Microorganisms Die-Off Rates in Urban Stormwater Runoff

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ABSTRACT

Stormwater best management practices (BMPs) are often considered effective tools to mitigate the effects of stormwater pollutants before they are discharged to receiving waters. However, BMP performance for microorganisms removal is not well documented. Microorganisms die-off in stormwater runoff is a complex process involving various types of environmental factors such as temperature, sunlight, dissolved oxygen, salinity, etc. This bench-scale study was designed to determine the effects of individual factors that influence the microorganisms die-off in BMPs. In this study, organism-specific die-off constants in stormwater were developed assuming temperature and sunlight as the major influential parameters. The temperature study indicated that the organisms persisted at higher levels at lower temperatures. The die-off rate constants increased with increasing temperatures. These observed temperature effects on die-off were well documented in other literatures as well. Out of all the organisms studied, total coliforms had a much slower die-off rate. Fecal coliforms, fecal streptococci, E. coli, and enterococci have similar die-off rate constants. The temperature coefficient values obtained in this study are similar to the ones reported in the literature. Except for total and fecal coliforms, the effect of sunlight on die-off constant is significant. The initial concentrations of organisms in the stormwater have an effect on the die-off rates.

INTRODUCTION

U.S. EPA identified urban stormwater runoff as one of the top four leading causes of water quality impairment related to human activities in lakes and reservoirs (U.S. EPA, 2002). Stormwater discharges can have significant negative impacts on receiving water bodies and create human health concerns since these waters may be used for drinking water resources, shellfish harvesting, and recreational purposes. They release pathogenic bacteria, protozoans, and viruses as well as a number of potentially toxic, bioaccumulative contaminants. Selvakumar and Borst (2006) reported concentration ranges for total coliforms (4.2 x 10^4 - 1.9 x 10^5 CFU/100 mL), fecal coliforms (5.6 x 10^3 - 2.2 x 10^4 CFU/100 mL), fecal streptococci (3.5 x 10^2 - 3.2 x 10^3 CFU/100 mL), enterococci (1.0 x 10^3 - 6.6 x 10^3 CFU/100 mL), Escherichia coli (E. coli) (1.5 x 10^3 - 8.5 x 10^3 CFU/100 mL), Pseudomonas aeruginosa (3.4 x 10^2 - 1.2 x 10^3 CFU/100 mL), and Staphylococcus aureus (4.6 x 10^1 - 1.8 x 10^4 CFU/100 mL) in urban stormwater runoff from the three different land uses in Monmouth County, New Jersey. Maestre and Pitt (2005) reported similar nationwide median concentrations for fecal coliforms, fecal
streptococci, total coliforms and *E. coli* using data from a number of National Pollutant Discharge Elimination System (NPDES) Municipal Separate Storm Sewer System (MS4) stormwater permit holders. A recent epidemiology study conducted at Santa Monica Bay adjacent to Los Angeles County, California found that untreated stormwater runoff poses symptoms of both upper respiratory and gastrointestinal illnesses for people swimming closer to storm drains (Haile *et al.*, 1999).

Once introduced into the environment, microorganisms can be affected by various types of environmental factors such as sunlight, temperature, turbidity, dissolved oxygen, salinity, predation, nutrient deficiencies, and toxic substances. A significant number of die-off rates for microorganisms in fresh, sea, rain, and marine waters have been reported in the literature. However, data on die-off rates for microorganisms in stormwater and effects of natural factors on survival rates are limited, except for one study by Geldreich *et al.* (1968).

**Effect of temperature**

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

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

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

Recent investigations considered bacterial removal as a more complex mechanism involving interactions between the physical, chemical, and biological systems present in wetlands and
retention ponds, although temperature clearly remains an important parameter. For example, Polprasert et al. (1983), Pearson et al. (1987a, b), Barzily and Kott (1991), Mara et al. (1992a, b), and Mezrioui et al. (1995a, b) all found that removal rates of fecal coliforms increased with increasing temperature.

**Effect of sunlight**

Numerous studies have shown sunlight as an important factor in microorganism die-off though it is difficult to separate these effects from other factors. Sinton et al. (1994) studied inactivation in sunlight of fecal coliforms and enterococci from sewage and meat works effluent concluding that the die-off rate of fecal coliforms was 2-4 times that of enterococci and inactivation was generally slower at lower light intensities. Alkan et al. (1995) found that variability of enteric bacteria (i.e., enterococci and E. coli) die-off due to the effect of sunlight depends on the variability of the intensity of light and other small-scale environmental factors such as turbidity, sewage content, and degree of mixing. They further reported that the die-off rates of E. coli and enterococci from exposure to light were similar. Canteras et al. (1995) reported that sunlight was the most important factor affecting die-off of E. coli with 90% concentration reductions within about 1 h (i.e., inactivation rate of about 0.89 h⁻¹) at 18°C, 8.5% of salinity, and greater than 120 W/m² (12 mW/cm²) light radiation. Yukselen et al. (2003) studied the effects of solar radiation and temperature on bacterial die-off rates in Black Sea coastal waters and found that solar radiation was the most significant factor affecting the mortality of coliform bacteria. No significant effect of temperature was observed in the presence of solar radiation by the variation of temperature from 9 to 26°C. However, the effect of temperature is significant in dark experiments with die-off taking approximately 20 times longer to reach 90% concentration reductions compared to values in the light. Davies-Colley et al. (1999) reported that sunlight is the main factor causing natural attenuation in waste stabilization ponds, although dissolved oxygen (DO) and pH can also influence the rate of attenuation. Die-off studies on E. coli and Salmonella were conducted in two different ecosystems: Morlaix estuary in the English Channel and Bay of Toulon on the Mediterranean Sea. In the Morlaix estuary, most of the bacteria were mixed with turbid waters and were able to survive several hours to several days as light penetration was prevented by suspended matter, lowering the effect of sunlight. On the contrary, through lack of nutrients and very high sunlight intensity due to climate, die-off rates in Mediterranean waters were high, with 90% mortality within 2 h near the water surfaces, and several hours in deep waters (Pommepuy et al., 1992).

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

The exact mechanism whereby microorganisms become non-viable after sunlight exposure is not entirely clear. Chamberlin and Mitchell (1978) and Eisenstark (1971) noted that the mechanism of light-induced bacterial decay depends on the presence of endogeneous sensitizers or chromophores, which adsorb light energy and cause cell damage directly or by reaction with
oxides to form superoxides, which in turn may cause damage to the cells.

Historically, total and fecal coliforms with fecal streptococcus have served as the preferred indicators, but recent efforts are leading to recommendations to substitute enterococci and \textit{E. coli} for water quality monitoring because of higher correlation with gastrointestinal illness (Gray, 2000). \textit{E. coli} and enterococci are the most representative of warm blooded animal fecal contamination in water. They also have the ability to survive and do not generally grow outside of the intestinal tract (Ashbolt \textit{et al.}, 2001). In 1976, the U.S. EPA recommended that states adopt as a recreational water quality standard, fecal coliforms not to exceed 200 organisms/100 mL (U.S. EPA, 1976). In 1986, the U.S. EPA revised its recreational water quality criteria to 33/100 mL for enterococci and 126/100 mL for \textit{E. coli} (U.S. EPA, 1986).

\textbf{OBJECTIVE}

The overall objective of this study was to determine the die-off rate constants for traditional and alternate microbial indicators in stormwater runoff. The research separately assessed the influence of time, temperature, and sunlight on the die-off rates to isolate the effects.

\textbf{MATERIALS AND METHODS}

\textbf{Sample collection}

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

After collection, the samples were transported to the on-site laboratory and allowed to quiescently settle for 10 to 20 min at room temperature to allow the larger solids to fall to the container bottom. The water from the settled collection container was transferred leaving about 2.54 cm in the composite container bottom to limit the potential effects of settleable particulates on the experiments and avoid interference with the enumeration process. While continuously stirring the container holding the decanted supernatant, a peristaltic pump transferred aliquots to 250-mL pre-cleaned HDPE bottles. All subsample bottles were completely filled leaving no headspace.
Experimental methods

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

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

The experimental design selected the controlled independent variables and their ranges based on the broad, literature-reported influence and the likelihood of the condition existing in structural BMPs (e.g., retention ponds and constructed wetlands). As widely reported in the literature, time, temperature, and light intensity are important environmental variables which determine the rate of change of indicator organism concentrations.

**Temperature Study** - The temperature-dependence die-off study targeted temperatures of 10°C, 20°C, and 30°C. The mean temperatures recorded by the data loggers were used in the analysis (usually slightly lower than the targeted temperatures), however, for simplicity, the descriptive target temperatures were used. Temperature monitoring was also included as part of the light study.

**Light Study** - The light-dependence study established a target temperature of 25°C. Samples were exposed to light at four intensities including one dark sample. The respective light intensities for different light conditions were established using Reptisun 5.0 UVB fluorescent bulbs (Zoo Med Laboratories, Inc., San Luis Obispo, CA). The manufacturer reports that the bulbs produce light consists of up to 30% UVA (320-400 nm) and 6% UVB (290-320 nm) wavelengths. Light intensities were controlled by adjusting the distance between the light source and the surface of the container. The distance was maintained at 12.7, 22.9 or 35.6 cm above the water surface to provide light intensities between 20 and 100 mW/cm² (200 to 1000 W/m²). Light intensities at the sample surface were measured using a light meter (International Light IL 1400A with thermopile detector) daily throughout the experiments. The measured light intensity varied about 5% during the experiment. Table 1 lists the average light intensities measured throughout the experiment.

The container used to hold the sample in the light study was 250-mL thin, flat-sided polystyrene flasks with canted neck and plug seal cap (BD Biosciences, Bedford, MA). This container was selected because it did not have any effect on the light attenuation and would reduce the depth of water in the container assuring a more homogenous light dose.
Table 1. Light Intensities Corresponding to the Height of Light Source above the Water Surface

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>Light Intensity Range (mW/cm²)</th>
<th>Average Light Intensity (mW/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.7</td>
<td>89.0 – 97.8</td>
<td>94.7±4.1</td>
</tr>
<tr>
<td>22.9</td>
<td>50.2 – 58.4</td>
<td>55.2±2.7</td>
</tr>
<tr>
<td>35.6</td>
<td>19.7 – 21.9</td>
<td>20.9±0.8</td>
</tr>
</tbody>
</table>

Analysis of microorganisms

All samples were examined using membrane filtration methods following Standard Methods for the Examination of Water and Wastewater (APHA et al., 1998). Total coliforms were determined by incubation on M-Endo agar (24 h at 35°C) and confirmed by gas formation in lauryl tryptose broth and brilliant green lactose broth. Fecal coliforms were incubated on M-FC agar (24 h at 44.5°C) and were confirmed by gas formation in lauryl tryptose broth and EC broth. *E. coli* levels were measured by transferring the membrane from the Endo-type medium to a nutrient agar containing 4-methylumbelliferyl-β-D-glucuronide (NA-MUG) and incubating 4 h at 35°C. Production of blue fluorescence on the periphery of colonies under long wavelength UV indicated *E. coli*. Fecal streptococci were determined by incubation on m-Enterococcus agar (48 h at 35°C). Colonies were transferred to brain heart infusion (BHI) agar. Transfers were made to BHI broth and incubated at 35°C for 24 h, with confirmations made by retransfer to bile esculin agar, BHI broth incubated at 45°C, and BHI with 6.5% NaCl. Growth on bile esculin agar, BHI broth verified that the colony was of the fecal streptococci group. Growth at 45°C and in BHI with 6.5% NaCl indicated that the colony belonged to the enterococci group.

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

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

Die-off rate models

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

\[ C_t = C_0 e^{-Kt} \]  

(1)
where: \( C_t \) = concentration of organism at time \( t \) (CFU/100 mL); 
\( C_0 \) = concentration of organism at time zero (CFU/100 mL); 
\( K_o \) = overall die-off rate constant at the environmental conditions (h\(^{-1}\)); and 
\( t \) = elapsed time since time zero (h).

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

\[
K_0 = K_T + K_l + K_f
\]

(2)

where:
- \( K_T \) = die-off rate constant due to temperature (h\(^{-1}\));
- \( K_l \) = die-off rate constant due to light (h\(^{-1}\)); and
- \( K_f \) = die-off rate constant due to other factors such as sorption, filtration, and sedimentation (h\(^{-1}\)).

**Temperature**

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

\[
K_T = K_{20} \Phi_T^{(T-20)}
\]

(3)

where:
- \( K_T \) = die-off rate constant due to temperature at \( T = T_0^0 \)C (h\(^{-1}\));
- \( K_{20} \) = die-off rate constant due to temperature at \( T = 20^0 \)C (h\(^{-1}\));
- \( T \) = temperature in \(^0\)C; and
- \( \Phi_T \) = temperature coefficient (dimensionless).

**Light**

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

\[
K_l = \Phi_l I_Z
\]

(4)

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

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

\[
I_Z = \frac{I_0}{\tau_Z} (1 - e^{-\tau_Z})
\]

(5)
where: $I_Z$ = light intensity at depth $Z$ below the surface (mW/cm$^2$); 
$I_o$ = light intensity at the earth surface (mW/cm$^2$); 
$\tau$ = vertical light extinction coefficient (1/m); and 
$Z$ = depth of water (m).

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

Combining equations (2), (3), and (4), the overall equation can be written as:

$$K_o = K_{zo} \Phi_T^{(T-20)} + \Phi_f I_Z + K_f$$

Data analysis/reduction

The data analysis used all incubated plates with colonies in the countable range. The count from each 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 was 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 1 colony.

The sample weighted-average concentration and associated uncertainty was calculated using the uncertainty in the individual estimates as the weighting factor. This approach reduces the multiple (5 to 12) results from the samples and dilutions associated with the original source (bottle) to a single concentration estimate for the organism representing the experimental value resulting from the environmental condition.

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

Effects of temperature

The regression used the proposed time-dependent die-off function, known as Chick’s Law (equation (1)). The concentrations at each elapsed time for a given temperature were used to estimate the die-off rate constant for the indicator organism at that temperature. Table 2 lists the regression results from the temperature study with the 25°C results from the light study (discussed below). These experiments were also conducted in salt-free, dark, isothermal conditions and represent data that was included in the analysis.

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

Table 2. Die-Off Rate Constants for Each Indicator Organism at Tested Temperatures in the Isothermal Experiments

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Total Coliforms</th>
<th>Fecal Coliforms</th>
<th>E. coli</th>
<th>Fecal Streptococci</th>
<th>Enterococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inactivation Rate Constants (h⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.07</td>
<td>0.007±0.010</td>
<td>0.03±0.025*</td>
<td>0.027±0.015*</td>
<td>0.027±0.022*Δ</td>
<td>0.021±0.014</td>
</tr>
<tr>
<td>19.87</td>
<td>0.017±0.035</td>
<td>0.01±0.140</td>
<td>0.085±0.033*</td>
<td>0.076±0.077</td>
<td>0.095±0.038*</td>
</tr>
<tr>
<td>29.32</td>
<td>0.016±0.045</td>
<td>0.76±0.510*</td>
<td>0.136±0.072*</td>
<td>0.100±1.300◊</td>
<td>0.150±0.420</td>
</tr>
<tr>
<td>26.17 1</td>
<td>0.013±0.020</td>
<td>0.07±0.060*</td>
<td>0.019±0.072*</td>
<td>0.039±0.055</td>
<td>0.027±0.015*</td>
</tr>
</tbody>
</table>

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

Δ The first concentration is omitted from the analysis as an apparent outlier

◊ ANOVA shows regression model is not significant

1 Data from light experiment

The results listed in Table 2 emphasizing temperature, generally demonstrate an increase in the calculated die-off constant with increasing temperature. The same analysis on the results from the subsequent investigations for the light study at roughly 25°C does not produce rate constants expected by interpolating between the bounding temperatures of 10 and 20°C. This suggests that differences in the stormwater concentration influence the die-off rates.

The experimental design pooled the results of the temperature study data across the experimental temperatures. The weighted-average concentration results were regressed on elapsed time using the same nonlinear procedures to test the model proposed by equation 3. As discussed above, the temperature study used a common stormwater source for the samples exposed to the selected
temperature conditions. These samples had uniform starting concentrations, allowing for the
time delays in reaching time zero. The studies for light effects used different stormwater
samples with dramatically different initial concentrations, as would be expected from samples
collected at different times of the year. To pool the data into a common set, the results were
normalized to the estimated initial concentration using the calculated value of the initial
concentration, \( \hat{C}_o \), for the specific source calculated above. After transforming the data to \( \frac{C_t}{\hat{C}_o} \)
the above-described procedures were applied to estimate the reference temperature die-off
constant and the temperature coefficient using equation 7:

\[
\frac{C_t}{\hat{C}_o} = e^{-K_{20} \Phi_T (T_{20} - T_{ct})} \tag{7}
\]

Table 3 lists the regression coefficients. In all cases the temperature coefficient (\( \Phi_T \)) and the
reference die-off constant at 20\(^{\circ}\)C (\( K_{20} \)) are statistically significant.

<table>
<thead>
<tr>
<th>Indicator Organism</th>
<th>Reference Temperature Rate Constant (( K_{20} )) (h(^{-1} ))</th>
<th>Temperature Coefficient (( \Phi_T ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>0.016±0.009(^*)</td>
<td>1.057±0.085(^*)</td>
</tr>
<tr>
<td>Fecal Coliforms</td>
<td>0.042±0.030(^*)</td>
<td>1.090±0.110(^*)</td>
</tr>
<tr>
<td>( E. coli )</td>
<td>0.036±0.019(^*)</td>
<td>1.023±0.072(^*)</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>0.047±0.031(^*)</td>
<td>1.044±0.040(^*)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.042±0.014(^*)</td>
<td>1.057±0.045(^*)</td>
</tr>
</tbody>
</table>

* Coefficient is statistically significant at \( \alpha=0.05 \).

The \( \Phi_T \) values for all the organisms range between 1.02 and 1.09, which is consistent with the
values reported in the literature. Mancini (1978) and Khatiwada and Polprasert (1999) suggested
a value of 1.07 for fecal coliforms which is similar to the calculated value in Table 3.

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

The analysis assumes the light intensity measured at the sample surface is representative of the exposure throughout the container. The limited water depth in the selected container bottles supports this assumption. The light experiment was conducted at 26.17°C. The analysis of the results of the light exposure experiments were examined in a two-step process. The weighted-average concentration was first used to calculate the overall coefficient under the established condition of light and temperature \((K_T=26.17,l)\) for each indicator at each exposure level using the model in equation (8).

\[
C_t = C_o e^{-K_T,l t} \quad (8)
\]

where:
- \(C_t\) = concentration of organism at time (t) (CFU/100 mL);
- \(C_o\) = concentration of organism at time zero (CFU/100 mL);
- \(K_T,l\) = overall die-off rate constant at the environmental conditions (h\(^{-1}\)); and
- \(t\) = elapsed time since time zero (h).

Table 4 lists the regression-estimated values of \(K_T=26.17,l\) for each indicator organism at each light level. The statistical quality of the fecal coliform and *E. coli* results is generally poor. The increased light intensity affects the calculated die-off constant confirming that light influences the die-off process. The calculated die-off constant values increase with increasing light intensity. These results support the presumptive additive effect of light on the overall rate constant, i.e., \(K_{T,l} = K_T + \Phi l\).

### Table 4. Regression-Estimated Values of Die-Off Rate Constants at 26.17°C for Experimental Light Intensities

<table>
<thead>
<tr>
<th>Indicator Organism</th>
<th>0</th>
<th>20.86</th>
<th>55.23</th>
<th>94.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>0.131±0.037*</td>
<td>0.21±0.16*</td>
<td>0.23±0.053*</td>
<td>0.32±0.12*</td>
</tr>
<tr>
<td>Fecal Coliforms</td>
<td>0.100±0.18</td>
<td>0.07±0.17(\circ)</td>
<td>0.08±0.25(\circ)</td>
<td>0.20±0.24</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.029±0.048</td>
<td>0.14±0.16</td>
<td>0.16±0.23</td>
<td>0.26±0.20*</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>0.056±0.029*</td>
<td>0.205±0.063*</td>
<td>0.254±0.022*</td>
<td>0.469±0.025*</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.044±0.038*</td>
<td>0.202±0.070*</td>
<td>0.19±0.15*</td>
<td>0.96±0.14*</td>
</tr>
</tbody>
</table>

* Coefficient is statistically significant
\(\circ\) Regression result is not statistically significant at \(\alpha=0.05\)

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

\[
C_t = C_o e^{-\left(K_{T=26.17} + \Phi l\right) t} \quad (9)
\]

Table 5 lists the estimated values of the constants for each organism. The effect of light on the
die-off coefficient varies by a factor of four across the organisms showing a difference in light sensitivity. As expected, the values of $K_{T=26.17}$ generally agree with the values listed in Table 4 for the dark experiments. The values also agree with the expected estimated values using $K_T = K_{20}\Phi^{(T-20)}$ evaluated at 26.17°C. The light-free exposure for this data set is included in the previous analysis. The added light has minimal effects on the die-off rates for total and fecal coliforms. There is an order of magnitude smaller than the effects on the other indicators.

Table 5. Regression-Estimated Coefficients from Light Experiments

<table>
<thead>
<tr>
<th>Indicator Organism</th>
<th>Die-Off Rate Constant ($K_{T=26.17}$) (h$^{-1}$)</th>
<th>Light Proportionality Coefficient ($\Phi_L$) (cm$^2$/mW-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>0.155±0.047*</td>
<td>0.0016±0.0012*</td>
</tr>
<tr>
<td>Fecal Coliforms</td>
<td>0.070±0.090</td>
<td>0.0130±0.0027</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.040±0.037*</td>
<td>0.0025±0.0019*</td>
</tr>
<tr>
<td>Fecal Streptococci</td>
<td>0.046±0.015*</td>
<td>0.0057±0.0018*</td>
</tr>
<tr>
<td>Enterococci</td>
<td>0.034±0.020*</td>
<td>0.0076±0.0038*</td>
</tr>
</tbody>
</table>

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

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

The pH of the samples varied between 6.5 and 7.5. The DO content of the samples varied between 8.2 and 12.3 mg/L. The difference in DO from the temperature study is noteworthy. The decline observed in the temperature was not observed in this effort. The light exposure experiments lasted nearly 50 h. The total suspended solids concentration in the sample was measured at 85 mg/L. While about double the concentration recorded in the temperature study, this is still in the typically reported range.

CONCLUSIONS

This experiment demonstrated that the concentration of the tested microorganisms decrease exponentially with time. The first-order die-off process reasonably models the concentration time series for the durations tested. The analysis of the weighted average concentrations enabled developing organism-specific die-off rate constants in stormwater assuming time, temperature,
and light intensity as the most significant parameters. The factors of light, time, and temperature influence processes in all wet ponds and wetlands constructed to mitigate the effects of stormwater runoff on the receiving waters.

BMPs were originally designed to control runoff volumes and rates by attenuating the flow. The attenuation increases the time between the rainfall-generated runoff and the water reaching the receiving water. The time lag serves to reduce the concentration of these indicator organisms.

The temperature study indicated that the organisms persisted at higher concentrations at lower temperatures. This would suggest that when attempting to mitigate bacteria in runoff, watershed managers construct BMPs to maximize the temperature increase from solar exposure. Similarly, the added effects of light, even at constant temperature, suggest that the shade provided by emergent plant in wetland will tend to reduce the performance. The extent to which the deeper water of wet ponds attenuates the effect of incident light will vary with the properties (e.g., turbidity, color) of the water in the BMP. This effect may reduce the difference in performance to a negligible level.

These results suggest that the regulatory bacterial indicator selected will influence the adequate design. The apparent insensitivity of coliforms to light levels suggests that the shading effects will be reduced when this is selected as the water quality indicator. When the monitored microorganism is *E. coli* or enterococci, the effect of light is more significant.

The temperature study results suggest that differences in the stormwater characteristics measured by the initial concentration influence the reference die-off rate. This further suggests that the constants measured in the bench-scale experiments must be viewed as the rate for the specific stormwater sample evaluated and cannot be extrapolated to all stormwater sources or other storm events in the same watershed. The variability of the constants between sources, if any, cannot be estimated from these data.

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DISCLAIMER

Any opinions expressed in this paper are those of the author(s) and do not, necessarily, reflect the official positions and policies of the U.S. EPA. Any mention of products or trade names does not constitute recommendation for use by the U.S. EPA.

REFERENCES


