Exploring fecal indicator bacteria in a constructed stormwater wetland


ABSTRACT

Microbial pollution in surface waters is a concern throughout the world, with both public health and economic implications. One contributing source to such pollution is stormwater runoff, often treated using various types of stormwater control measures. However, relatively little is known regarding microbe sequestration in constructed stormwater wetlands (CSWs), one type of commonly installed stormwater control measure. In this study, indicator bacteria concentrations in both the water and sediment of a CSW were evaluated at multiple locations. Results suggested that fecal coliform concentrations in stormwater runoff decrease through the system, with relatively consistent concentrations noted throughout the second half of the wetland. This potentially indicates a baseline concentration of fecal coliform is present due to internal processes such as animal activity and microbial persistence. However, wetland sediments showed little E. coli present during most sampling events, with minimal patterns existing with respect to sediment sampling location. CSW designs should promote optimization of hydraulic retention time and minimization of stormwater velocities to promote sedimentation and degradation of microbes by way of wetland treatment functions.

Key words | fecal coliform, microbe, sediment, stormwater, wetland

INTRODUCTION

Contamination of surface waters by pathogens is a concern in North America, Europe, Australasia, and other regions throughout the world. Microbial quality in surface waters is typically evaluated based on target concentrations of fecal indicator bacteria such as fecal coliform, E. coli, and enterococci. Such indicator bacteria denote the presence of fecal matter and, thus, the possible existence of pathogens.

Stormwater runoff has been identified as a contributor to indicator bacteria in surface waters, with microbial concentrations commonly exceeding surface water quality standards (McCarthy et al. 2008; Hathaway et al. 2010). Stormwater runoff is often treated by implementing stormwater control measures (SCMs). SCMs are also known as sustainable urban drainage systems (SUDS), and water sensitive urban designs (WSUDs).

One commonly utilized SCM is the constructed stormwater wetland (CSW). While not yet thoroughly studied for fecal indicator bacteria sequestration, CSWs are understood to treat nutrients, sediment, and metals (Greenway 2004; Kohler et al. 2004; Hathaway & Hunt 2010). Further, Vymazal (2005) showed wetlands receiving wastewater to be effective for indicator bacteria treatment, finding 95 to >99% removal of total and fecal coliforms for 60 constructed wetlands. CSWs differ from wastewater wetlands as they accept lower concentrations of pollutants, receive inflow inconsistently, and experience more frequent drying-wetting cycles. Research performed on CSWs by Birch et al. (2004), Davies & Bavor (2000), and Hathaway et al. (2009) showed fecal coliform removal ranging between 56 and 98%, with a wide range of mean effluent concentrations (between 106 and 41,369 fecal coliforms/100 ml). Hathaway et al. (2009) reported E. coli removals of 56% and 33% for two stormwater wetlands, with geometric mean effluent concentrations of 106 and 864 MPN/100 ml, respectively. Despite somewhat favorable results from these studies, high variability in results was noted. Indicator bacteria fate and transport in CSWs is not well understood. For instance, most studies establishing CSW performance for indicator bacteria have involved only collecting samples at the wetland inlet and outlet. Understanding changes in...
concentrations throughout the wetland may lead to more effective designs for CSWs and a better understanding of the variability in performance noted in literature.

There are numerous removal mechanisms and environmental factors in CSWs which influence microbial dynamics. Temperature, exposure to sunlight, predation, sorption, and sedimentation all contribute to wetland sequestration of indicator bacteria and pathogens (Vymazal 2005; Struck et al. 2008; Kadlec & Wallace 2009). However, persistence of indicator bacteria has been noted in wetland sediments (Stenström & Carlander 2001; Karim et al. 2004). Thus, indicator bacteria may be available for resuspension and release from wetland systems (Perkins & Hunter 2000; Stenström & Carlander 2001; Ghermandi et al. 2007). Resuspension is of particular concern in CSWs, where erosive flows are common for high energy influent stormwater. Indicator bacteria concentrations in CSW sediments were explored by Stenström & Carlander (2001) and Davies & Bavor (2000); however, no analysis was provided as to differences in concentrations per characteristics of the sampling location (i.e. wetland zone). Common CSW zones are described by Hunt et al. (2007) as pools (water deeper than 30 cm), shallow water (water depth between 0 and 15 cm), and temporary inundation (water level only reaches zone during storm events). These wetland zones may differ in their ability to promote microbial die-off due to differences in drying/wetting cycles, soil moisture, and sunlight exposure. Design modifications to enhance die-off may be possible if a given wetland zone is less favorable for indicator bacteria persistence.

Further exploration of CSW dynamics may lead to more effective designs for microbe sequestration. The purpose of this study was to (1) determine the overall microbe sequestration efficiency of a CSW in Lenoir, NC, USA, and explore changes in indicator bacteria concentrations at various sampling points within the wetland, (2) explore relationships between total suspended solids and indicator bacteria to evaluate sedimentation and resuspension in CSW systems, and (3) evaluate differences in soil indicator bacteria concentrations based on location within the wetland.

**MATERIALS AND METHODS**

**Site description**

The CSW evaluated herein was located in Lenoir, NC, USA. The drainage catchment was approximately 36 ha and consisted primarily of residential area. The wetland was 0.9 ha with a design based on guidance by Hunt et al. (2007), including varied topography and emergent vegetation. The site was operational in January 2009, and was planted with vegetation in April 2009. Soils in the project location and in the surrounding area were predominately loamy (loams and fine sandy loams). A 10 cm layer of topsoil was added to the wetland bottom after final grading to provide substrate for vegetative growth.

**Monitoring methods**

**Surface water**

Grab samples were collected at the inlet, outlet, and three additional points along the flow path of the wetland. All samples were typically collected over a 20- to 30-minute period. Samples were collected during both storm flow and base flow, and sterile vessels were utilized for all microbial samples. Samples were placed on ice and delivered to the City of Lenoir Wastewater Treatment Plant for analysis of fecal coliform and total suspended solids (TSS) within 24 hours of collection. Fecal coliform was determined using a membrane filter methodology (SM 9222d) and TSS was determined using a gravimetric methodology (SM 2540d – APHA, AWWA & WEF 1998). The wetland inlet received constant base flow, allowing grab samples during base flow conditions. Samples were deemed base flow if collected at least 2.5 days after any rainfall greater than 1.3 mm. Storm flow samples were collected from approximately the thalweg of the wetland at all locations. Sampling points inside the wetland were all located immediately after a pool. The distance from the wetland inlet to points 1, 2, 3, and the outlet were approximately 64, 244, 345, and 438 m, respectively. Time of sampling, in context of the storm event, varied; however, all samples considered in the analyses were taken either during a storm or within 6 hours of the end of rainfall, when storm-related inflow was still occurring.

**Soil sampling**

Soil samples were collected on four occasions, once per season. A 10 cm auger equipped with clean plastic sampling tubes was used to take soil cores from the wetland bottom. Samples were collected from the inlet pool, outlet pool, and from each of the three internal monitoring points. At each internal monitoring point, separate samples were collected from a pool, shallow water, and temporary inundation zone. Equipment was washed with sterile water between sampling locations. 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 min 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 min at 4°C. After centrifugation, 100 ml of supernatant from each sediment sample was analyzed for most probable number (MPN) concentrations of E. coli using the Colilert defined substrate method with the Quantitray/2000 format (Idexx Corporation, Westbrook, ME). All analyses were performed per manufacturer instructions. Analyses included suitable blanks and standard positive cultures (E. coli, ATCC 25922) for quality control purposes.

Statistical evaluations

Highly variable fecal coliform concentrations were present in the surface water during this study. Such variations necessitated changes in sample dilution to achieve detection. As such, maximum and minimum reporting limits varied based on dilution. Such changes in detection limits precluded use of any technique to normalize data with such reporting limits for the purpose of analysis. Thus, surface water samples below detection limits or above reporting limits for either TSS or fecal coliform were excluded from analysis. This typically resulted in exclusion of high readings of fecal coliform, representing a source of error in the analysis. To mitigate the influence of high and low readings, analysis included the use of appropriate summary statistics such as geometric means. Further, non-parametric statistical procedures such as Wilcoxon Rank Sum and Spearman Correlation were utilized (Hollander & Wolfe 1999).

RESULTS AND DISCUSSION

Summary statistics

Samples were collected at the wetland between June 2009 and June 2010. Six to 9 base flow samples (which were within detection limits) were collected at each monitoring location. An additional 15 to 24 samples were collected during storm events, depending on monitoring location. Storm events ranged from 0.5 to 4.1 cm with a median of 1.4 cm. The geometric mean fecal coliform concentration at the wetland inlet was 7,980 CFU/100 ml, which is in the range of fecal coliform concentrations reported at the inlet of SCMs by Hathaway et al. (2009).

Comparison of base and storm flow concentrations

Base flow concentrations were typically lower than storm flow concentrations at all sample points (Figure 1). Geometric mean concentrations of fecal coliform at the wetland inlet and outlet during base flow were 1,857 and 1,029 CFU/100 ml, respectively. During storm flow, inlet and outlet concentrations increased to geometric means of 7,980 and 1,931 CFU/100 ml, respectively. Geometric mean outlet fecal coliform concentrations during storm flow were similar to those reported for a CSW studied by Davies & Bavor (2000). The base flow geometric mean outlet concentration was high relative to locations 1, 2, and 3. There is a possibility that a nearby municipal wastewater conveyance influenced concentrations at the outlet, but an exact cause for the increase is unknown. Wilcoxon rank sum analysis indicated significant differences between base and storm flow at all sampling points other than the outlet (p < 0.05). A lack of statistical difference between storm and base flow at the outlet is likely due to the high variance noted for data at the outlet.

Wetland function during storm flow

General wetland function

Concentration reductions (based on geometric mean values) of 76% and 56% were noted between the inlet and outlet for fecal coliform and TSS, respectively. A downward trend in
Retention time has been identified as an important factor influencing wetland efficiency for enteric bacteria by Vymazal (2005). Further, (re)introduction of fecal coliform into the wetland flow stream from sources such as animal defecation and from microbes persisting in the system may result in a plateau of fecal coliform concentrations as noted in Kadlec & Wallace (2009).

**Correlation analysis**

Fecal coliform concentrations were significantly correlated between adjacent sampling sites ($p < 0.05$). Spearman correlation coefficients ranged from 0.70 to 0.88 for all pairs of sites. Thus, it appears similar factors influence changes in the magnitude of fecal coliform concentrations in CSWs. Possible explanations include wetland-wide changes due to seasonal effects, consistent resuspension of microbes, and consistent differences in retention time among locations (Vymazal 2005; Ghermandi et al. 2007). Fecal coliform and TSS concentrations at each monitoring point were also analyzed for correlation. Correlations were poor for all locations other than site 1. Site 1 is located after the wetland’s initial settling basin (forebay) and a pool. It is possible that this location is influenced by resuspension of sediments due to both their availability and the likelihood of high velocity at this first internal wetland sampling point. Bavor et al. (2001) suggested fecal coliform were typically attached to particles less than 2 μm; however, these TSS measurements include a wide range of particle sizes, potentially making significant correlations impossible.

**Analysis of soil indicator bacteria concentrations**

*E. coli* concentrations in wetland sediments are shown in Table 1. Concentrations were typically much lower than those reported in CSW sediment by Stenström & Carlander (2001) and Davies & Bavor (2000). At the Lenoir Wetland, a surprising number of bacteria concentrations were low and/or at the detection limit. Elevated *E. coli* concentrations were noted for sediment samples only during some sampling events. On these occasions, the concentrations observed in the sediment appeared high enough to influence wetland microbe sequestration should they become resuspended. Boutilier et al. (2009) found similarly small numbers of *E. coli* in the sediment of a wetland treating domestic wastewater, hypothesizing that sedimentation was not a major pathway of *E. coli* sequestration in the wetland.

Some limited observations were made from the collected data. Concentrations were commonly higher in the top 8 cm
of the soil sample than in the bottom. Similar observations have been noted in river bank soils (Desmarais et al. 2002). This is potentially due to greater access to oxygen and nutrients (Coyne 1999). Additional trends appear possible in regard to seasonal differences in E. coli concentrations in sediment. The winter sampling date (16-Mar-2010) generally provided the lowest E. coli concentrations for most sampling locations. Seasonality of microbial data has been also shown in stormwater runoff (Selvakumar & Borst 2006; Hathaway et al. 2010), but seasonal differences in E. coli concentrations in wetland sediments warrant further study.

A sequential decline in sediment E. coli concentration was not noted between sampling locations. However, concentrations were higher at the inlet than outlet on every sampling occasion. This may occur for numerous reasons, including sedimentation of microbes at the inlet forebay, or the presence of excess nutrients and organic matter at the inlet (due to settling) providing a favorable environment for microbes. No wetland zone (pool, shallow water, or shallow land) appeared to have substantially higher E. coli concentrations than other zones. Thus, no wetland zone appeared to be more conducive to microbial die-off, making design suggestions impossible and suggesting the need for further research.

### Table 1 | E. coli concentrations in CSW sediments (MPN/20 g)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet</td>
<td>14.5</td>
<td>36.4</td>
<td>67.7</td>
<td>2,419.6</td>
</tr>
<tr>
<td>Bottom</td>
<td>30.5</td>
<td>4.1</td>
<td>4.1</td>
<td>104.3</td>
</tr>
<tr>
<td>Site 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>53.8</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>1</td>
</tr>
<tr>
<td>SW</td>
<td>&lt;1.0</td>
<td>16.1</td>
<td>&lt;1.0</td>
<td>95.9</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>2.0</td>
<td>&lt;1.0</td>
<td>3.1</td>
</tr>
<tr>
<td>P</td>
<td>9.7</td>
<td>28.5</td>
<td>3.1</td>
<td>90.6</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>1.0</td>
<td>&lt;1.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Site 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>137.4</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Bottom</td>
<td>1</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>SW</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>1</td>
<td>5.2</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>P</td>
<td>3.1</td>
<td>1.0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Site 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Top</td>
<td>20.6</td>
<td>&lt;1</td>
<td>2</td>
<td>32.7</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>3.1</td>
</tr>
<tr>
<td>SW</td>
<td>25.9</td>
<td>14.6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>15.8</td>
<td>&lt;1.0</td>
<td>1</td>
</tr>
<tr>
<td>Bottom</td>
<td>&lt;1.0</td>
<td>&lt;1</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Outlet</td>
<td>5.2</td>
<td>1.0</td>
<td>1</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Bottom</td>
<td>1</td>
<td>&lt;1</td>
<td>4.1</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

*a* top and ‘bottom’ refer to location of sample soil column from which measurements were taken.


CSWs appear capable of reducing indicator bacteria concentrations. Concentrations declined by mid-wetland and then remained relatively consistent from mid-wetland to the outlet (i.e. the rate of decline dropped substantially). This may indicate a baseline concentration of microbes to be released by CSWs, an important observation when considering the ability of wetlands to achieve surface water standards for indicator bacteria. If baseline concentrations of indicator bacteria are above surface water quality targets, the benefits of implementing CSWs are constrained. As distance from the inlet increased, microbe concentrations generally decreased. This implies that CSW designs should seek to optimize hydraulic retention time. Such designs would promote sedimentation and allow ample microbe exposure to wetland treatment mechanisms. Because this wetland was studied during the first two years of operation, it is possible that functionality will change with time.

E. coli concentrations in sediments were lower than reported in other studies performed on CSWs, but similar to results found by Boullier et al. (2009) in a wastewater treatment wetland. Concentrations were variable between locations and sampling dates. Indicator bacteria concentrations in CSW sediments likely vary based on season, location, and antecedent dry period. More robust studies are required to determine how these concentrations change over time. Determining the extent to which CSW sediments act as storage zones for indicator bacteria is important, as reducing scour of indicator bacteria-laden sediment would be a viable option to improve sequestration of microbes.

Although this study provides some insight into CSW functionality for microbes, there are numerous areas of research which remain. Understanding specific microbial removal mechanisms in CSWs will aid in more effective designs. Modifications to vegetative density, percentage of each wetland zone, and length to width ratio are all possible. Further, understanding relationships between sediment and microbes is critical to understanding sedimentation, resuspension, and wetland functionality during storm events.

### ACKNOWLEDGEMENTS

The authors acknowledge the Clean Water Management Trust Fund for funding the design and construction of this wetland. The authors also acknowledge the City of Lenoir for their contributions to all facets of this study, Seth Nagy for providing assistance throughout the project, and Joel...
Ballestero for major contributions to the design of the system. Last, the authors acknowledge the staff of the City of Lenoir Wastewater Treatment Plant for analysis of fecal coliform and TSS samples.

REFERENCES


Coyne, M. S. 1999 Introduction to Soil Microbiology. Delmar Cengage Learning, Albany, NY.


