Prediction of Effluent Quality from Retention Ponds and Constructed Wetlands for Managing Bacterial Stressors in Storm-Water Runoff

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Abstract: Microbial indicator organisms make up the greatest number of reported receiving water impairments, resulting in many questions on the fate of indicator bacteria passing through storm-water best management practices (BMPs). Storm-water BMPs are often considered effective tools to mitigate the effects of urbanization on receiving waters. The USEPA's, Office of Research and Development investigated the processes occurring within two commonly used BMPs, constructed wetlands and retention ponds. This research focused on creating pilot-scale systems to determine the environmental mechanisms that affect effluent indicator bacteria concentrations and to provide better information for the prediction of bacterial indicators for models when developing and meeting total maximum daily loads. Research results indicate water temperature, light, and a combination of other environmental factors influence bacteria indicator concentrations. Results from this research suggest that both constructed wetlands and retention ponds lower microbial concentrations in urban storm-water runoff. Bacteria inactivation generally followed the first-order, KC* model, which includes irreducible or background concentrations of a stressor. Sediment analyses indicate bacteria accumulated in sediments which may maintain background concentrations could be reintroduced into the effluent of these BMPs by turbulent flow causing resuspension or by accumulation through lack of maintenance. First-order models that do not consider irreducible concentrations may underestimate actual bacterial concentrations. The relationship between turbidity and bacteria suggests storm-water management practices that substantially reduce turbidity may also provide the greatest improvement in reducing concentrations of bacteria in storm-water runoff.

DOI: 10.1061/(ASCE)0733-9437(2008)134:5(567)

CE Database subject headings: Bacteria; Stormwater management; Best Management Practice; Wetlands; Retention basins; Receiving water; Water quality.

Introduction

Microbial contamination from fecal origins in storm-water runoff poses a risk to human health through the consumption of drinking water and recreational and bathing contact with surface waters. Indicator bacteria serve as the regulatory meter by which water quality is measured and water quality standards (WQS) must be met. Research on constructed wetlands inactivation of fecal indicators in secondary and animal wastewater is well documented (Bavor et al. 1987; Gersberg et al. 1987; Ottová et al. 1997). Removals of fecal streptococci and coliforms generally exceeded 80 and 90%, respectively, in a study by Kadlec and Knight (1996). Gersberg et al. (1987) and Garcia and Bécares (1997) concluded that extensively vegetated systems remove indicator bacteria at significantly higher rates from wastewater than unvegetated systems. However, because of the potentially high indicator bacteria concentrations in storm-water runoff, the untreated fraction in effluent may increase receiving water concentrations beyond WQS. This is in contrast to separate sanitary systems and combined storm-water and sanitary systems which, other than during sewer overflow, chemically treat the wastewater routed to treatment plants.

Experiments to evaluate the use of the first-order decay function for predicting indicator bacteria (total and fecal coliform, *Escherichia coli*, and enterococci) concentrations in effluents from best management practices (BMPs) were designed and completed by U.S. EPA's Urban Watershed Research Facility in Edison, N.J. Two studies, one at the bench-scale and the other at the pilot scale, were completed to determine similarities and differences in inactivation rate constants, coefficients, and effects of environmental conditions on bacterial indicator concentrations. The focus of this technical paper is on the results of the pilot-scale studies which specifically explored the environmental factors that influence the rate of microbial inactivation as urban storm water passes through retention ponds and constructed wetlands. The mesocosms designed and constructed for this project offered a unique setting allowing many characteristics associated with storm water and flow to be held constant (i.e., influent characteristics, residence time, and pollutant loading). By varying testing dates with climatic conditions experienced throughout the

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Note. Discussion open until March 1, 2009. Separate discussions must be submitted for individual papers. The manuscript for this paper was submitted for review and possible publication on August 14, 2007; approved on February 27, 2008. This paper is part of the ***Journal of Irrigation and Drainage Engineering***, Vol. 134, No. 5, October 1, 2008. ©ASCE, ISSN 0733-9437/2008/5-567–578/$25.00.

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(a)

(b)

Fig. 1. Pictures of the retention pond (a) and constructed wetland (b) treatment systems

year, an assessment of the impact of the environmental change on bacterial inactivation rates could be assessed. The results allowed comparison of rates of inactivation with seasonal wet-weather events and were used to determine new inactivation coefficients based on environmental variables. More information is needed to determine whether models that use first-order decay functions when predicting bacteria effluent concentrations from field BMPs (usually as a point source) are accurately providing effluent predictions and concomitant loads.

Methods

Study Site and Experimental Design

Two rectangular mesocosms of the same size with separate storm-water BMP treatments (constructed wetland and retention pond) were constructed at the Urban Watershed Research Facility in Edison, N.J. The experimental designs are detailed in Struck et al. (2006). Fig. 1 provides a picture of each mesocosm used in the study. Tanks had a length, width, and depth of 1.78, 0.74, and 0.65 m, respectively, with a volume of approximately 227 L. Both systems were constructed in August of 2002.

Initial concentrations in the storm-water runoff collected on-site were low (10^1–10^3 colony forming units CFU/100 mL) compared to many urban watersheds. To achieve a higher loading concentration (10^4–10^6 CFU/ 100 mL) for this study, a 500 mL aliquot of storm-water runoff was collected on-site, placed in growth media to encourage growth of representative bacterial strains and then reintroduced, producing higher densities of bacteria in the storm water.

Preparation of Bacterially Loaded Storm Water

Target indicator bacteria concentrations in mesocosms following addition of the enriched storm water were 10^4–10^7 CFU/100 mL. The cultured storm water was introduced over a 30–45 min time period (to limit shock) to a common supply tank that contained approximately 1,000 L of recently captured storm water and mixed for 30 min. Constructed wetland, retention pond, and control mesocosms were filled from the same supply source.

Water Quality Monitoring, Solids, and Light

The constructed wetland, retention pond, and control tank each had a water quality sonde (YSI Incorporated, Yellow Springs, Ohio) was placed on the sediment surface at a depth of 6 cm in the constructed wetland and 25 cm in the retention pond. These sondes recorded in situ temperature, dissolved oxygen (DO), pH, conductivity, and turbidity averaged over 10 min intervals.

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

Bacterial Indicator Analyses

Effluent from retention pond and constructed wetland riser pipes were collected in prewashed 1 L HDPE bottles placed at the effluent drainpipe (to collect enough volume for the sample but subject to continuous replacement). Microbiological and total suspended solids (TSS) samples were collected from these 1 L containers using automatic samplers (Hach Company, Loveland, Colo.) and placed into cooled prewashed 1 L high-density polyethylene (HDPE) bottles within the sampling device at preprogrammed times. Timed samples were also collected at a depth of 5 cm below the water surface in the control tank. If discrete samples were necessary due to autosampler failure samples using precleaned polyvinyl chloride (PVC) bottles and tubing were collected.

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

Six 500 mL PVC bottles were wrapped in aluminum foil filled with 400 mL of inoculated storm water, sealed, and placed in the control container to duplicate environmental conditions (other than sunlight) in the mesocosms and control tank. These samples were collected daily and with extended times (beyond 90 h) along

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with the "light" control samples as necessary and termed "dark controls." These samples separate light and dark affects on the microbial population in the control groups.

All samples were transferred to appropriate sized precleaned PVC containers for analyses depending on the type of analyses and the source of the sample. Samples were transported to the laboratory for splitting, filtering, and preservation as necessary within specified holding times. If storage was necessary, samples were stored in a refrigerator at 4°C. Bacteria indicator samples were analyzed for four indicator organisms (total and fecal coliforms, enterococci, and *E. coli*) using membrane filtration methods following *Standard Methods for the Examination of Water and Wastewater* (APHA et al. 1998).

Data Analysis and Model/Equation Development

A simple first-order decay model of $C_t = C_0 e^{-Kt}$ where C_t=concentration of organism at time t (CFU/100 mL); C_0=concentration of organism at time zero (CFU/100 mL); inactivation rate constant for the present environmental conditions (h^{-1}); and t=elapsed time since time zero (h), has commonly been used to predict the effluent concentrations in many systems. This study used this model as a basis for predicting effluent concentrations and evaluated the effect of environmental variables on the inactivation rate, K.

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

The sample weighted-average concentration and associated uncertainty was calculated using the uncertainty in the individual estimates as the weighting factor. This approach reduces the multiple (5–12) results from the samples and dilutions associated with the original source (bottle) to a single concentration estimate for the organism representing the experimental result resulting from the environmental condition. This data reduction approach relies on regression analysis of the concentration time series developed under the environmental conditions. The approach estimates the effect of the various conditions (treatments). No attempt was made to establish why the environmental exposure reduces the measured concentration, e.g., cellular wall degradation or DNA damage. Generally, the concentration time series developed for the individual experiments showed undetectably low concentrations for the final analyses.

The weighted-average concentration for each organism was regressed on the independent variables using nonlinear least-squares regression techniques. The nonlinear regression method used the Levenberg–Marquardt technique (a modified algorithm of the Gauss–Newton least-squares technique) in Statistica software package (version 7.1, Statsoft, Inc., Tulsa, Okla.). All regressions were run at the 95% level of confidence (α=0.05). The reported uncertainty in the calculated coefficients is the confidence interval reported by the Statistica software package. After the regression was complete, an ANOVA was run to test the significance of the proposed model. The regression coefficient of the controlled environmental variables determined to be significant were accepted as the coefficient of the inactivation rate for that individual or grouped variable.

Results

Physical and Chemical Properties of the Pilot-Scale Systems

Physical and chemical parameters measured in the study are listed in Table 1. Water temperatures averaged 2.15°C lower in the constructed wetland compared to the retention pond. This difference was likely due to shading from the macrophytic vegetation (*Typha latifolia*, average stem density=39.3 stems/m^2). This temperature difference was more notable in September and July sampling events (difference of 3.08 and 1.82°C, respectively) compared to the November sampling event (difference of 1.0°C).

Dissolved oxygen (DO) was higher in the retention pond compared to the constructed wetland with the highest temperatures recorded in May, July, and September. The process of decomposition of organic matter in the constructed wetland may consume some of the DO (with some values near 0 mg/L), especially during the warmer periods in which greater rates of decomposition are expected. Also, diurnal fluctuations in DO and temperature tended to be reduced during initial storm event loading. Values for these parameters did not generally reach preevent diurnal fluctuations until after 48 h of detention for most events.

Conductivity was nearly the same in the two systems, whereas pH was neutral to alkaline in the retention pond but tended to be acidic in the constructed wetland. This pattern was observed by Mitch and Gosselink (2000) in constructed wetlands with mineral soils and in some lake sediments by Stumm and Morgan (1996). These differences were attributed to the organic matter build-up in sediments and corresponding decomposition causing greater quantities of organic acid in the constructed wetland system, reducing the pH. The oxygen-reduction potential (ORP) was much lower (and often negative) in the retention pond compared to the constructed wetland.

The depth of inundation of the free water in the retention pond was generally three times that of the constructed wetland. A greater water depth and the lack of aquatic vegetation would substantially increase the potential for reducing conditions, resulting in lower ORP values, through both reduced oxygen diffusion and lower photosynthetic oxygen production.

The difference in light intensity, recorded with a hand-held meter under several light intensities, was used to calculate a corrected irradiance expected at the surface of the constructed wetland to compare irradiance values between the wetland and retention ponds. Light intensity in the constructed wetland was consistently 9–10% of that measured in the retention pond.

Most storm events had maximum initial TSS and turbidity values below 100 mg/L and 150 NTU, respectively, upon storm-water loading to the mesocosms. As expected, turbidity and TSS values decreased with residence time in each system. Geometric mean turbidity values for sampling events before October 2005 are shown in Fig. 2. Turbidity values were averaged for each time step and then over each sampling event.

The October 2005 experimental run had starting TSS values near 3,000 mg/L, and average turbidity values of 849–937 NTU, 50–70 times greater than typical conditions. Active construction in the watershed was evident in the storm-water runoff during this

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Table 1. Average Event In Situ Physical and Chemical Results

		Retention pond						Retention pond					
Date	Parameter	Valid *N*	Mean	Min.	Max.	SD	SE	Valid *N*	Mean	Min.	Max.	SD	SE
June 2004	Temp (°C)	36	27.0	21.9	32.2	2.9	0.5	36	25.5	22.2	29.4	2.2	0.4
	Cond (mS/cm)	36	0.316	0.296	0.345	0.016	0.003	36	0.284	0.265	0.316	0.014	0.002
	D0 (mg/L)	36	5.8	1.9	11.4	2.6	0.4	36	3.6	0.8	9.2	2.3	0.4
	pH	36	8.0	7.3	9.1	0.6	<0.1	36	6.7	6.6	7.0	0.1	<0.1
	ORP (mV)	36	364	200	465	93	15	36	465	317	529	67	11
	Turbidity (NTU)	36	11.6	5.1	31.1	5.7	0.9	36	10.3	6.5	23.8	4.8	0.8
	Irradiance (kJ/m^2)	12	43.9	<0.1	139.1	48.9	14.1	12	4.1	<0.1	12.8	4.5	1.3
September 2004	Temp (°C)	36	22.8	18.7	27.3	2.8	0.5	42	19.4	16.1	22.1	1.8	0.3
	Cond (mS/cm)	36	0.217	0.196	0.237	0.011	0.002	42	0.204	0.190	0.237	0.013	0.002
	D0 (mg/L)	36	8.5	5.8	11.5	1.9	0.3	42	4.1	0.7	13.4	3.6	0.5
	pH	36	7.9	7.5	8.3	0.2	<0.1	42	6.4	6.3	6.7	0.1	<0.1
	ORP (mV)	36	−288	−354	−15	95	16	42	555	524	591	19	3
	Turbidity (NTU)	36	11.0	7.9	20.7	3.2	0.5	42	6.6	3.7	18.5	3.8	0.6
	Irradiance (kJ/m^2)	12	31.6	<0.1	125.0	45.1	12.0	14	3.4	<0.1	11.5	4.7	1.3
November 2004	Temp (°C)	42	11.1	5.6	16.1	3.3	0.5	42	10.1	4.6	15.9	3.2	0.5
	Cond (mS/cm)	42	0.189	0.159	0.216	0.018	0.003	42	0.179	0.130	0.208	0.021	0.003
	D0 (mg/L)	42	9.5	6.2	13.9	2.0	0.3	42	9.0	2.3	14.3	4.1	0.6
	pH	42	7.2	7.0	7.3	0.1	<0.1	42	6.3	6.1	6.8	0.2	<0.1
	ORP (mV)	42	−285	−322	−211	29	4	42	403	376	452	19	3
	Turbidity (NTU)	42	7.8	4.0	11.5	1.9	0.3	42	8.4	0.4	27.6	6.5	1.0
	Irradiance (kJ/m^2)	14	19.0	<0.1	84.4	26.5	7.1	14	1.9	0.0	7.8	2.4	0.7
May 2005	Temp (°C)	33	19.9	16.6	27.0	3.2	0.6	39	17.1	15.3	22.3	1.9	0.3
	Cond (mS/cm)	33	0.447	0.394	0.555	0.047	0.008	39	0.680	0.585	0.771	0.052	0.008
	D0 (mg/L)	33	10.5	8.5	15.6	1.8	0.3	39	1.4	0.1	4.4	1.2	0.2
	pH	33	8.3	7.3	9.3	0.5	0.1	39	6.8	6.8	7.0	0.1	<0.1
	ORP (mV)	Not recorded											
	Turbidity (NTU)	33	1.8	<0.1	11.2	2.8	0.5	39	4.4	2.5	12.0	2.4	0.4
	Irradiance (kJ/m^2)	12	26.9	<0.1	119.0	40.2	11.1	13	2.6	<0.1	17.4	5.6	1.6
July 2005	Temp (°C)	24	26.7	24.0	31.2	2.2	0.4	24	24.5	23.1	27.1	1.3	0.3
	Cond (mS/cm)	24	0.253	0.235	0.297	0.018	0.004	24	0.259	0.197	0.371	0.054	0.011
	D0 (mg/L)	24	5.5	3.0	9.5	1.9	0.4	24	1.3	0.1	3.7	1.3	0.3
	pH	24	7.5	7.2	8.2	0.3	0.1	24	6.4	6.2	6.6	0.1	<0.1
	ORP (mV)	Not recorded											
	Turbidity (NTU)	24	26.0	6.5	92.0	20.2	4.1	24	61.0	13.5	167.2	50.9	10.4
	Irradiance (kJ/m^2)	8	29.4	<0.1	119.0	40.3	14.3	8	2.7	<0.1	10.9	3.7	1.3
October 2005	Temp (°C)	48	14.6	12.6	17.6	1.6	0.2	48	14.7	11.6	18.4	1.7	0.3
	Cond (mS/cm)	48	0.156	0.066	0.240	0.048	0.007	48	0.184	0.092	0.273	0.046	0.007
	D0 (mg/L)	48	3.2	<0.1	6.3	1.6	0.2	48	2.8	<0.1	9.1	3.0	0.4
	pH	48	7.0	6.1	7.5	0.4	0.1	48	6.0	5.8	6.5	0.1	<0.1
	ORP (mV)	Not recorded											
	Turbidity (NTU)	48	849.1	<0.1	1236.5	396.9	57.3	48	937.4	11.2	2141.4	782.5	112.9
	Irradiance (kJ/m^2)	16	18.7	<0.1	135.3	39.8	10.0	16	1.9	<0.1	12.4	3.7	0.9

sampling event. Inclusion of this storm-water runoff greatly increased the variability in solids concentration, overwhelming the smaller concentrations found in the previous and subsequent runoff events. Thus some analyses occurred with the exclusion of this event as noted.

Samples were not analyzed for enterococci in the first sampling event (June 2004) but resumed for all subsequent events. Temperature appeared to affect bacterial indicator organism concentrations in the retention pond. The optimal temperature range that resulted in the greatest number of observed bacteria colony forming units was between 11 and 26°C. A similar trend was noticed in the constructed wetland for a temperature range between 11 and 23°C (Fig. 3). Conductivity and DO did not appear to significantly affect bacterial concentrations over the ranges observed. ORP may have moderately affected fecal coliforms and *E. coli* concentrations around 200 mV in the retention pond, whereas densities of fecal coliforms, *E. coli*, and enterococci decreased between 500 and 600 mV in the constructed wetland. However, only three events were monitored for this parameter. Densities of fecal coliforms and *E. coli* decreased above a pH of 8.5 in the retention pond but remained unaffected over the range of observed pH values in the constructed wetland.

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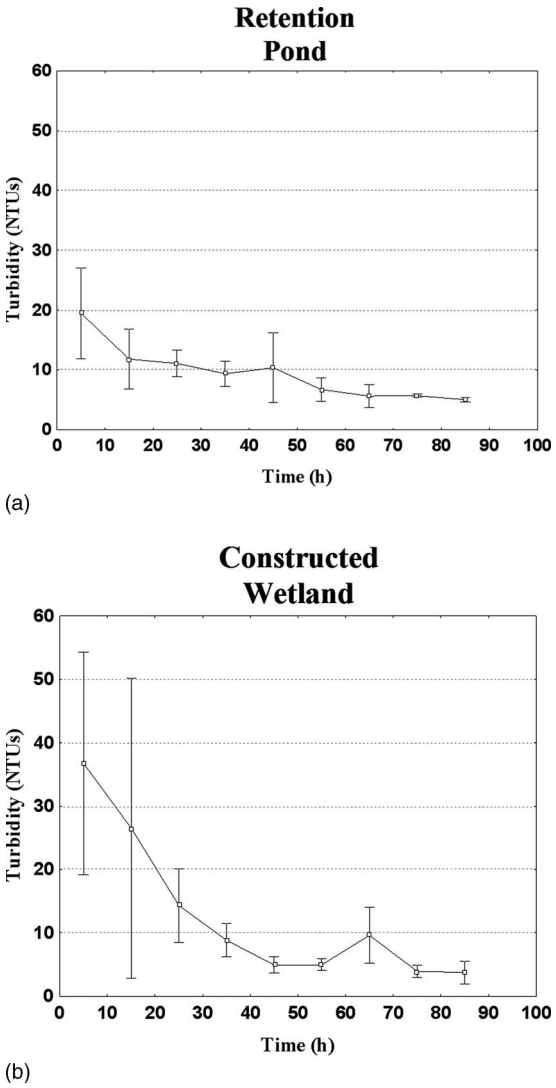

Fig. 2. Mean turbidity in the retention pond and constructed wetland in all storm events except October 2005. Vertical bars indicate 95% confidence intervals.

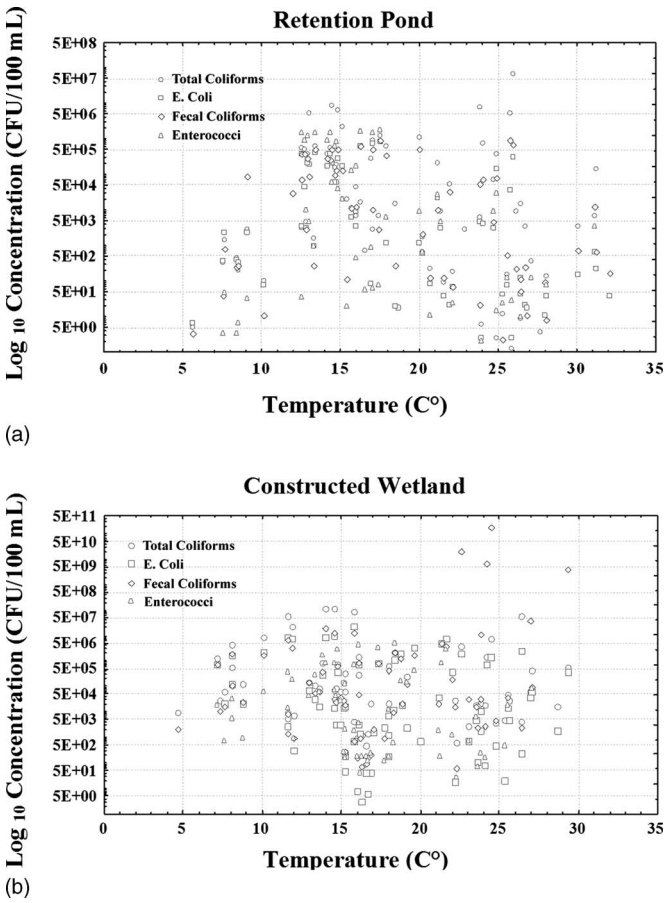

Fig. 3. Effluent concentrations of indicator organisms with temperature

There was a distinct relationship between concentration of indicator organisms and turbidity during this study. Although there was no visible trend in turbidity at concentrations less than 20 NTU, turbidities greater than 100 NTU exhibited a predictable increase in bacteria organism concentrations with increasing turbidity in both the retention pond and constructed wetland (Fig. 4). The USGS reported similar results in larger rivers in northern and central Virginia and the EPA in smaller streams in northern Virginia (Hyer and Moyer 2003; Struck et al. 2008). These solids can potentially affect rates of bacteria attenuation via obscuring ultraviolet light penetration and occlusion or protection from predators through particle-bacteria agglomeration.

Overall bacteria indicator inactivation rates for all simulated storm events are shown in Table 2. Significant differences were observed between the constructed wetland and retention pond in bacteria indicators for six runoff events. The retention pond had significantly higher inactivation rates for total coliforms in June and July; *E. coli* in May; fecal coliforms in July; and enterococci in May and November compared to the constructed wetland. However, the constructed wetland had significantly greater bacterial inactivation rates compared to the retention pond for fecal coliforms in June and enterococci in July. Both treatments had significantly greater inactivation rates compared to the light and dark controls in September and November for total coliforms, *E. coli*, and fecal coliforms. Retention pond inactivation rates were also greater than controls for total coliforms in June and July, *E. coli* in May and July, and fecal coliforms in May, June, and July. Constructed wetlands inactivation rates were greater for *E. coli* in July, fecal coliforms in May and June, and enterococci in July. Light controls were greater than dark controls in nine instances, including May and June for total coliforms and *E. coli*, July for fecal coliforms, and May, July, September, and November for enterococci. This suggests light does have an impact on bacteria indicator organisms.

The exponential regression coefficients shown in Fig. 5 are the calculated inactivation rates from the data. A two-step process of generating an overall inactivation value for each bacterial indicator organism from 0 to 50 h and from 50 to 100 h was used to generate a best fit relationship. This time frame was determined by maintaining R^2 values of regressions greater than 0.70 and varying the time interval between 0 and 100 until the difference in slope (inactivation rate) was maximized for the majority of the bacteria indicator organisms.

In most instances, the R^2 values improved when dividing the duration of the experiment into the two time frames, suggesting that inactivation rates vary as a function of time with greater rates of inactivation during the first 50 h time period compared to the second 100 h time frame. Fecal coliforms and enterococci in the

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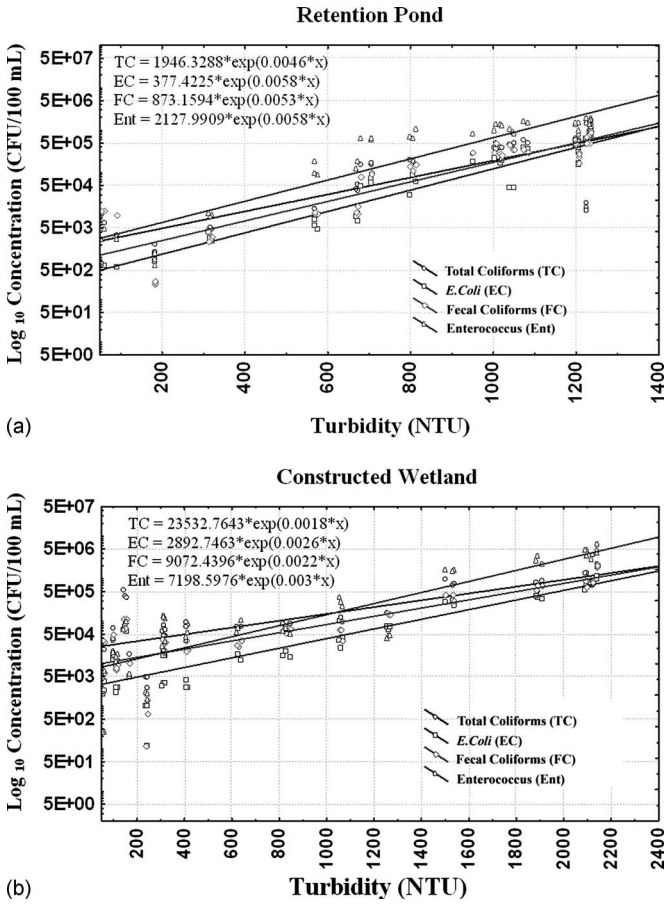

Fig. 4. Effluent indicator bacteria concentrations with in situ turbidity

retention pond were an exception to this generalization. Several of the inactivation rates during the 50–150 h time period had values nearing zero suggesting that these organisms may have reached or nearly reached background concentrations after 50 h. The average pre-event background concentrations in the retention pond and constructed wetland (Table 3) also support this.

Bacteria Concentrations in Sediment

Results from the sediment bacteria indicator concentrations collected and analyzed 1 day before and 2 days after the November 2004 storm event are shown in Table 4. Sediment bacteria increased by an order of magnitude for total coliforms for both the constructed wetland and retention pond. In the constructed wetland, *E. coli* increased by one order of magnitude but the retention pond had over a three orders of magnitude increase after the storm event. However, concentrations of enterococci decreased one to two orders of magnitude in these systems. The initial bacteria indicator organism concentrations measured before the storm event were considered as background concentrations as the previous addition of indicator organisms through storm-water runoff was more than 60 days prior to the November event.

Discussion

Effects of Environmental Variables

Results from the pilot-scale studies show environmental conditions affect indicator bacteria concentrations in retention ponds and constructed wetlands. Attempts have been made to single out environmental variables such as temperature and sunlight. Other variables in which separation was not possible were considered as a group.

Table 2. Inactivation Rates for the Constructed Wetland, Retention Pond, and Dark and Light Controls for All Indicator Bacteria Organisms for Each Sampling Event

		Retention pond				Constructed wetland			
Month	Year	Total coliforms (h^{-1})	*E. coli* (h^{-1})	Fecal coliforms (h^{-1})	Enterococci (h^{-1})	Total coliforms (h^{-1})	*E. coli* (h^{-1})	Fecal coliforms (h^{-1})	Enterococci (h^{-1})
June	2004	0.2419[a,b]	0.1484	0.1814[b]		0.1529	0.1651	0.3277[a,b]	
September	2004	0.144[b]	0.1164[b]	0.1192[b]	0.2030	0.1204[b]	0.1204[b]	0.1515[b]	0.1786
November	2004	0.1653[b]	0.1164[b]	0.1485[b]	0.1730[a]	0.1235[b]	0.1157[b]	0.1137[b]	0.1245
May	2005	0.0949	0.3350[a,b]	0.1417[b]	0.1717[a]	0.1090	0.0919	0.1233[b]	0.0852
July	2005	0.1811[a,b]	0.1957[b]	0.2610[a,b]	0.1240	0.0733	0.1894[b]	0.1025	0.2112[a,b]
October	2005	0.0437	0.0524	0.0566	0.0512	0.0427	0.0597	0.0536	0.0594
		Dark control				Light control			
Month	Year	Total coliforms (h^{-1})	*E. coli* (h^{-1})	Fecal coliforms (h^{-1})	Enterococci (h^{-1})	Total coliforms (h^{-1})	*E. coli* (h^{-1})	Fecal coliforms (h^{-1})	Enterococci (h^{-1})
June	2004	0.0247	0.0276	0.0249		0.1390[c]	0.1502[c]	0.0242	
September	2004	0.0700	0.0563	0.0527	0.0773	0.0588	0.0789	0.0760	0.2027[c]
November	2004	0.0815	0.0480	0.0445	0.0711	0.0658	0.0724	0.0692	0.1787[c]
May	2005	0.0258	0.0725	0.0514	0.0351	0.0679[c]	0.1158[c]	0.0828	0.0884[c]
July	2005	0.0637	0.0509	0.0619	0.0944	0.0720	0.0712	0.1136[c]	0.1681[c]
October	2005	0.0538	0.0605	0.0514	0.0194	0.0676	0.0862	0.0822	0.0316

[a]A significantly higher value between retention pond and constructed wetland.

[b]A significantly higher value between retention pond or constructed wetland values and control values.

[c]A significantly higher value between light and dark control values.

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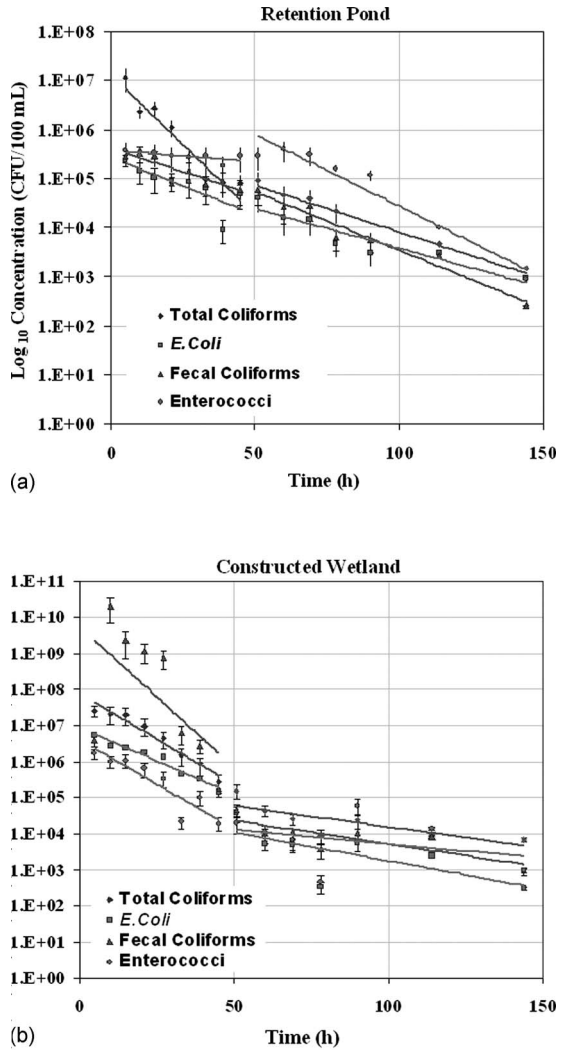

Fig. 5. Indicator organism concentrations with time. Regressions fits are for time=0–50 and 50–150 h. Regression coefficients (k values) of the exponent (slope) are shown below the graphs.

Effects of Temperature

Generally, the results are similar to those of other studies (e.g., Easton et al. 2005; Ferguson et al. 2003; Geldreich et al. 1968; Medema et al. 1997; Canteras et al. 1995). Calculated inactivation rate constants increased with increasing temperature. Similarly, inactivation rates were lower at lower temperatures. This trend was most notable during the October 2005 sampling event. Selvakumar et al. (2004) noted that concentrations of organisms did not change significantly when the samples were stored at 4 °C beyond the standard holding time of 24 h. Geldreich et al. (1968) noted that organism persistence remained at higher levels at 10 compared to 20 °C. In the pilot-scale experiment the optimal temperature range for growth (as indicated by overall indicator bacteria concentrations) was similar to values reported in the literature with indicator concentrations increasing with temperature, reaching a maximum concentration from 20 to 25 °C in both the retention pond and constructed wetland. Medema et al. (1997) found that inactivation was faster at 15 than at 5 °C. Canteras et al. (1995) noted a clear positive correlation between inactivation and temperature. In their study, when test conditions were at 10 °C, 36 h was necessary to reduce the population of *E. coli* to 10% of the original as opposed to 8.4 h at 42 °C. Greater inactivation was also noticed in the range between 10 and 18 °C than between 18 and 42 °C.

The study also found indicator organism inactivation rates were much greater over the first 50 h as compared to the following 100 h. Although over a longer period of time, Easton et al. (2005) reported that the 0–7 day inactivation rates were much larger than the 7–21 day rates. While not measured, it may be possible that during the 0–7 days studied by Easton et al. (2005) rates inactivation were higher during the first few days compared to the later days of the time period. In this study, the temporal resolution measured 16 times over 150 h, compared to 4 times over 168 h (7 days) by Easton et al. (2005), likely provides better differentiation in short-term bacteria concentration trends.

Table 3. In Situ Indicator Organisms Average Background Concentrations

Indicator organism	Background concentration (CFU/100 mL ± SE)	
	Retention pond	Constructed wetland
Total coliforms	$1.39\times10^4\pm3.85\times10^3$	$3.37\times10^4\pm4.04\times10^3$
E. coli	$6.42\times10^0\pm7.22\times10^0$	$2.55\times10^1\pm6.21\times10^0$
Fecal coliforms	$1.02\times10^4\pm2.55\times10^3$	$8.09\times10^3\pm1.12\times10^3$
Enterococci	$3.70\times10^1\pm8.29\times10^0$	$2.01\times10^1\pm5.46\times10^0$

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

	Indicator organism	Concentrations before storm-water loading (MPN)	Concentrations after storm-water loading (MPN/100 mL)
Retention pond	Total coliforms	2.25×10^4	1.94×10^5
	E. coli	<MDL	7.41×10^4
	Enterococci	1.62×10^4	1.88×10^3
Constructed wetland	Total coliforms	3.70×10^4	$>2.41\times10^5$
	E. coli	2.37×10^4	$>2.41\times10^5$
	Enterococci	2.02×10^5	8.67×10^3

Effect of Sunlight/Light Intensity

Many studies have shown that sunlight is an important factor in bacteria indicator inactivation (Sinton et al. 1994; Canteras et al. 1995). In this study, statistically lower inactivation rate constants in the dark control compared to the light control for total coliforms and *E. coli* were found for the months of May and June. June had the greatest irradiance of any of the dates sampled, whereas May ranked fourth in light intensity (because of cloudy conditions throughout the duration of the experiment) (Table 2). Enterococci showed the greatest difference in inactivation rate constants between light and dark controls followed by total coliforms and *E. coli*. The primary difference between these controls was the exposure to sunlight. The difference in rate constants, up to 0.12 h^{-1} for enterococci and *E. coli*, is substantial.

Effects of Sedimentation, Sorption, and Filtration

Sedimentation, sorption, and filtration processes are generally accepted as the dominant mechanisms for removal of solids and

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other sediment-related stressors such as heavy metals. Settling velocity has been used as an approximation of the overall removal (settling) rate constant in storm-water treatment systems (Wong and Geiger 1997). Other environmental factors such as nonideal flow conditions, though, could increase solids in the water column through resuspension.

Indicator bacteria concentrations and the inactivation rate constants between the constructed wetland and retention pond in this study support settling as a contributing but not primary factor in bacterial inactivation. It is possible that a large fraction of the influent indicator bacteria were unassociated (free) with solids or associated with only very fine particles and would not settle during the period of sampling. This may be a real phenomenon or an artifact of the manner in which the enriched storm water was created.

Wong and Geiger (1997) suggest, when selecting an appropriate K value for sedimentation, filtration, and sorption using the settling velocity of the 50th percentile sediment grade with adjustments for increased effectiveness for wetlands having higher vegetation density. However, as experienced in this study, this may not adequately predict the effluent concentrations of storm-water runoff passing through passive treatment systems if bacteria are either unassociated with settleable particles or if they are associated with the fine particle fraction, i.e., less than 2 μm in size (Davies and Bavor 2000). In addition, bacteria indicator organisms present in the sediments have the potential to be resuspended in the water column with turbulent flow or disturbance and may contribute to increased effluent bacteria indicator organism concentrations in future events.

Effects of Predation

Previous research has suggested bacterivory can significantly reduce indicator bacteria organism concentrations (Green et al. 1997; Mandi et al. 1993; Decamp and Warren 1998; Pretorius 1962; Fernandez et al. 1992; Troussellier et al. 1986). However, because of the difficulty in measuring predation, this parameter was not assessed for this technical paper.

Effects of Other Potential Factors

The many other factors (i.e., DO, pH, conductivity, ORP) that may contribute to inactivation rates of indicator bacteria were not directly assessed in this study. Instead, rather than individually assessing these factors, their effects were grouped into the overall inactivation rates and treated as collective environmental factors.

Evaluation of the First-Order Decay Equation

This pilot-scale study demonstrated the first-order decay function adequately models indicator bacteria concentrations in the short term. However, during longer periods, the first-order decay equation may not provide an accurate prediction of indicator bacteria (or other stressors of concern) in effluent from these types of BMPs. This equation also fails to account for background concentrations that may limit attenuation of stressors to levels predicted. Literature has reported that the assumptions for a first-order decay function (i.e., steady flow conditions) may seldom be met in studies concerning storm-water runoff in constructed wetlands and retention ponds (Wong and Geiger 1997).

Other researchers have suggested using surface area based models for wetlands constructed for secondary treatment of wastewater (Kadlec and Knight 1996). One of these models, the K-C^* model, incorporates a concentration term, C^*, that represents the background concentration often present in natural systems. The formula is

$$\frac{(C_{\text{out}} - C^*)}{(C_{\text{in}} - C^*)} = e^{-Kt} \tag{1}$$

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

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

They recommend an adaptation of Kadlec and Knight's K-C^* model to

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

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

It should be noted that Eqs. (1) and (2) have attempted to incorporate conditions that meet the assumptions for the first-order decay equation or include environmental realities such as background concentrations of indicator organisms (C^*) to improve prediction of this stressor. The rate constant K, which governs inactivation rate determinations in the first-order decay equation, is the only means of incorporating environmental variables to better predict effluent concentrations in surface water quality models when taking this approach. To provide more information on the effects of environmental variables, the present study estimated inactivation rate constants for indicator bacteria from environmentally influenced systems. Further, constant coefficients were calculated to improve predictions of the effects that environmental factors have on overall indicator bacteria inactivation rates.

Khatiwada and Polprasert (1999) and Canteras et al. (1995) proposed the following formula for overall inactivation rate constants:

$$K_{\text{overall}} = K_{20}\Phi_T^{T-20} + \Phi_l l + K_f + K_p \tag{3}$$

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

With the inability to separate sorption, sedimentation, predation, and other environmental factors in this study, the variable K_{other} was substituted for K_f and K_p. As a result, the inactivation rate equation from Eq. (3) can be written as

$$K_{\text{overall}} = K_{20}\Phi_T^{T-20} + \Phi_l l + K_{\text{other}} \tag{4}$$

where definitions are as noted earlier and K_{other}=inactivation rate

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Table 5. Inactivation Rate Coefficients from the Mesocosm Studies

Indicator organisms	Φ_L ($cm^2/mW\,h$)	K_{20} (h^{-1})	Φ_T	Φ_L ($cm^2/mW\,h$)
Total coliforms	0.0016	0.066 ± 0.007[a]	1.005 ± 0.011	0.0092
Fecal coliforms	0.0130	0.053 ± 0.006[a]	1.017 ± 0.004[a]	0.0047
E. coli	0.0025	0.057 ± 0.008[a]	1.013 ± 0.002	0.0022
Enterococci	0.0076	0.054 ± 0.006[a]	1.050 ± 0.014	0.0070

[a]Coefficient is statistically significant at $\alpha = 0.05$.

constant due to other factors such as sorption, filtration, sedimentation, predation, pH, DO, conductivity, ORP, etc.

Table 5 lists coefficients for light and temperature from the light and dark controls of this study. Using the light and dark control inactivation rates, the inactivation rate due to other parameters was calculated by subtracting the $K_{temperature}$ value from the $K_{light+temperature}$, resulting in a calculated K_{light} value. The result of subtracting K_{light} and $K_{temperature}$ from the $K_{overall}$ values that were measured for the retention pond and constructed wetland gave the K_{other} values.

All K values for the retention pond and constructed wetlands are in Tables 6 and 7, respectively. The $K_{light+temperature}$ values for the constructed wetland were not directly measured but were calculated. To calculate these values, the K_{light} value from the light control was multiplied by the weighted average of light intensity expected at the surface of the constructed wetland. The weighted average was calculated as 10% of the light that reached the retention pond surface (based on light meter measurements) for 6 out of 24 h of effective light exposure, multiplied by 18 out of 24 h in which the exposure was relatively the same as in the retention pond. This resulted in a multiplication factor of $0.775K_{light}$ of the retention pond. All negative calculated values were assumed to be a propagation of error and were therefore expected to be within the range of error for the respective inactivation rate constant.

Inactivation rate constants had differing values throughout the year based on varying effects of environmental factors. With the exception of enterococci, the combination of "other factors" had the greatest impact on inactivation rates in the retention pond. Temperature was found to have a greater effect than light on inactivation rates of indicator bacteria, with the exception of enterococci in which light had the greatest influence. Light, however, was still a statistically significant factor and should be considered when using the first-order equation.

Table 6. Retention Pond Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

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

[a]Coefficient is statistically significant at $\alpha = 0.05$. Small errors in measured $K_{overall}$ and K_{temp} resulted in several negative values when calculating K_{light}.

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Table 7. Constructed Wetland Overall, Temperature, Sunlight, and Other Factors Rate Coefficients

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

[a]Coefficient is statistically significant at $\alpha = 0.05$ Small errors in measured $K_{overall}$ and K_{temp} resulted in several negative values when calculating K_{light}.

In the constructed wetland, temperature was found to have the greatest effect on inactivation rates for the indicator bacteria, with the exception of fecal coliforms. The combination of other factors had the greatest influence on fecal coliforms and was similar or slightly greater in influence to light for the other indicator bacteria. Again, enterococci were an exception to this, where light and temperature had a similar influence on inactivation rates.

Application of the inactivation rate constants found in Tables 6 and 7 can provide an overall inactivation rate constant incorporating temperature, light intensity, and a grouped factor for other environmental variables. The overall inactivation rate constant can be applied to Eq. (2) to determine the required area necessary to achieve water quality standards when designing constructed wetland or retention ponds.

If a background concentration exists, the overall rate constant can also be applied to Eqs. (1) and (2) to predict the expected event mean concentration of BMP effluent. The first-order decay equation is most accurate when used with the inactivation rates and uncertainties in short-term models to predict storm-water runoff effluent quality from constructed wetland and retention pond BMPs to improve or prevent further degradation of water quality. Longer-term modeling would benefit from applying separate inactivation rates for periods immediately following storm-water runoff and periods unaffected by storm-water runoff.

Scaling Consideration

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

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

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Conclusions

This study demonstrated that the concentration of the tested indicator organisms decrease exponentially with time. The first-order decay model is a simple and efficient means of predicting indicator bacteria concentrations in storm-water runoff effluent from BMPs such as retention ponds and constructed wetlands for shorter durations (about 50 h). Results from this study provide new data on inactivation rate constant coefficients, and uncertainties used in the first-order equation. The factors of light, time, and temperature influence bacterial life processes in retention ponds and wetlands constructed to mitigate the effects of storm-water runoff on the receiving waters. A combination of other factors (e.g., predation, sedimentation, sorption, filtration, pH, BOD, and DO) can also contribute significantly to the inactivation of indicator bacteria in these BMPs. The determination of reliable rates, coefficients, and uncertainties expected in reported values will add to the accuracy of surface water quality models. Water quality models are a primary tool for evaluating permit applications (e.g., National Permit Discharge Elimination System permits) and have an important regulatory role in developing total maximum daily load (TMDL) allocations. These models should incorporate the affects of BMPs to better model their potential for improving water quality in the watershed. Results suggest the basic first-order decay equation may not adequately consider background concentrations when calculating dry-weather loading. Similarly, predicting loading over longer durations (e.g., annually) and ignoring seasonal changes may result in less accurate predictions. Both scenarios may affect calculations of bacterial loading and potentially increase the risk to human health with exposure to bacterially contaminated storm-water runoff. Improving predictive models is an important step in assuring TMDL allocations meet the state of the science and protect human and environmental health.

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