50% for all SCMs. Non-exceedance probabilities for *E. coli* were higher than 50% for Wet Pond 1, Wet Pond 2, and Bioretention-D. The Bioretention-D non-exceedance probability for *E. coli* approached that observed for two

bioretention areas evaluated by Li and Davis (2009), where non-exceedance probabilities for *E. coli* were estimated as > 65% and >75%.

Table 1.8: USEPA targeted concentration non-exceedance probabilities

SCM Type	Approximate Non-exceedance Probability (%)	
	E. coli	enterococci
Wet Pond 1	57	29
Wet Pond 2	60	49
Bioretention-D	63	47
Bioretention-S	43	9
Wetland 1	26	26
Wetland 2	32	16

1.1.8 Seasonal Impacts on SCM Effluent Concentrations

The public health impacts of urban stormwater runoff are of particular interest during periods of the year when water related recreational activities are most common. Studies such as Hathaway and Hunt (in review), Selvakumar and Borst (2006), and Line et al. (2008) suggest indicator bacteria concentrations in stormwater runoff may increase with warmer seasons/temperatures. Data were separated into swimming and non-swimming periods based on dates used as guidelines for compliance sampling by the NCDEH (2010). The non-swimming period is from November to the end of March, when average daily temperatures are lowest. For each SCM, both swimming and non-swimming seasons were represented by at least 5 samples (Table 5.9). The influent and effluent concentrations of *E. coli* and enterococci for each SCM are illustrated in Figures 5.5 and 5.6 for the two periods (swimming and non-swimming).

Table 1.9: Number of swimming and non-swimming samples for each SCM for both indicator bacteria

Location	Number of Samples	
	swimming	non-swimming
Wet Pond 1	10	5
Wet Pond 2	10	8
Bioretention-D	10	10
Bioretention-S	10	10
Wetland 1	10	8
Wetland 2	10	8*

* 9 non-swimming enterococci samples

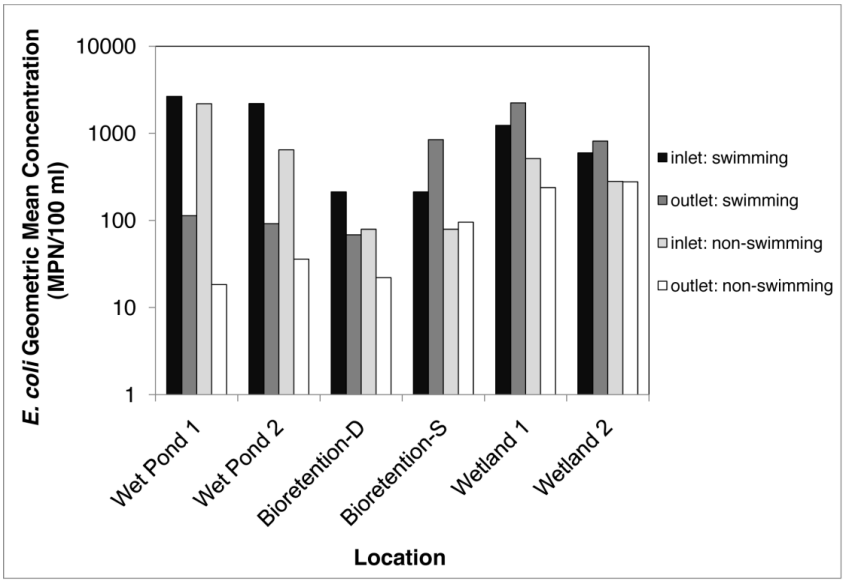


Figure 1.5: Geometric mean influent and effluent *E. coli* concentrations for swimming and non-swimming seasons for each SCM

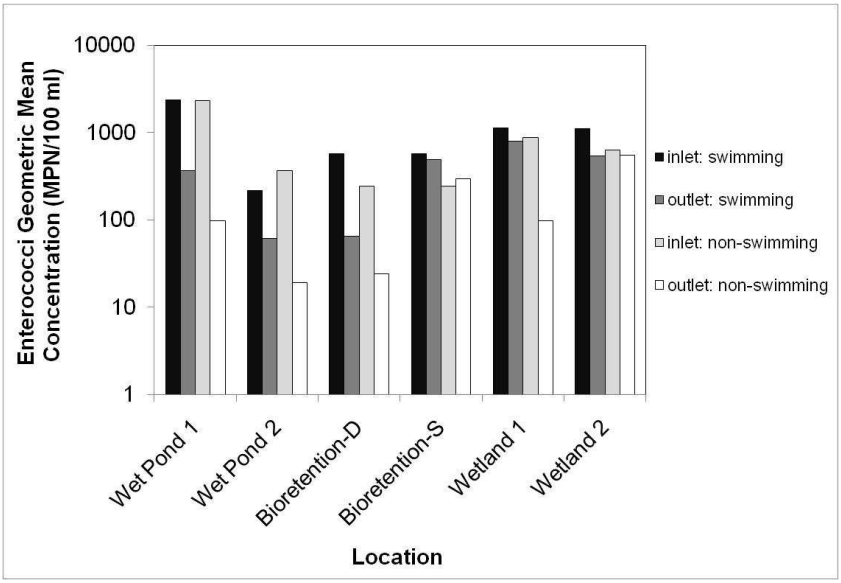


Figure 1.6: Geometric mean influent and effluent enterococci concentrations for swimming and non-swimming seasons for each SCM

Geometric mean concentrations of *E. coli* were higher during the swimming season for both the inlet and outlet of each SCM. For enterococci, this was also true for the majority of SCMs. Geometric mean inlet enterococci concentrations were found to be higher during the non-swimming season for Wet Pond 2, and geometric mean outlet enterococci concentrations were found to be higher during the non-swimming season for Wetland 2. Table 5.10 shows the

difference in geometric mean indicator bacteria concentrations between the swimming and non-swimming seasons as calculated by equation 3. Effluent concentrations decreased by more than 60% for both indicator bacteria in all SCMs (other than enterococci in Bioretention-S and Wetland 2).

Table 1.10: Analysis of seasonal differences in effluent concentrations

Location	Effluent concentration reduction - swimming to non-swimming (%)	
	E.coli	enterococci
Wet Pond 1	84	73
Wet Pond 2	61	69
Bioretention-D	68	63
Bioretention-S	89	40
Wetland 1	89	88
Wetland 2	66	-2

Despite apparent differences between swimming and non-swimming periods, Wilcoxon Rank Sum tests showed no statistically significant differences between inlet and outlet concentrations for the two seasons ($p < 0.05$). It is possible the high variability common in microbiological data resulted in statistically insignificant results; further, a relatively low number of non-swimming samples (5) was available for Wet Pond 1. Nonetheless, these data suggest effluent indicator bacteria concentrations may vary seasonally for SCMs. A study on two bioretention areas by Li and Davis (2009) identified summer as the season when the highest influent concentrations of *E. coli* and fecal coliforms were found for each system; however, removal performance could not be correlated to temperature. Similar observations of higher effluent enterococci concentrations during the summer and early fall were made by Jones et al. (2008) on a wet pond in New Hampshire.

Temporal changes in both influent and effluent indicator bacteria concentrations could also lead to differences in SCM removal efficiency throughout the year. Table 5.11 shows indicator bacteria concentration reductions in SCMs during the swimming and non-swimming seasons. Concentration reductions are typically higher during the non-swimming season, although systems which performed well overall (Wet Pond 1, Wet Pond 2, and Bioretention-D) provided relatively high concentration reductions throughout the year. Nonetheless, these data suggest SCM effectiveness for indicator bacteria may vary throughout the year, but more data are

needed to strengthen this postulation. This represents a future research need in the stormwater management field, and could have implications for both public health and watershed management.

Table 1.11: Indicator bacteria concentration reductions in SCMs during swimming and non-swimming seasons

Location	*E.coli*		Enterococci	
	Concentration Reduction - swimming (%)	Concentration Reduction - non-swimming (%)	Concentration Reduction - swimming (%)	Concentration Reduction - non-swimming (%)
Wet Pond 1	96	99	84	96
Wet Pond 2	96	94	71	95
Bioretention-D	68	72	89	90
Bioretention-S	-297	-21	15	-20
Wetland 1	-81	53	30	89
Wetland 2	-36	1	52	14

Conclusions

Six Stormwater Control Measures were evaluated for *E. coli* and enterococci removal over 15 to 20 storm events in Wilmington, NC. Both wet ponds and the deep bioretention cell were effective at removing both *E. coli* and enterococci, with concentration reductions exceeding 70% for both indicator bacteria in each SCM. However, the shallow bioretention cell and both stormwater wetlands did not perform well in comparison, particularly for *E. coli*. These data suggest some SCMs can export indicator bacteria, as two of the six SCMs showed negative removal of *E. coli*. Similar results have been seen in such studies at Krometis et al. (2009), Li and Davis (2009), Jones et al. (2008), and Hathaway et al. (2009). These results are not illogical, as indicator bacteria have been shown to persist in sediments of streams and estuaries (Sherer et al. 1992, Jeng et al. 2005). Further, studies by Davies and Bavor (2000) on wet pond sediments indicated the persistence of indicator bacteria even after 28 days. SCMs may also attract wildlife, leading to direct addition of indicator bacteria into the system through defecation.

These data have some similarity to other studies which evaluated microbial reductions in SCMs; however, some differences in performance may occur based on geophysical region. This is possibly due to differences in particle-microbe interactions in sandy watersheds and/or dilution from the water table. In particular, wet ponds evaluated in this study and one of three

evaluated by Mallin et al. (2002) performed well in comparison to wet ponds studied in clayey watersheds by Krometis et al. (2009) and Davies and Bavor (2000). Also, further study is needed to determine how soil type and design configuration affect indicator bacteria removal in bioretention areas. Despite a larger watershed, Bioretention-D performed well in comparison to Bioretention-S. Both the depth and type of fill media likely influence the ability of Bioretention-S to sequester bacteria.

SCMs which performed well in Wilmington, NC, showed promise in meeting USEPA target *E. coli* concentrations for surface waters. Both wet ponds and Bioretention-D had geometric mean effluent *E. coli* concentrations lower than USEPA target values. Enterococci target values were not achieved by any SCM; however, both Wet Pond 2 and Bioretention-D had geometric mean effluent concentrations which approached target concentrations. Although this creates some concern as to the benefit of SCMs in watersheds impacted by microbial pollution, a SCM's contribution to watershed restoration cannot be evaluated based on concentration reduction alone. Reductions in indicator bacteria mass entering surface waters may be achieved through such mechanisms as infiltration. Evaluation of the impacts of infiltration on groundwater microbial quality represents another need within the field of stormwater management, particularly as infiltration-based SCMs become increasingly implemented.

SCM effluent indicator bacteria concentrations appear to vary throughout the year. Specifically, effluent concentrations may elevate during the swimming season from April to October. Further research is needed to verify this observation due to the variability in these data and lack of statistically significant results. Elevated concentrations during the period of the year when water related recreational activities are most frequent causes some concern in regard to public health. Understanding these changes is important in determining how to manage watersheds for indicator bacteria. TMDLs for microbial pollution are required to account for seasonal variability. Seasonal variability has been shown for indicator bacteria in urban stormwater runoff in such studies at Selvakumar and Borst (2006) and Hathaway and Hunt (in review). Further research is needed to determine if there are also seasonal differences in SCM indicator bacteria sequestration. This could be included in watershed TMDLs, as varied load reductions could be applied to SCMs based on season.

Despite a recent increase in the number of studies evaluating indicator bacteria performance of SCMs, data are variable. Further, there are limited data in regard to SCM performance for enterococci, the USEPA recommended indicator bacteria for marine environments. A relatively limited amount of scientific literature has shown differences in performance of SCMs for fecal coliform, *E. coli*, and enterococci. The data presented herein have added to the limited scientific knowledgebase for SCM performance for enterococci. Removal of enterococci was found to vary substantially from removal of *E. coli* for a number of the six SCMs evaluated. Differences in removal of *E. coli* and enterococci were not consistent, as some SCMs performed better for *E. coli* and others for enterococci. However, effluent enterococci concentrations did not approach USEPA target concentrations even in SCMs which had effluent *E. coli* concentrations less than USEPA targeted concentrations. Further, non-exceedance probabilities were lower for enterococci for all SCMs other than Wetland 1. It is unknown if these differences are due to variations in the magnitude of influent and effluent microbe populations, or due to differences in indicator persistence. For instance, enterococci are typically regarded as being more resistant to environmental conditions (USEPA 2001). At this time, it does not appear that similar performance can be assumed for a given SCM for all indicator bacteria.

Perhaps the most important need in examining indicator bacteria removal in SCMs is an understanding of mechanisms which control indicator bacteria persistence and sequestration. Such understanding will: (1) help determine which SCMs should be used in watersheds impacted by microbial pollution, (2) allow a greater understanding of the public health implications of indicator bacteria persistence in SCMs, (3) explain the variability noted in this study and others with regard to SCM removal efficiency of indicator bacteria, and (4) potentially lead to design modifications which can be used for SCMs in an effort to enhance removal of indicator bacteria.

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2 Analysis of Factors Influencing Bioretention Performance for Indicator Bacteria in Wilmington, NC

Abstract

Although bioretention has been shown to remove or sequester a wide range of pollutants, relatively little study has been performed to evaluate its ability to sequester indicator bacteria. Two bioretention areas in Wilmington, NC, were studied in a paired-watershed experimental design. The primary difference in the design of the two systems was soil depth. One bioretention cell was constructed with 25 cm of fill soil (Bioretention-S) and one with 60 cm of fill soil (Bioretention-D). The systems were found to perform differently for indicator bacteria based on multiple performance evaluation metrics. Bioretention-D showed concentration reductions of 70% and 89% for *E. coli* and enterococci, respectively. Effluent concentrations from Bioretention-D compared well to EPA target values and other studies in literature. Conversely, Bioretention-S showed concentration "reductions" of -119% and -102% for *E. coli* and enterococci, respectively. Effluent concentrations from Bioretention-S were substantially higher than USEPA target values and other studies in literature. Multiple factors were evaluated to determine the cause of performance differences between the two cells. Soil depth was identified as the most important factor. The 25 cm of fill soil in Bioretention-S exhibited poorer runoff detention and theoretically resulted in higher soil water flux and decreased contact time relative to Bioretention-D. These differences seemingly led to diminished indicator bacteria sequestration. The results of this study suggest soil depth is an important design parameter for bioretention which should be carefully selected. Further, minimum soil depths appear to exist, below which decreased sequestration of indicator bacteria may be experienced.

Introduction

Low Impact Development (LID) is increasingly utilized as a technique to mitigate the impact of stormwater runoff on surface waters (USEPA 2000). As part of LID, infiltration based SCMs (Stormwater Control Measures – also known as Best Management Practices, "BMPs," Water Sensitive Urban Designs, and "WSUDs") are implemented to facilitate stormwater treatment, groundwater recharge, and stormwater volume and peak reductions. One commonly utilized

SCM as part of LID is bioretention (also known as biofiltration, or bio-infiltration when underdrains are not employed).

Bioretention has been shown effective at reducing runoff volumes, peak flows, and numerous pollutants ranging from nutrients to metals (Hunt et al. 2006, Dietz and Clausen 2005, Davis et al. 2006, Davis et al. 2009, Roseen et al. 2006). However, until recently, little was known regarding bioretention sequestration of indicator bacteria. Indicator bacteria denote contamination from fecal matter and thus the possible presence of pathogens. In a review of bioretention literature and future needs, Davis et al. (2009) identified research on bioretention removal of pathogenic bacteria as a need for the stormwater management community. Indicator bacteria are a common source of impairment in surface waters in North America, Europe, Australia, and elsewhere. In the United States, there are more Total Maximum Daily Loads (TMDLs) in place for indicator bacteria than any other pollutant (USEPA 2010). Stormwater runoff from urban watersheds has been shown to have substantial concentrations of indicator bacteria (Selvakumar and Borst 2006, McCarthy et al. 2007, Hathaway et al. accepted), contributing to microbial pollution in surface waters.

Bioretention has numerous treatment mechanisms for indicator bacteria. In addition to filtering bacteria as stormwater passes through the system, microbes may sorb to organic particles and soils. Such mechanisms result in sequestration of microbes; however, die-off of captured microbes is controlled by other factors. Exposure to sunlight (UV radiation), desiccation, predation, temperature, and nutrient availability can all influence microbial survival (Ferguson et al. 2003, Arnone and Walling 2007). Further, Indicator bacteria have been shown to persist in natural systems. Studies by Sherer et al. (1992) and Jeng et al. (2005) suggest indicator bacteria can persist in sediments from 7 to 30 days given suitable environmental conditions. Therefore, despite treatment mechanisms within bioretention areas to facilitate indicator bacteria removal, microbial persistence within bioretention areas may limit overall effectiveness.

Laboratory analyses emulating bioretention function have been utilized to evaluate the potential for indicator bacteria removal in these systems. Rusciano and Obropta (2007) observed a 91.6% mean reduction of fecal coliform concentrations through bioretention columns receiving diluted swine manure. Similarly, column studies using conventional

bioretention fill media by Zhang et al. (2008) showed an 80% *E. coli* reduction. Zhang et al. (2008) also analyzed bacteria concentrations in the bioretention fill media, observing a 99.9% die off of *E. coli* cells one week after synthetic stormwater runoff was applied to the columns.

Field studies on bioretention have also evaluated indicator bacteria removal. Some studies showed either concentration reductions of indicator bacteria greater than 85% (Hathaway et al. 2009, Passeport et al. 2009) or effluent indicator bacteria concentrations below detectable limits (Dietz and Clausen 2005). Conversely, an analysis of two bioretention cells in Maryland by Li and Davis (2009) yielded somewhat different results. *E. coli* concentration reductions in the two cells were 57% and 0%, while fecal coliform reductions were 0% and 50%. Li and Davis (2009) also observed export of indicator bacteria during some monitored events. Thus, although studies such as Hathaway et al. (2009) have proposed the effectiveness of bioretention for indicator bacteria sequestration, variability exists among field collected performance data. It should be noted that other than Hathaway et al. (2009), field studies performed on bioretention for indicator bacteria have involved seven or fewer samples.

Although there are a growing number of studies evaluating bioretention performance for indicator bacteria, relatively little is understood regarding microbial dynamics within bioretention fill media (Li and Davis 2009). No studies have been performed to evaluate which environmental conditions within bioretention areas can influence indicator bacteria performance. Such data will result in a refined understanding of differences in performance observed for infiltration-based SCMs, and may lead to revised design standards for bioretention being implemented in watersheds with microbial TMDLs.

The objectives of this study were to build upon the current understanding of indicator bacteria removal in bioretention by: (1) evaluating the performance of bioretention areas with varied depths of fill soil, and (2) characterizing physical and chemical properties which may potentially lead to differences in performance between the two bioretention cells.

Materials and Methods

2.1.1 Site Descriptions

The experimental site was located in Wilmington, North Carolina (Figure 6.1). Two bioretention areas were constructed adjacent to one another within a parking lot (Figure 6.2). A paired-watershed experimental design was desired, but watershed areas differed due to microtopography within the parking lot. The surface areas of the bioretention cells differed by only 1 m^2. One bioretention was constructed with an average soil depth of approximately 60 cm (Bioretention-D), the other had an average soil depth of approximately 25 cm (Bioretention-S). All fill soil for the bioretention areas came from on-site sandy soil, which was classified as Baymeade fine sand (NRCS 2010). Clay and silt comprised 8 to 10 percent of the soil used as fill for the bioretention areas (Table 6.1). This is a lower percentage than bioretention areas evaluated by Dietz and Clausen (2005) and Li and Davis (2009), where fines comprised 16 to 46 percent of the soil media. However, this percentage of fines is acceptable per North Carolina SCM design regulations (NCDENR 2007).

Each cell was constructed with a 10-cm underdrain to facilitate sample collection. It should be noted that underdrains are typically not required for bioretention areas in the sandy soils of coastal areas, thus this design differs from standard practice in the region. Runoff entered each bioretention cell as sheet flow. A small flume was installed at the pavement edge in a location presumed to be representative of the entire watershed. This allowed some pooling of runoff as it entered the bioretention cell, facilitating sampling of the inlet. A similar sampling strategy was used in such studies as Hunt et al. (2006). The bioretention areas were vegetated with turf grass and a small number of shrubs. It should be noted that bypass of the shallow cell occurred on some occasions due to watershed topography routing water around the cell. This bypass was judged to not substantially influence the results of this study. General characteristics of each SCM are given in Table 6.1.

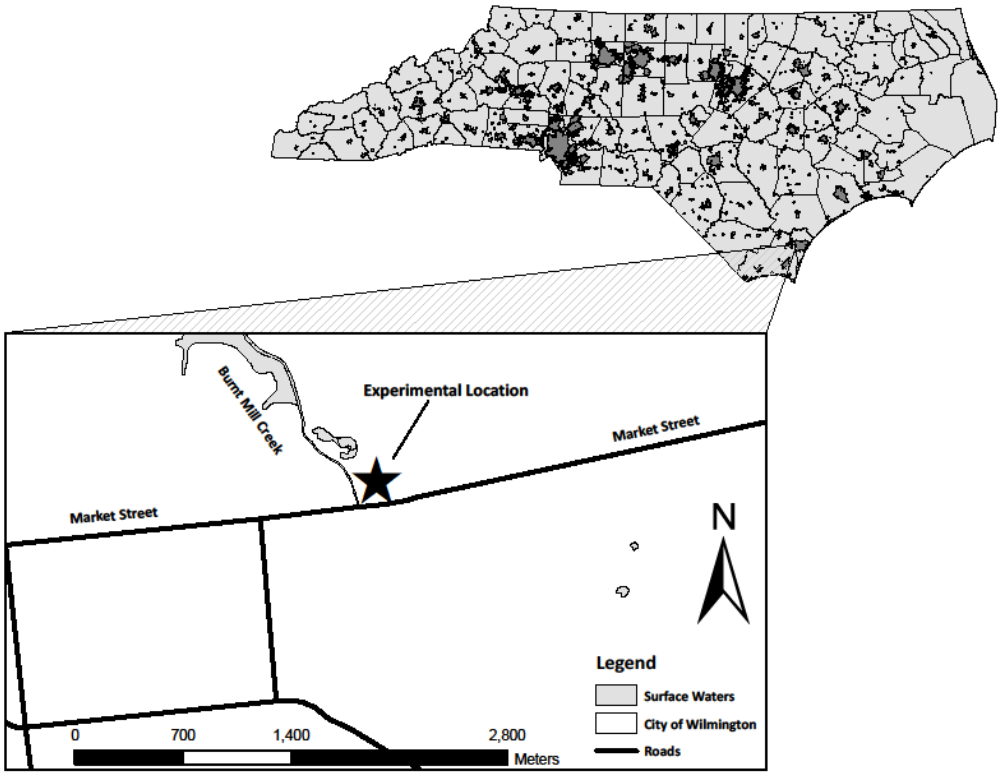

Figure 2.1: Experimental location in Wilmington, NC

Table 2.1: General characteristics of Wilmington SCMs

Characteristic		Bioretention-D	Bioretention-S
Drainage Area (ha)		0.10	0.05
Watershed Composition		Commercial (parking lot)	Commercial (parking lot)
Estimated Imperviousness		100%	98%
Surface Area (m^2)		55	54
Surface Area: Drainage Area Ratio		0.054	0.110
Storage Depth (cm)		28 (1% slope on cell)	28 (1% slope on cell)
Estimated Average Soil Depth (cm)		25	60
Soil Texture[3]	Sand (%)	88	87
	Silt (%)	5	4
	Clay (%)	5	4

1. NRCS 2010 – Soil Data Mart
2. Average depth represents soil depth for bioretention cells
3. Does not add to 100% due to some gravel particles

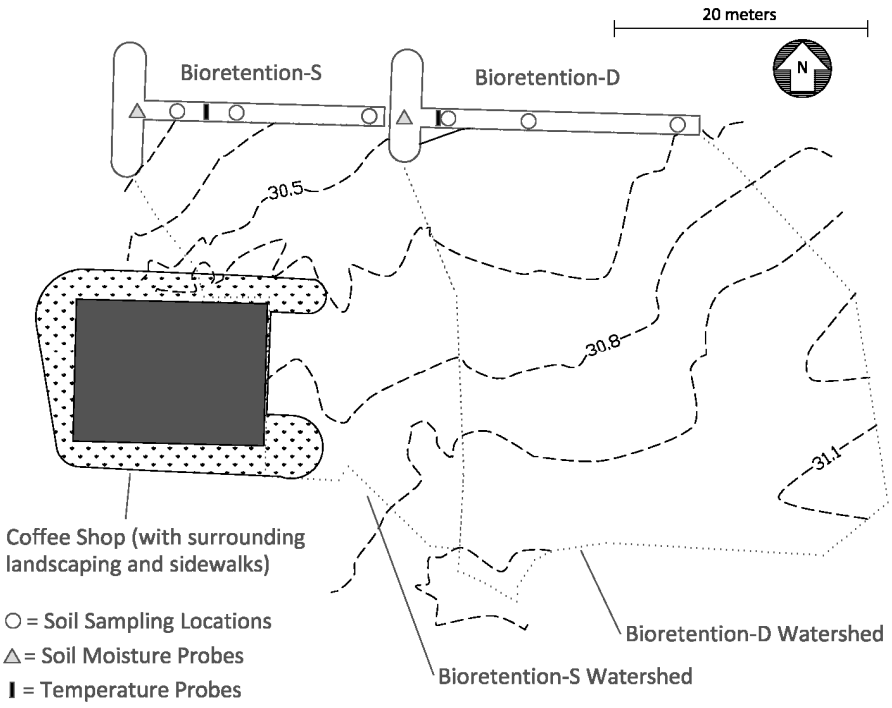

Figure 2.2: Schematic of SCMs and associated watersheds (elevations based on relative datum)

2.1.2 Monitoring Methods – Flow and Rainfall Monitoring

Bioretention underdrains were hydrologically separate and each routed to a nearby catch basin. Two weir boxes fitted with 30-degree, v-notch weirs were installed in the catch basin to receive discharge from each underdrain (independently). Flow was monitored by measuring stage within each weir box using ISCO 6712 autosamplers equipped with 730 bubbler modules. Data were recorded on 5 minute intervals from January 2007 to December 2008, after which data were recorded on 2 minute intervals. An ISCO 674 tipping bucket rain gage was installed at the site, away from the tree canopy, to monitor rainfall. An additional Davis tipping bucket rain gage equipped with a HOBO event logger was installed nearby (approximately 60 m) to provide back-up rainfall data. The ISCO rain gage was not operational until July 2007, leading to the use of data from the Davis rain gage from January 2007 to July 2007. After July 2007, data from the ISCO rain gage was primarily used for rainfall characterization.

Inflow was estimated using the initial abstraction methodology, similar to that employed for estimating inflow to bioretention areas by Li et al. (2009). The Bioretention-D watershed was

100% impervious, while the Bioretention-S watershed was approximately 98% impervious. An initial abstraction of 1 mm (CN of 98) was applied to all impervious areas, and an initial abstraction of 24 mm (CN of 68) was applied to all pervious areas. The amount of rainfall directly falling onto each cell was also calculated and included in the inflow volume.

2.1.3 Monitoring Methods – Indicator Bacteria Monitoring

Grab samples were taken to characterize bioretention performance for indicator bacteria. This is a common methodology for sampling surface waters for indicator bacteria (USEPA 2002, Burton and Pitt 2002). There are valid concerns over the use of grab samples due to potential variations in microbial concentrations during the course of a stormwater runoff event. However, short hold times, increased man-hours, and the technical difficulty of using automatic samplers for microbial analyses led to the use of grab samples for this study. Further, studies such as McCarthy et al. (2008) have illustrated the uncertainties present in indicator bacteria field monitoring, which potentially overshadow the negative impacts of using single grab samples to some degree. Inlet samples were collected for both bioretention areas from the inlet flume discussed previously. Outlet samples were collected from each respective bioretention cell's weir box effluent. Each sample set consisted of two sterile bottles for two bacterial analyses (*E. coli* and enterococci).

Samples were transported to Tritest, Inc for analysis. Hold times were generally less than 6 hours. Samples were analyzed for both *E. coli* and enterococci. *E. coli* were enumerated using Colilert® and enterococci were enumerated using Enterolert®. Each methodology is based on the use of a defined substrate media (IDEXX Laboratories Inc., Westbrook, Maine). Sample dilutions were performed as needed to adequately characterize bacteria concentrations. The Limit of Detection (LOD) was typically either 2 or 10 MPN / 100 ml depending on the dilution utilized. A Maximum Reporting Limit (MRL) was only reached on one occasion and only for enterococci. The MRL was 4839 MPN / 100 ml. Data are analyzed herein using the values at the reporting limit without adjustment.

2.1.4 Monitoring Methods – Physical Measurements

Physical characteristics which could influence bacteria sequestration and persistence in the two bioretention areas were examined. These characteristics included soil temperature, soil

moisture, soil chemical properties, and soil physical properties. Soil temperature was monitored in each bioretention cell using temperature sensors (TMCX-HD, accuracy: ±0.5°C at 20°C) connected to HOBO 4-channel data loggers (H08-008-04). Soil moisture was monitored using soil moisture sensors (S-SMC-M005, accuracy: ±0.031 m^3/m^2) connected to HOBO Micro Station Data Loggers (H21-002). Both temperature and soil moisture sensors were placed at approximately 10 and 20 cm in the shallow cell and at 20 cm and 61 cm in the deep cell. Sensors were placed in relatively similar locations in each cell as shown in Figure 6.2. Temperature and soil moisture data were collected on 5-minute intervals and averaged hourly to facilitate analysis.

On 12/15/2008, soil samples were collected from three locations in each bioretention cell at 2 to 3 depths per location (2 for the shallow cell, 3 for the deep cell). Samples were taken to the North Carolina Division of Agriculture and Consumer Services for analysis. Samples were measured for cation exchange capacity (CEC), pH, and other chemical properties. Soil sampling locations are shown in Figure 6.2.

Worm burrows could serve as conduits to facilitate runoff travel through soil media, thereby reducing contact time. A methodology to determine worm burrow abundance in agricultural fields was employed by Fox et al. (2008), and a similar approach was applied at the SCMs in Wilmington, NC, in October 2008. A large fan was attached to the underdrain outlet of both bioretention cells, blowing air upstream into the bioretention soil (Figure 6.3). A smoke generating firework (known as a “smoke bomb”) was released between the fan and the underdrain, causing smoke to be pushed into the cell’s fill media. The smoke bomb lasted approximately 90 seconds. As smoke passed upward through conduits connecting the underdrain to the surface, visible smoke streams emerging from the soil surface were flagged and counted.

Figure 2.3: Fan attached to bioretention underdrain to blow smoke into the bottom of a bioretention cell

2.1.5 Monitoring Methods – Soil Bacteria Analysis

Soil samples were collected for bacteria analyses once per season. A 10 cm auger equipped with clean plastic sampling tubes was used to take soil cores at incremental depths through the soil profile at three locations per bioretention cell (Figure 6.2 – Approximately the same as chemical analysis sampling locations). Approximately 2.5 – 5 cm of turf and soil were removed from the bioretention surface prior to sampling to ensure proper auger function. Equipment was washed with sterile water at each new sampling location. Cores (intact in plastic sleeves) were placed in plastic bags and stored on ice during transport to the Department of Soil Science at North Carolina State University.

Twenty mg of soil from both the top and bottom of each soil column were removed and analyzed separately. Soil samples were suspended in Winogradsky salt solution (10 ml/g soil) (Pochon 1954) and shaken for 15 minutes at 250 rpm on a G10 Gyrotory Shaker (New Brunswick Scientific Company Inc, Edison NJ) at room temperature. The soil suspension was centrifuged (model RC5C, Sorvall Instruments, DuPont) at 2,500 rpm for 10 minutes at 4°C. After centrifugation, 100 ml of supernatant from each sediment sample was analyzed for most probable number (MPN) concentrations of *E. coli* and enterococci by use of the Colilert and Enterolert defined substrate method, respectively, using the Quantitray/2000 format (Idexx Corporation, Westbrook, ME). All analyses were performed per manufacturer's instructions.

Analyses included suitable blanks and standard positive cultures (*E. coli,* ATCC 25922 and *Enterococcus faecium* ATCC 35667) for quality control purposes. Results were reported as MPN of *E. coli* or enterococci per gram of sediment.

2.1.6 Statistical Evaluations

Multiple analyses were utilized to establish the efficiency of indicator bacteria sequestration in the bioretention cells including: (1) removal percentages, (2) effluent concentration comparisons, and (3) probability plots. Removal percentages (Concentration Reduction "CR") were calculated for each SCM using Equation 1. Geometric mean effluent indicator bacteria concentrations from each cell were compared to values from literature and USEPA target concentrations for indicator bacteria in fresh waters. Last, concentration data were used to generate effluent probability plots using methodologies by Burton and Pitt (2002).

$$CR = \left(1 - \frac{Geometric\_Mean_{outlet}}{Geometric\_Mean_{inlet}}\right) \times 100\% \qquad (1)$$

All statistical analyses were performed using SAS 9.1 (SAS 2001). Non-parametric Wilcoxon Signed Rank tests were used to determine differences among paired observations. Non-parametric analyses also lessen the influence of high and low concentrations, which is important when data sets contain values below the MDL or above the MRL.

Results and Discussion

2.1.7 Bioretention Performance for Indicator Bacteria

Twenty storm events were monitored for indicator bacteria between February 2008 and February 2010. Geometric mean influent *E. coli* and enterococci concentrations were 130 MPN / 100 ml and 375 MPN / 100 ml, respectively. The inlet *E. coli* concentration was higher than median values reported at the inlets of two bioretention areas studied by Li and Davis (2009), and less than the geometric mean inlet *E. coli* concentration for a bioretention area evaluated in Hathaway et al. (2009). The inlet enterococci concentration was similar to that reported by Jones et al. (2008) for a parking lot in New Hampshire. To validate the inlet sampling location, composite grab samples were also collected from multiple inflow points on 18 occasions for *E. coli* and 17 occasions for enterococci. There was no significant difference between the

composite samples and samples collected from the inlet flume ($p < 0.05$). Summary statistics are presented in Table 6.2.

Bioretention-S geometric mean effluent concentrations were higher than those from Bioretention-D for both *E. coli* and enterococci, leading to dissimilar concentration reductions. Concentration reductions of *E. coli* and enterococci for Bioretention-D were 70% and 89%, respectively. Concentration "reductions" of *E. coli* and enterococci for Bioretention-S were -119% and -102%, respectively. A statistically significant difference between inlet and outlet concentrations was only found for enterococci in Bioretention-D ($p < 0.05$). No significant difference in inlet and outlet *E. coli* concentrations was found for either SCM ($p < 0.05$). However, significant differences were also found between Bioretention-D and Bioretention-S effluent concentrations for both *E. coli* and enterococci ($p = 0.0158$ and $p = 0.0005$, respectively).

Comparisons to USEPA target indicator bacteria concentrations in fresh waters were performed. The USEPA target concentration for *E. coli* in fresh water is 126 / 100 ml, and the target concentration for enterococci in fresh water is 33 / 100 ml (USEPA 1986). The Bioretention-D geometric mean effluent concentration was lower than the USEPA target concentration for *E. coli* and slightly higher than the target concentration for enterococci. Bioretention-S had a geometric mean effluent concentration higher than the USEPA allowable concentration for both *E. coli* and enterococci. Thirteen of 20 *E. coli* samples and 10 of 20 enterococci samples were lower than USEPA target values at the Bioretention-D outlet. Conversely, at the outlet of Bioretention-S, only 7 of 20 *E. coli* samples and 3 of 20 enterococci samples were lower than USEPA target values. Wilcoxon Signed Rank analyses showed no statistical difference between USEPA target concentrations and effluent indicator bacteria concentrations for Bioretention-D and statistically significant differences for both indicator bacteria for Bioretention-S ($p < 0.05$).

Table 2.2: Comparison of Wilmington, NC, bioretention cells to other field-analyzed sites

Cell Name	Statistic	# of Samples	fecal coliform (per 100 ml)		*E. coli* (per 100 ml)		enterococci (per 100 ml)		Reference
			inlet	outlet	inlet	outlet	inlet	outlet	
Bioretention - D	Geometric Mean	20	-	-	130	39	375	39	-
Bioretention - S	Geometric Mean	20	-	-	130	284	375	378	-
CP	Median	8	140	290	92	90	-	-	Li and Davis (2009)
SS	Median	5	8	2	5	58	-	-	Li and Davis (2009)
Bioretention	Geometric Mean	19 (14 for *E. coli*)	2420	258	241	20	-	-	Hathaway et al. (2009)
Rain Garden 1	n/a	6	< 10	< 10	-	-	-	-	Dietz and Clausen (2005)
Rain Garden 2	n/a	6	< 10	< 10	-	-	-	-	Dietz and Clausen (2005)
North Cell	Mean	7	4172	125	-	-	-	-	Passeport et al. (2009)
South Cell	Mean	4	4172	646	-	-	-	-	Passeport et al. (2009)
Bioretention Area	Median	9	-	-	-	-	400	20	Jones et al. (2008)

Effluent concentrations from Bioretention-D and Bioretention-S were also compared to those reported in other field studies in Table 6.2. Relatively few field studies have been performed for indicator bacteria removal in bioretention areas, particularly for *E. coli* and enterococci. However, Bioretention-D compared well to effluent concentrations reported in literature for both *E. coli* and enterococci. Conversely, Bioretention-S had substantially higher concentrations for both *E. coli* and enterococci.

Effluent probability plots were used for detailed comparison of indicator bacteria concentrations from all monitoring points (Figures 6.4 and 6.5). From these probability plots, differences in performance between Bioretention-D and Bioretention-S are observed, particularly in comparison to USEPA target indicator bacteria concentrations for fresh waters. Estimations of non-exceedance probability were made based on these probability plots. For Bioretention-D, non-exceedance probabilities for *E. coli* and enterococci were 63% and 47%, respectively. For Bioretention-S, non-exceedance probabilities for *E. coli* and enterococci were 42% and 10%, respectively.

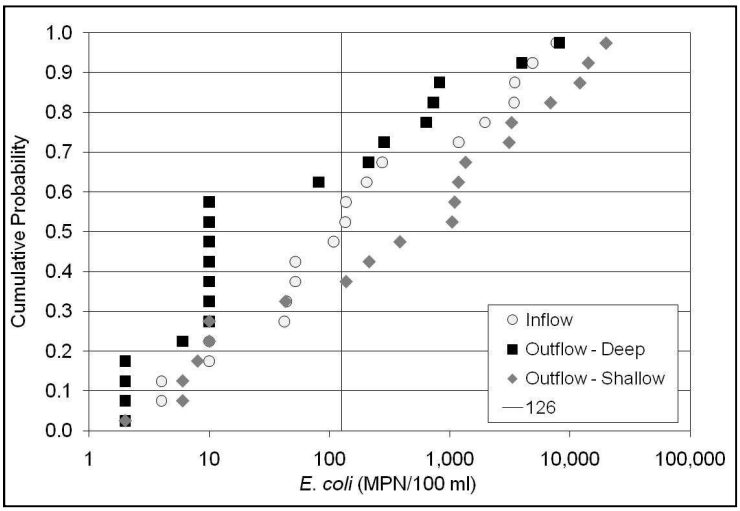

Figure 2.4: *E. coli* cumulative probability plot

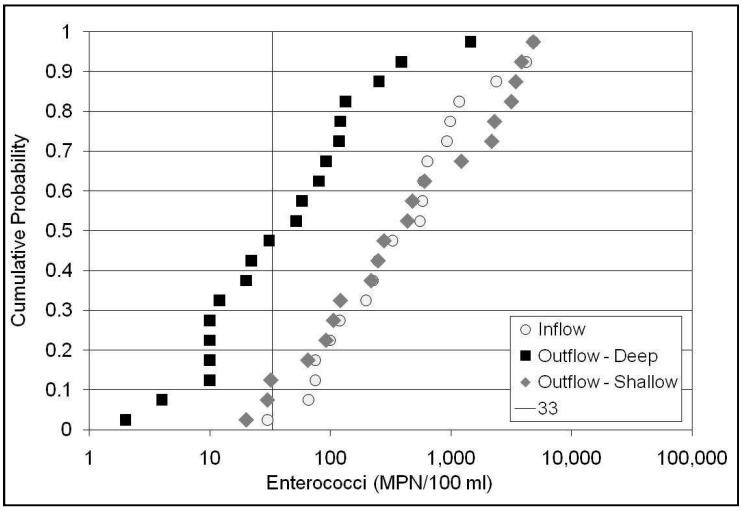

Figure 2.5: Enterococci cumulative probability plot

Based on concentration reductions, comparison to values reported in literature, and comparisons to USEPA standards, Bioretention-D clearly performed well relative to Bioretention-S. Differences in performance could be due to numerous factors which influence microbial survival, including temperature, soil moisture, soil chemistry, and hydrology (Ferguson 2003, Garbrecht et al. 2009). Thus, efforts were made to characterize various aspects of each bioretention area to determine potential explanations for the difference in performance between the two cells.

2.1.8 Hydrology

Hydrology within the two bioretention cells was characterized over 110 storm events from February 2007 to December 2009. Storm events ranged from 0.03 to 8.7 cm. Outflow typically did not occur for storms less than 1 mm. Qualitative observation of the effluent hydrographs from each of cells indicated differences in functionality between the two systems. Bioretention-S commonly exhibited a sharp spike in effluent flow soon after outflow began, followed by a rapid decline after peak flow was reached. Bioretention-D also exhibited relatively sharp spikes in outflow soon after outflow began; however, Bioretention-D typically had a longer time to peak and outflow continued after Bioretention-S flow had ceased. Obviously, variations in hydrographs were noted based on rainfall patterns. Such variations in outflow hydrograph are logical given the difference in soil depth in the two systems. The deeper Bioretention-D would provide better detention of runoff, allowing slow release over a longer period of time relative to Bioretention-S.

Similar volume reductions were noted for the two bioretention cells after summing total estimated inflow and outflow over the storms monitored. Bioretention-D and Bioretention-S provided infiltration of approximately 63% and 61% of monitored runoff, respectively. Outflow volume was normalized by watershed area for direct comparison of the two systems. Figure 6.6 shows the cumulative probability plot for effluent volume (normalized by area) for the two cells. Functionality of the two systems was similar throughout much of the probability plot, and a Wilcoxon Signed Rank analysis indicated no significant difference among normalized outflow from the two SCMs ($p < 0.05$). Some variation existed during smaller flow events, where the deep system produced outflow more often. This may be due to measurement errors at low flows or a proportionally higher volume delivery from the larger watershed during small events.

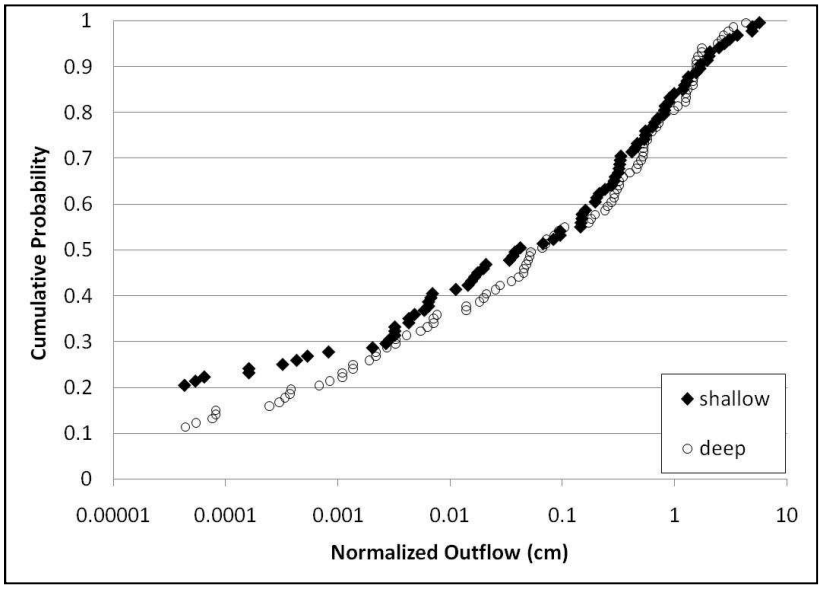

Figure 2.6: Effluent volume cumulative probability plot

Effluent peak flows from each cell were also normalized by watershed area to account for hydrologic differences in the influent runoff. The cumulative probability plot for normalized peak flow from the two bioretention cells is presented in Figure 6.7. Hydrologic differences between the two cells are more apparent based on Figure 6.7. Normalized peak flow from Bioretention-S was higher than that from Bioretention-D for a substantial proportion of storm events. This was supported by Wilcoxon Signed Rank analyses where statistical differences were found between normalized peak flow from the two cells ($p < 0.05$). Similar observations of increased peak flow mitigation with increased soil media depth in bioretention were made by Li et al. (2009).

These analyses are consistent with qualitative observations of the effluent hydrographs, where sharp peaks were noted for Bioretention-S. Thus, Bioretention-S seemed to support little detention of stormwater runoff. Studies such as Li and Davis (2009) suggest bioretention hydrology can influence pollutant removal by affecting such factors as contact time. Further, assuming similar infiltration capacities of the soils and ponding depths in the two cells, the substantially shallower Bioretention-S likely supports a higher flux and consequently, higher interstitial velocity. This may be important in explaining differences in performance between the two cells as retention of indicator bacteria has been shown to decrease as flow velocities increase in soil columns (Garbrecht et al. 2009).

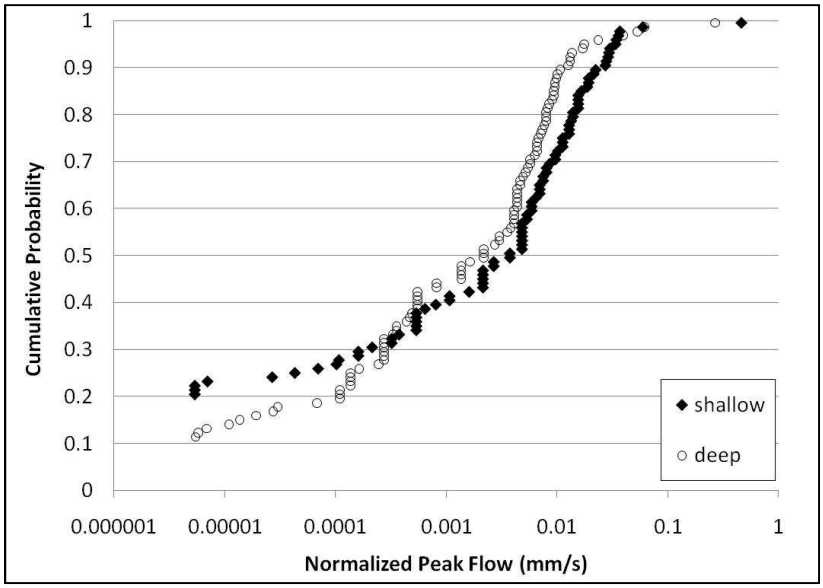

Figure 2.7: Effluent peak flow cumulative probability plot

2.1.9 Worm Hole Presence

While a study of worm presence was not conducted, potential worm burrows were quantified by filling the underdrains with smoke and identifying locations where smoke was leaving the soil surface in concentrated streams. Very few worm holes, or other conduits, were identified. Eight smoke releases were identified in Bioretention-S and 0 were found in Bioretention-D. This indicates Bioretention-S had slightly more direct connection from the surface to the underdrain through which water could short circuit, thus negating much of the filtering benefit offered by the soil media. However, the abundance of holes was not particularly high, and the results suggest only a modest potential for flow-through in the more shallow Bioretention-S.

2.1.10 Soil Temperature and Moisture

Temperature data were collected between January 2009 and October 2009. Equipment malfunctions left missing data for some portions of the monitoring period. Characterization of temperature and soil moisture probes was performed by averaging data collected at probes from two depths in each bioretention cell. Average monthly temperatures for Bioretention-D and Bioretention-S are presented in Table 6.3. As expected, the deeper Bioretention-D showed a lower, more buffered average temperature throughout the majority of the study. Average temperatures in Bioretention-S reached as high as 34.4^{o}C and as low as 7.0^{o}C, while

Bioretention-D reached as high as 31.3°C and as low as 7.6°C. Similarly, Jones and Hunt (2009) suggest deeper bioretention areas are more conducive to thermal control due to the temperature stability in deeper soils. Jones and Hunt (2009) also showed maximum soil temperatures above 30°C in monitored bioretention areas.

Table 2.3: Average monthly soil temperatures in Bioretention-S and Bioretention-D

Month	Bioretention-S Average Temperature (°C)	Bioretention-D Average Temperature (°C)
January	11.1	10.7
February	12.3	11.2
March	15.6	14.0
April	21.3	19.3
Mid-July[1]	28.5	26.3
August	31.0	28.7
Beginning-September[2]	28.8	27.2
End-October[3]	22.4	20.7

1: 7/7/2009 – 7/13/2009
2: 9/1/2009 – 9/4/2009
3: 10/24/2009 – 10/29/2009

It should be noted that some variability was observed between temperature probes placed at a depth of approximately 20 cm in each cell. This may be due to the presence of an underdrain immediately under a depth of 20 cm in Bioretention-S, potentially allowing warming of the soils due to ambient air entering the underdrain. Temperature variations may have also occurred due to measurement accuracy of the probes themselves. Nonetheless, the average temperature in the Bioretention-S cell over the entire monitoring period was found to be only 1.5 °C higher than Bioretention-D. The difference in hourly average temperature between the two SCMs was statistically significant ($p < 0.05$).

Soil moisture data were collected from January 2009 to January 2010. Equipment malfunctions left missing data during some portions of the monitoring period. Figure 6.8 shows the hourly average soil water content in both bioretention cells. The decline in soil moisture following storm events followed a similar pattern in both bioretention cells. Bioretention-S typically had higher soil moisture content throughout the study. Despite a smaller watershed area, Bioretention-S had a higher average runoff loading when inflow was normalized by soil volume.

Thus, more runoff was available to saturate soils within Bioretention-S. Average Bioretention-S water content was approximately 0.04 m^3/m^3 higher than that of Bioretention-D. The difference in average hourly soil moisture between the two SCMs was statistically significant ($p < 0.05$).

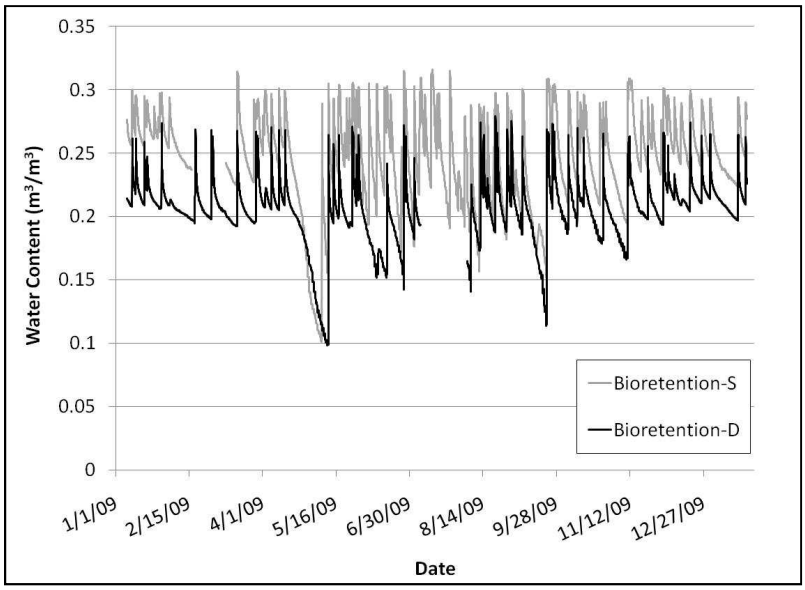

Figure 2.8: Average hourly soil moisture in Bioretention-S and Bioretention-D

A laboratory evaluation of *E. coli* in soils by Chandler and Craven (1980) identified moisture availability as a crucial factor affecting survival. Byappanahalli et al. (2006) analyzed beach sand amended with plankton in laboratory studies, showing significant increases in *E. coli* over the first 24 hours of the study followed by a gradual return over the next 6 days to levels approximately 1-log over initial concentrations. Byappanahalli et al. (2006) theorized that indicator bacteria in moist soils have the potential to grow during warm summer months when nutrients are available. Similar assertions were made by Byappanahalli and Fujioka (1998) regarding *E. coli* in Hawaiian soils, where adequate temperature, moisture, and nutrients conditions appeared to allow growth. Thus, the warmer and wetter soil environment present in Bioretention-S (relative to Bioretention-D) may provide a more favorable environment for indicator bacteria growth. However, influences on microbial growth are complex and involve numerous factors. In a review of transport and fate processes involving microbes, Ferguson et al. (2003) concluded that few studies have examined more than one or two factors simultaneously. Further, confounding relationships in literature are present in regard to microbial persistence vs. microbial growth. Kibbey et al. (1978) showed S. faecalis survival

(persistence) was prolonged as soil temperature *decreased* and soil moisture increased. Thus, it is unknown to what degree relatively small variations in soil temperature and water content influence indicator bacteria concentrations in bioretention areas. Additionally, soil moisture conditions likely vary spatially within bioretention areas depending on underdrain location, bioretention slope (if present), and inlet location. Overall, differences in temperature and soil moisture in the two systems appeared relatively minor, but slightly favored microbial growth and/or persistence in Bioretention-S.

2.1.11 Soil Properties

Although soils were characterized for numerous soil properties (Table 6.4), not all are directly related to microbial performance. Microbial sequestration is influenced by soil properties such as pH and soil type (Ferguson et al. 2003). Soil pH varied from 6.4 to 8.0 in Bioretention-S and from 7.6 to 8.0 in Bioretention-D, with average pH being 7.5 and 7.9 in Bioretention-S and Bioretention-D, respectively. Thus, differences in soil pH appeared relatively minor. Coyne (1999) describes a pH range of 6 to 7 as optimal for *E. coli*, with a pH of 9 as a maximum. Similar data were reported for soils from two bioretention areas studied by Li and Davis (2009), where pH for the two cells was reported as 7.3 and 7.7. Overall, soils were similar between the two cells with regard to phosphorus index, cation exchange capacity, humic matter, and pH, all of which are common parameters used to describe soils.

As previously noted, bioretention cells studied in Wilmington, NC, had high sand content and relatively low fines (clay and silt) relative to studies by Li and Davis (2009) and Dietz and Clausen (2005). This likely influenced the performance of the cells in regard to indicator bacteria sequestration. Column studies by Mankin et al. (2007) utilized sand and silt loam soils to test *E. coli* sorption, finding greater sorption for silt loam soils. Studies by Garbrect et al. (2009) also evaluated differences in soil type with regard to *E. coli* sequestration. Garbrect et al. (2009) demonstrated enhanced performance by loamy sands (84.5% sand) in comparison to course sands (100% sand). Soils in Bioretention-S and Bioretention-D were very similar in regard to distribution between sand, silt, and clay. Thus, differences in performance based on soil type do not seem to be logical.

Table 2.4: Soil properties of Bioretention-S and Bioretention-D

Location		Approximate Depth Range Collected From (cm)	Cation Exchange Capacity (meq / 100 cm^3)	Soil pH	Phosphorus Index	Humic Matter (%)
Bioretention-S	Site 1	0 - 7.6	11.4	6.4	34	0.3
		20.3 - 30.5	11.2	7.4	41	0.3
	Site 2	0 - 7.6	8.4	7.6	23	0.3
		14.0 - 24.1	4.1	7.8	13	0.1
	Site 3	0 - 7.6	14.8	7.8	24	0.2
		10.2 - 20.3	8.7	8	12	0.2
Average =			**9.8**	**7.5**	**24.5**	**0.2**
Bioretention-D	Site 1	0 - 7.6	7.2	8	20	0.3
		17.8 - 22.9	6	8	17	0.3
		48.3 - 53.3	32.6	8.2	9	0.1
	Site 2	0 - 7.6	12.9	7.7	41	0.5
		17.8 - 22.9	3.2	7.6	21	0.4
		58.4 - 63.5	7.1	8	19	0.2
	Site 3	0 - 7.6	13.2	7.8	56	0.5
		22.9 - 30.5	11.1	7.9	49	0.4
		55.9 - 61.0	9.2	7.9	31	0.4
Average =			**11.4**	**7.9**	**29.2**	**0.3**

2.1.12 Soil Indicator Bacteria

Multiple collection tubes were used to sample the soil profile in each bioretention cell. Indicator bacteria were measured at the top and bottom of each tube; however, data presented in Tables 6.5 and 6 include only the top and bottom of the first tube and the bottom of each tube thereafter. Approximate depths are presented for each reading, but actual depths varied based on sampling date.

Data were spatially and temporally variable for both *E. coli* and enterococci, making trends difficult to identify. However, some observations can be made. First, enterococci soil bacteria concentrations were consistently and markedly higher than *E. coli* concentrations. Consistent relationships were even more difficult to find in enterococci data. Enterococci are known to be more persistent in the environment (USEPA 2001), potentially leading to higher concentrations. It should also be noted that the geometric mean inlet enterococci concentration was higher than that of *E. coli*. Additionally, geometric mean Bioretention-S effluent enterococci

concentrations were higher than those of *E. coli*. Studies such as Hartel et al. (2005) showed the ability of enterococci to regrow after desiccation and rewetting, which would commonly occur in bioretention systems.

Table 2.5: Soil enterococci concentrations in Wilmington, NC, bioretention areas

SCM	Location	Approximate Depth (cm)	enterococci (MPN / g)			
			12/15/2008[1]	3/5/2009[2]	6/1/2009[3]	8/4/2009[4]
Bioretention-S	Site 1	5 - 7.5	> 242.0	101.1	> 242.0	> 242.0
		20	> 242.0	101.1	> 242.0	14.6
		30	> 242.0	96.1	> 242.0	130.0
	Site 2	5 - 7.5	> 242.0	31.3	> 242.0	11.0
		23	92.1	25.1	43.5	3.7
	Site 3	5 - 7.5	43.5	101.1	1.7	4.5
		23	32.6	0.3	8.7	17.3
Bioretention-D	Site 1	5 - 7.5	> 242.0	101.1	> 242.0	6.5
		24	> 242.0	24.0	11.6	21.4
		44	242.0	91.4	3.4	242.0
		53	> 242.0	33.0	3.4	7.4
	Site 2	5 - 7.5	> 242.0	101.1	4.4	25.0
		25	> 242.0	68.9	5.3	64.9
		47	62.9	57.5	16.8	77.0
		61	96.1	96.1	4.2	46.1
	Site 3	5 - 7.5	> 242.0	101.1	> 242.0	> 242.0
		22	198.6	101.1	> 242.0	> 242.0
		39	> 242.0	96.1	196.6	34.5
		51	> 242.0	101.1	-	-

1: Antecedent dry period: 3.5 days, Average temperature preceding 7 days: 11.8^{o}C

2: Antecedent dry period: 4 days, Average temperature preceding 7 days: 6.3^{o}C

3: Antecedent dry period: 2.5 days, Average temperature preceding 7 days: 23.7^{o}C

4: Antecedent dry period: 0.5 days, Average temperature preceding 7 days: 27.3^{o}C

E. coli concentrations were commonly higher in the upper portion of the soil column. Analysis of indicator bacteria in river bank soils by Desmarais et al. (2002) also suggested a decline in *E. coli* concentration with soil depth, yet a less appreciable decline for enterococci. This is consistent with the general understanding that microbial abundance is highest in the top of the soil profile due, in part, to availability of oxygen and nutrients (Coyne 1999). Despite some patterns which could roughly be identified, there is not sufficient evidence to suggest indictor bacteria concentrations are substantially different in the soil of either bioretention cell.

Table 2.6: Soil *E. coli* concentrations in Wilmington, NC, bioretention areas

SCM	Location	Approximate Depth (cm)	*E. coli* (MPN / g)			
			12/15/2008[1]	3/5/2009	6/1/2009	8/4/2009
Bioretention-S	Site 1	5 - 7.5	> 242.0	0.5	2.8	< 0.1
		20	242.0	0.9	0.3	< 0.1
		30	173.3	1.7	0.2	0.1
	Site 2	5 - 7.5	11.9	0.6	0.6	< 0.1
		23	4.6	0.4	1.9	< 0.1
	Site 3	5 - 7.5	0.2	< 0.1	< 0.1	0.1
		23	< 0.1	< 0.1	1.1	< 0.1
Bioretention-D	Site 1	5 - 7.5	0.1	0.5	8.4	< 0.1
		24	< 0.1	0.2	2.5	< 0.1
		44	< 0.1	< 0.1	0.1	0.1
		53	< 0.1	0.2	1.1	< 0.1
	Site 2	5 - 7.5	0.5	0.5	1.4	0.2
		25	0.4	0.1	1.0	0.5
		47	< 0.1	1.4	2.9	0.1
		61	0.3	2.4	0.6	< 0.1
	Site 3	5 - 7.5	< 0.1	0.1	24.9	< 0.1
		22	< 0.1	1.7	36.5	< 0.1
		39	< 0.1	< 0.1	1.2	< 0.1
		51	< 0.1	< 0.1	-	-

1: Antecedent dry periods and temperatures for each sampling date presented in Table 5

Laboratory analysis of bioretention soil columns by Rusciano and Obropta (2007) indentified fecal bacteria primarily in the top 5.1 cm of soil. However, indicator bacteria were present throughout the soil profiles on some sampling dates at the Wilmington, NC, SCMs. No other field studies have been performed where indicator bacteria concentrations were evaluated within bioretention soils, making comparisons to other field sites impossible. However, it appears that although variability in concentrations were noted between sampling dates, indicator bacteria can be present in the soils of bioretention areas after storm events. Similarly, persistence has been show in stream and estuarine sediments by Sherer et al. (1992) and Jeng et al. (2005). Thus, bacteria are likely present and available to persist, potentially regrow, and be exported during subsequent events. Regrowth studies performed on indicator bacteria in river bank soils by Desmarais et al. (2002) showed increases in concentrations during the first 24 hours of laboratory experiments. Conversely, studies such as Zhang et al. (2008) showed rapid declines in *E. coli* concentration in bioretention columns studied at the laboratory scale. Thus, understanding microbial ecology within bioretention systems is an area of research need within the stormwater management community (Li and Davis 2009). Microbial survival in soils is

dependent on numerous environmental factors including (but not limited to) temperature, soil moisture, and predation (Ferguson 2003, Desmarais et al. 2002). Thus, soil conditions after a rain event likely influence persistence and may also influence the ability to identify relationships in these data. It is apparent that higher resolution field studies are required to investigate microbial relationships in bioretention soil than available in these data. Characterizations cannot be made based on only one or a limited number of sampling dates based on the variability present in these data. Also, there are likely environmental phenomena which occur in field studies that cannot be easily replicated in laboratory analyses.

2.1.13 Synthesis of Data and Design Implications

Bioretention-D and Bioretention-S showed differing levels of indicator bacteria sequestration based on multiple performance analysis metrics. Soil chemistry and other soil properties of the two bioretention cells were similar. Higher average temperature and soil moisture within the shallower Bioretention-S suggests the potential for greater regrowth; however, because differences were relatively minor, the authors minimize the significance of these differences in explaining the contrast in performance between the systems. Further, no substantial differences in indicator bacteria concentrations within the two system's soils could be established, suggesting neither is more prone to microbial persistence and/or regrowth (although more research is needed to study microbial interactions in bioretention).

Thus, the difference in soil depth was identified as the most likely factor affecting performance of the two SCMs. Soil depth can influence multiple facets of bioretention function. In addition to reductions in contact time as suggested by Li and Davis (2009), higher soil water flux prevails in shallow systems (assuming similar hydraulic head). Bioretention-S was found to exhibit poorer mitigation of peak flow despite relatively similar performance between the two systems in regard to volume reduction. This suggests poorer detention of flow relative to Bioretention-D. These physical characteristics have implications for microbial performance, as column studies by Garbrect et al. (2009) showed decreased sequestration of *E. coli* occurred in coarse sand columns with increased flow velocity, and column studies by Bright et al. (accepted) demonstrated that total coliform concentrations leaving sand columns decreased over time as infiltration rates decreased.

The influence of hydrologic function in bioretention cells likely varies based on soil type. For instance, observations on *E. coli* sequestration in soil columns by Garbrect et al. (2009) indicated varied performance based on soil type (coarse sand vs. loamy sand). Similar observations were made on soil columns by Mankin et al. (2007), where silt loam was found to have a greater capacity for *E. coli* retention than sand. Therefore, it should be restated that the SCMs studied in Wilmington, NC, had a lower percentage of soil fines than studies by Li and Davis (2009) and Dietz and Clausen (2005). However, soil fines were found to be 8 to 10%, which is within the State of North Carolina's acceptable range for bioretention media (NCDENR 2007).

Determining appropriate bioretention media depth and composition is an ongoing area of research (Davis et al. 2009). Shallow media depths offer economic benefits, as soil media can be a substantial portion of the cost associated with bioretention construction. Further, design guidance such as Hunt and Lord (2006) suggested that shallow bioretention depths are adequate for pathogen/indicator bacteria sequestration due to the supposition that they are removed at the bioretention surface. This assertion was supported by such studies as Rusciano and Obrotpa (2007), where fecal bacteria were only found in the top 5.1 cm of bioretention soil columns in laboratory analyses. However, the data herein suggest indicator bacteria can be present lower in the soil profile, leaving them available for later export from the system. Nonetheless, as bioretention media depth becomes more shallow, a higher fraction of the soil profile consists of depths generally considered to have the greatest abundance of microbes (Coyne 1999). This, in turn, leaves less soil available for indicator bacteria capture prior to their exiting the bioretention system.

Economics, hydrologic performance, and water quality function must be balanced when determining appropriate bioretention depth. For indicator bacteria sequestration, these data suggest a minimum bioretention depth does exist. Specifically, the authors suggest bioretention should not be less than 60 cm deep when indicator bacteria are a concern. This depth will provide better detention of runoff and decrease soil water flux. It is possible that inclusion of a higher percentage of fines would lead to better performance for indicator bacteria even in very shallow bioretention; however, hydrologic function may be compromised.

Conclusions

Two bioretention cells were monitored in Wilmington, NC, in a paired-watershed experimental design. The two cells had two different soil depths: Bioretention-D with a soil depth of 60 cm and Bioretention-S with a soil depth of 25 cm. Concentration "reductions" in Bioretention-D and Bioretention-S were 70% and -119% for *E. coli* and 89% and -102% for enterococci, respectively. Geometric mean effluent *E. coli* and enterococci concentrations from Bioretention-D compared relatively well to USEPA target values and to values reported in literature for bioretention. Conversely, geometric mean effluent *E. coli* and enterococci concentrations from Bioretention-S did not compare well to USEPA target values or those reported in literature. Thus, based on multiple performance evaluation metrics, Bioretention-D clearly outperformed Bioretention-S.

Evaluation of multiple factors which could impact performance in the systems was conducted. Soil type and soil chemistry were found to be quite consistent between the two bioretention cells. Conversely, soil temperature and soil moisture were found to be slightly higher in Bioretention-S, potentially leading to slightly more favorable conditions for regrowth. However, no substantial differences in indicator bacteria abundance could be identified in bioretention soils over 4 sampling events. Thus, the most logical difference between the two SCMs, which could lead to differences in performance, is soil depth. Bioretention-S was found to have poorer detention of runoff, and the shallow system would logically result in higher soil water flux. Higher velocity of runoff passing through the system should result in less contact time and potential stripping of bacteria from the soil matrix.

Although shallow bioretention cells offer numerous design and economic benefits, they may have functional limitations with respect to indicator bacteria. These data suggest design of bioretention for indicator bacteria removal should include a soil media depth of at least 60 cm. This will result in greater indicator bacteria sequestration and runoff detention.

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A. Appendix A: Bioretention performance for indicator bacteria in Raleigh, NC

Introduction

Bioretention areas have shown promise in treating stormwater runoff for indicator bacteria; however, these practices have not been studied in depth in peer reviewed literature, particularly with flow weighted monitoring. An evaluation of bioretention using flow weighted samples will provide more accurate insight into their ability to remove indicator bacteria. The objective of this study was to evaluate the performance of a bioretention area receiving runoff from a stormwater "hot spot" utilizing a flow paced sampling methodology.

Site Description

The research site is located in Raleigh, NC, at the City of Raleigh Street Maintenance Yard (SMY). The bioretention area treats a watershed of approximately 1.24 acres. The watershed is nearly 100% impervious and incurs heavy traffic from street maintenance vehicles. The bioretention has a depth of 4 feet with media adhering to typical specifications set forth by the State of North Carolina (Figure A.1). Underdrains were placed in the bioretention area to provide drawdown of the system.

Figure A.1: SMY bioretention area

Monitoring Scheme

Due to the relatively high length to width ratio of the system, and perceived heterogeneity of runoff from the SMY watershed, two inlet monitoring locations were utilized to characterize stormwater runoff entering the system. One inlet was considered to drain a relatively "clean" region of the watershed (inlet 1), while the other inlet was fed by a portion of the watershed perceived as more polluted (inlet 2). Each inlet monitoring point was equipped with a small, metal flume to facilitate pooling of stormwater runoff and allow sample collection (Figure A.2). Sampler tubing was secured into the flume and directed to an ISCO Avalanche refrigerated sampler. A plastic container was placed in each sampler to collect samples. Samplers were initialized and paced by way of ISCO rain gages installed on site.

Figure A.2: Sample collection flume at inlet 2

The outlet of bioretention area was a 12-inch corrugated plastic pipe. The pipe was fitted with a v-notch weir to allow flow measurements (Figure 3). Flow was determined using standard weir equations and stage data collected via a bubbler fixed in the outlet pipe. An Avalanche refrigerated sampler was utilized for sample collection at the outlet. A plastic container was placed in each sampler to collect samples. Flow pacing was adjusted prior to storms to allow adequate sample collection over the entire rainfall event.

Tubing and sample collection bottles from all samplers were removed in between storm events, cleaned, and autoclaved to maintain sterility. Samples were transported to the Department of Biological and Agricultural Engineering for microbial analysis. Bacteria analyses were performed

within 24 hours of sample collection. Fecal coliform and *E. coli* were enumerated using Colilert (defined substrate technologies; IDEXX, Westbrook, Maine). The Colilert method was modified to detect fecal coliform and *E. coli* by incubating the samples at 37°C for 1 to 3 hours followed by incubation at 44.5°C for 21 to 23 hours. Positive (stock culture) and negative (dilution blank) controls were used during laboratory analyses. Samples required between a 10:1 and 10,000:1 dilution due to high bacteria counts.

Figure A.3: Weir installed at bioretention outlet

Results and Discussion

Samples were collected between July 2009 and September 2009. Ten storms were monitored over this period, ranging from 0.25 to 1.20 inches in depth. Although sample pacing utilizing rainfall is a justifiable methodology for collecting runoff from systems with sheet flow, the inlet samplers did not perform well at the SMY in terms of collecting multiple samples throughout the course of each storm. At inlet 1, two or more aliquots of runoff were typically collected. At inlet 2, more than 4 aliquots were typically collected. Geometric mean fecal coliform and *E. coli* concentrations were 9654 MPN/100 ml and 213 MPN/100 ml for inlet 1, respectively. Geometric mean fecal coliform and *E. coli* concentrations were 626,161 MPN/100 ml and 182,026 MPN/100 ml for inlet 2, respectively. Concentrations at inlet 1 were comparable to microbial concentrations in stormwater runoff studied by Hathaway et al. (2009). However, microbial concentrations at inlet 2 were high, as was expected due to the contributing area to that monitoring location. Outlet concentrations fell between those of inlet 1 and inlet 2, with fecal coliform and *E. coli* geometric mean concentrations of 53294 MPN/100 ml, and 2099

MPN/100ml, respectively. Concentrations for all storms and summary statistics are presented in Table A.1 and Table A.2.

Table A.1: Fecal coliform concentrations for the SMY bioretention area

Date	rainfall (in)	fecal coliform (MPN / 100 ml)		
		inlet	inlet 2	outlet
7/13/2009	0.34	240,000	240,000	79,265
7/17/2009	0.85	n/a	n/a	240,000
7/23/2009	0.49	9,208	240,000	32,095
7/31/2009	0.41	14,881	3,778,760	72,287
8/6/2009	0.25	10,958	410,741	67,392
8/22/2009	0.30	4,994	1,137,610	662,953
8/28/2009	0.43	1,990	3,386,785	2,747
8/31/2009	0.36	36,501	2,137,627	100,322
9/7/2009	1.20	1,181	32,095	27,952
9/16/2009	0.31	4,722	n/a	12,168
Geometric mean =	**0.44**	**9654**	**626161**	**53294**
St. Dev. =	**0.30**	**77230**	**1500305**	**199143**

Table A.2: *E. coli* concentrations for the SMY bioretention area

Date	rainfall (in)	*E. coli* (MPN / 100 ml)		
		inlet	inlet 2	outlet
7/13/2009	0.34	55,875	240,000	8,439
7/17/2009	0.85	n/a	n/a	9,006
7/23/2009	0.49	100	399,452	336
7/31/2009	0.41	273	3,581,132	1,990
8/6/2009	0.25	351	110,642	1,470
8/22/2009	0.30	20	70,280	3,994
8/28/2009	0.43	41	1,616,136	12,070
8/31/2009	0.36	2,077	160,523	3,387
9/7/2009	1.20	10	1,740	555
9/16/2009	0.31	98	n/a	244
Geometric mean =	**0.44**	**213**	**182026**	**2099**
St. Dev. =	**0.30**	**18513**	**1249149**	**4215**

Effluent concentrations of both fecal coliform and *E. coli* were high relative to other bioretention studies (See Table 2.2). These high concentrations may be related to the very high concentration of microbes entering the bioretention cell as noted for inlet 2. Bioretention areas

receiving a high magnitude of microbes have not been evaluated in scientific literature, making comparisons impossible. However, these observations warrant further study. Effluent microbe concentrations were substantially lower than those at inlet 2, and typically higher than those at inlet 1. Inlet 2 is located closer to the outlet that inlet 1. Thus, stormwater entering in this location potentially received less treatment (due to a smaller contact time). Due to the mixing of runoff representative of inlets 1 and 2, it is also possible that dilution played an important role in "reducing" effluent concentrations from the concentrations of inlet 2.

It should be noted that the concentrations in Tables A.1 and A.2 vary from grab sample collection of influent and effluent flows by the City of Raleigh. Grab sampling performed by the city from July 2008 to October 2010 resulted in geometric mean *E. coli* concentrations of 3833 cfu/100ml at inlet 2 and 95 cfu/100ml at the outlet. Such differences cannot be fully explained; however, possible explanations include (1) collection of samples in this study only during the summer when bacteria concentrations have been shown to be higher, and (2) differences in collection methods (composite vs. grab sampling).

Conclusions

Based on sampling performed by the Department of Biological and Agricultural Engineering at North Carolina State University, the SMY bioretention area received higher microbial concentrations than other bioretention areas evaluated in scientific literature. Likewise, effluent concentrations from the bioretention area were higher than observed in other studies. The change in functionality of bioretention areas with varied influent concentration is not well understood, particularly for indicator bacteria. Thus, further study is warranted in this area. The degree to which bioretention can mitigate high concentrations of indicator bacteria influences it's ability as a stormwater control measure for stormwater "hot spots."

B. Appendix B: Raw data from Wilmington, NC, stormwater control measures

Table B.1: Wet Pond 1 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
6/23/2008	988	148	5475	10462
8/13/2008	2851	31	504	63
8/27/2008	> 24196	19863	> 24196	> 24196
9/25/2008	6310	41	> 24196	388
1/13/2009	4839	40	4,839	2
2/18/2009	651	< 2	330	13
4/2/2009	403	2	> 4839	83
5/14/2009	255	40	278	12
8/12/2009	15531	521	496	62
8/14/2009	1226	387	6940	3106
9/22/2009	5794	731	344	234
10/5/2009	3466	8	2098	168
11/10/2009	4611	2	12997	3973
11/11/2009	6488	19863	10112	4374
2/2/2010	1633	8	1314	32
2/9/2010	2092	1633	2599	2755

Table B.2: Wet Pond 2 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
2/18/2008	2909	697	8664	1483
2/22/2008	< 10	520	52	20
3/7/2008	3130	10	160	20
6/23/2008	613	399	364	97
8/13/2008	3649	31	31	< 10
8/27/2008	> 24196	521	> 24196	1240
9/25/2008	8840	< 10	1414	< 10
1/13/2009	1095	< 10	134	< 8
2/18/2009	3106	6	88	< 2
4/2/2009	582	6	2	4
5/14/2009	3106	1373	2240	1633
8/12/2009	81640	24	242	120
8/14/2009	192	120	197	49
9/22/2009	2068	3466	97	870
10/5/2009	90	8	27	< 2
11/10/2009	> 24196	172	11199	159
11/11/2009	19863	14136	1212	725
2/2/2010	55	< 2	150	< 2
2/9/2010	74	37	245	6

Table B.3: Wetland 1 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
2/18/2008	785	1017	> 24196	1657
2/22/2008	697	41	467	146
3/7/2008	697	61	723	30
6/23/2008	75	752	12033	29090
8/13/2008	2760	311	61	< 10
8/27/2008	9210	10500	8660	> 24196
9/25/2008	14136	2909	4360	3609
1/13/2009	870	3973	2407	1632
2/18/2009	158	51	225	14
4/2/2009	1160	303	135	21
5/14/2009	1540	1317	821	284
8/12/2009	403	1633	86	139
8/14/2009	3973	1842	6870	5640
9/22/2009	409	9804	1373	333
10/5/2009	259	36540	1540	2755
11/10/2009	6488	> 24196	2827	1785
11/11/2009	2613	6488	935	957
2/2/2010	75	49	74	< 2
2/9/2010	182	17	387	15

Table B.4: Wetland 2 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
1/17/2008	-	-	866	172
2/18/2008	256	199	1892	1594
2/22/2008	41	41	738	1198
3/7/2008	160	317	842	512
6/23/2008	771	723	1350	3950
8/13/2008	< 10	52	201	< 10
8/27/2008	5790	3870	> 24196	> 24196
9/25/2008	323	323	4360	2500
1/13/2009	731	449	2599	449
2/18/2009	2599	731	250	86
4/2/2009	2240	110	3973	22
5/14/2009	456	76	227	29
8/12/2009	690	11199	166	108
8/14/2009	403	521	3973	8350
9/22/2009	2092	4839	690	690
10/5/2009	651	11199	241	2105
11/10/2009	9804	> 24196	1373	9804
11/11/2009	960	933	250	448
2/2/2010	26	6	140	12
2/9/2010	46	279	182	1842

Table B.5: Bioretention – raw data

Date	*E. coli*			enterococci		
	inlet	outlet - Bioretenion-S	outlet - Bioretention-D	inlet	outlet - Bioretenion-S	outlet - Bioretention-D
2/22/2008	203	3255	< 10	591	2187	31
3/72008	10	1043	< 10	75	279	< 10
6/23/2008	1187	12033	8164	983	249	134
8/13/2008	135	384	< 10	328	480	< 10
8/27/2008	< 10	213	< 10	552	3870	121
9/25/2008	52	1350	< 10	638	2310	52
11/3/2008	108	< 10	638	119	20	389
11/13/2008	3433	19863	211	30	1223	20
1/13/2009	42	137	6	197	437	4
2/18/2009	< 2	6	10	225	32	10
4/2/2009	52	43	< 2	> 4839	30	12
5/14/2009	44	1095	3973	75	3466	118
8/12/2009	275	3106	81	99	92	58
8/14/2009	3466	14136	2	4210	3185	80
9/22/2009	7701	6867	731	247	218	92
10/5/2009	137	10	284	922	106	253
11/10/2009	1961	< 2	821	2382	4839	1454
11/11/2009	4884	1178	< 10	1174	605	< 10
2/2/2010	4	8	< 2	582	65	< 2
2/9/2010	4	6	< 2	66	121	22