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Mortality rates of pathogen indicator microorganisms discharged from point and non-point sources in an urban area

Geonha Kim[1,*], Jin Hur[2]

1. Department of Civil and Environmental Engineering, Hannam University, Ojungdong, Daedukku, Daejeon 306-791, Korea. E-mail: kimgh@hnu.kr
2. Department of Earth and Environmental Sciences, Sejong University, Seoul 143-747, Korea

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Abstract

This research measured the mortality rates of pathogen indicator microorganisms discharged from various point and non-point sources in an urban area. Water samples were collected from a domestic sewer, a combined sewer overflow, the effluent of a wastewater treatment plant, and an urban river. Mortality rates of indicator microorganisms in sediment of an urban river were also measured. Mortality rates of indicator microorganisms in domestic sewage, estimated by assuming first order kinetics at 20°C were 0.197 day^{-1}, 0.234 day^{-1}, 0.258 day^{-1} and 0.276 day^{-1} for total coliform, fecal coliform, *Escherichia coli*, and fecal streptococci, respectively. Effects of temperature, sunlight irradiation and settlement on the mortality rate were measured. Results of this research can be used as input data for water quality modeling or can be used as design factors for treatment facilities.

Key words: coliform; mortality rate; non-point source pollution (NPS); indicator microorganisms; pathogens

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Introduction

As recreational use of urban streams increases, concerns have arisen regarding personal hygiene and waterborne pathogens. Pathogens associated with waterborne disease include enteric viruses derived from human fecal contamination; bacterial pathogens, represented by *Escherichia coli* O157:H7; and the protozoan pathogens *Cryptosporidium* and *Giardia* (Ferguson et al., 1996; Gagliardi and Karns, 2000). Potential point pathogen sources are waste water treatment plants (WWTPs), separated sewer overflows (SSOs), slaughterhouses, and animal feedlots (Lipp et al., 2001; Exall et al., 2004; Charles et al., 2003; Gessel et al., 2004). Potential non-point sources are illicit sewage connections, wildlife, septic systems, livestock, landfills, combined sewer overflows (CSOs), and pastures (Doran et al., 1981; Ellis and Yu, 1995; Smith and Perdek, 2004).

Pathogen levels in water can be estimated by measuring pathogen indicator microorganism concentration. Pathogen indicator organisms, often called indicator organisms, refer to pathogen-associated microorganisms, typically chosen for easier isolation and identification of contamination (Thomann and Mueller, 1987; USEPA, 2001). Total coliform, fecal coliform, and *E. coli* are the most commonly used indicators. Enterococci and streptococci have also been used in studies (Baudisova, 1997; Jin et al., 2004; Murray et al., 2001).

* Corresponding author. E-mail: kimgh@hnu.kr

If pathogens are discharged into bodies of water, mortality rates are affected by many physical and biological factors, including sunlight, temperature, salinity, predators, nutrients, toxicity, settling/scouring, and aftergrowth. First order mortality rates of bacteria or other microorganisms can be represented by K_B (day^{-1}) and can be expressed as following Eq. (1) (Thomann and Mueller, 1987):

$$K_B = K_{B1} + K_{BL} + K_{BS} + K_A \quad (1)$$

where, K_{B1} is basic death rate as a function of temperature, salinity, predation; K_{BL} is death rate due to sunlight; K_{BS} is net loss (gain) due to settling (resuspension); and K_A is aftergrowth rate.

Pathogens of fecal origin decay rapidly upon leaving their hosts. However, the decay rate varies depending on certain circumstances in nature related to contaminant concentration and *E. coli* concentration (Kim and Kim, 1985). As pollution rate increases, the number of predators also increases, while *E. coli* using pollutants as substrates resulted in a lower mortality rate. In addition, mortality rate is closely related to temperature, turbidity, and suspended solids (Jang and Lee, 2003). However, few efforts have measured mortality rates of the indicator microorganisms discharged from various points and diffuse sources.

As total coliform among pathogen indicator microorganisms is included in water quality criteria and considering the increased recreational use of surface water, the mortality rate of indicator microorganisms is important parameter for water quality models. The objective of this research were to measure mortality rates of pathogenic indicator microorganisms originating from various point sources and non-point sources in an urban area for effective surface water quality management.

1 Materials and methods

1.1 Sampling sites

Water samples for measuring mortality rates were collected from urban wastewater, combined sewer overflows, effluents from wastewater treatment plants, urban stream water, and sediments of an urban stream.

1.2 Measurement methods of mortality rates

The following analyses were performed in this research: (1) to establish the organic content of the waters sampled, BOD (biochemical oxygen demand), SBOD (soluble BOD), COD (chemical oxygen demand), SCOD (soluble COD), SS (suspended solids), and VSS (volatile SS) concentrations were measured; (2) to measure the removal rate of indicator microorganisms by gravity, settling experiments were conducted; (3) mortality rates due to sunlight were measured; (4) to understand the impact of sunlight on mortality rates, temperature was varied during measurement of the mortality rate to obtain empirical equations relating the mortality rate and the temperature range.

Live microorganism count N, decayed as a function of time, t, can be expressed as Eq. (2):

$$N = N_0 e^{kt} \quad (2)$$

where, N_0 is live microorganism concentration at time 0; N is live microorganism concentration at time t; and k is mortality rate. The 90% decay time, t_{90}, can be represented as follows:

$$t_{90} = \frac{2.3}{K_B} \quad (3)$$

The mortality rates were measured at 20°C and can be represented as time-dependent equations as follows:

$$K_{temp} = K_{20} \times c^{(temp - 20)} \quad (4)$$

where, K_{temp} is mortality rate in the range of 10–20°C; K_{20} is mortality rate measured at 20°C, c is constant.

For settling experiments, urban sewage was put in 1 L bottles and settled for 5, 10, 30, 60, 120 min and analyzed for COD, SS, and total coliform (TC) concentrations. Separate columns were used for each constituent and settling time and each experiment was performed in triplicates and the measurements averaged.

To measure mortality rate due to sunlight, urban sewage and urban stream water were put into four transparent flasks and stored in 20°C water bath. The flasks were placed on the roof with no shade and exposed to sunlight for five hours and sampled at predetermined time intervals. The ultraviolet ray intensity of the sunlight was obtained from the local meteorological bureau office and was in the range of 1680–1890 J/m^2.

1.3 Analysis of samples

Analysis for BOD_5, $SBOD_5$, COD_{Cr}, $SCOD_{Cr}$, SS, VSS, and indicator microorganism concentrations was performed in accordance with Standard Methods (APHA, 1988). TC was measured by standard methods 9222B. An M-Endo medium was used and incubated for 24 hours at 35°C. Fecal coliform was measured using Standard Methods 9222D. An M-fecal coliform medium was used and incubated for 24 hours at 44.5°C. *E. coli* was measured using Standard Method 9230C. A nutrient agar-MUG was used and incubated for 24 hours at 35°C. Fecal streptococci was measured by the Standard Methods mE method, and incubated for 48 hours at 35°C.

2 Results and discussion

2.1 Mortality rate of urban wastewater during dry weather

Figure 1 presents the results of mortality measurement experiments as a function of temperature and time. The data indicated that mortality rates were very sensitive to changes in temperature. Table 1 summarizes the mortality rates of indicator microorganisms in domestic sewage.

Figure 2a shows the settling test results of domestic wastewater discharged from the study watershed for SS, COD, and TC concentrations. TC concentration was decreased 41% by 10-min settlement while SS was decreased 33%, and COD was decreased 23%, which implies that settling effectively removes indicator microorganisms. Previous research has reported that coliform among rainfall runoff discharged in association with suspended particles (Borst and Selvakumar, 2003; Crainiceanu et al., 2003; Ferguson et al., 2003; Schillinger and Gannon, 1985). Figure 2b shows that TC removal rate by settling, and the varying removal rate depending on the characteristics of raw sewage. Mortality rate was measured as 9.2×10^3–20.0×10^3 day^{-1}. This indicates that settling is an effective measure for removing pathogens from surface water. This also implies that discharged pathogens settled prior to exposure to sunlight and can survive for extended periods of time. Thus, resuspension of sediment could be an acute threat for degrading surface water quality during high flow conditions.

Figure 3 illustrates the determination of empirical equations for estimating mortality rates considering temperature effects. Overall, it can be observed from Fig. 3 that the mortality rate was very sensitive to temperature.

Table 2 summarizes mortality rates of indicator microorganisms in various water samples measured at 20°C.

Table 1 Mortality rate of indicator microorganisms in domestic sewer (day^{-1})

	Sewer temperature		
	10°C	15°C	20°C
Total coliform	0.048	0.088	0.197
Fecal coliform	0.069	0.173	0.234
E. coli	0.072	0.201	0.258
Fecal streptococci	0.079	0.202	0.276

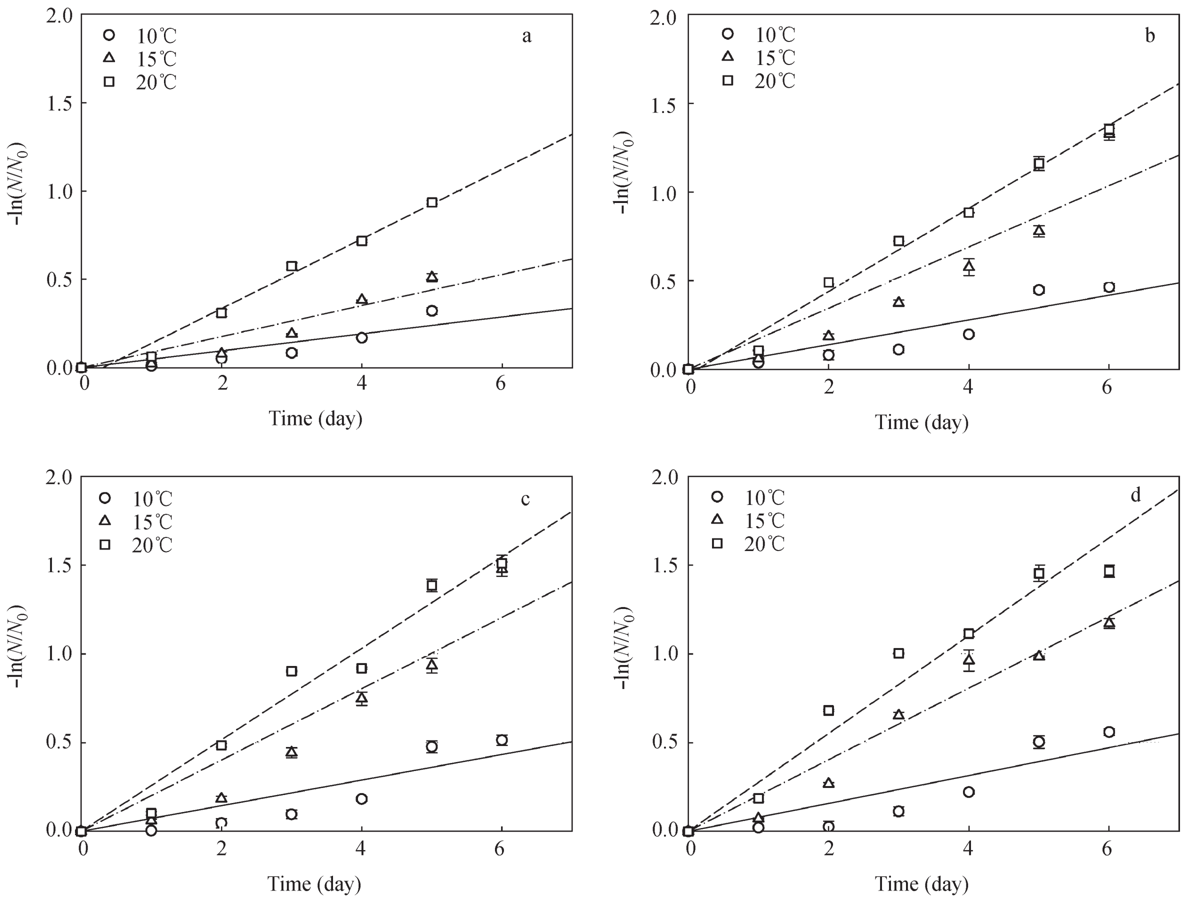

Fig. 1 Determination of mortality rate of wastewater by Chick's law as functions of initial enumeration count (N_0) and enumeration count (N). (a) total coliform; (b) fecal coliform; (c) *E. coli*; (d) fecal streptococci.

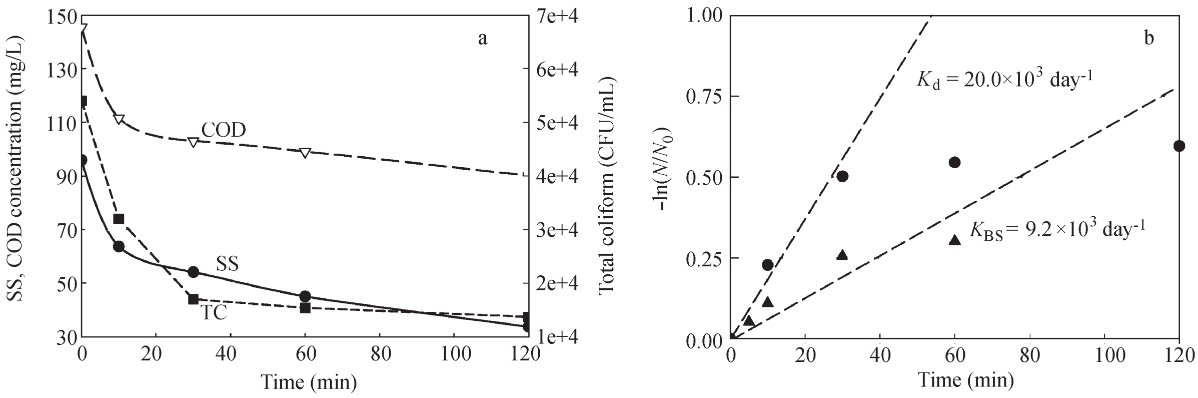

Fig. 2 Settling test results of domestic wastewater discharged from the study watershed for SS, COD, and TC concentrations variations as a function of time (a); determination of die-off rate of total coliform in domestic wastewater by Chick's law (b).

Indicator microorganisms in effluents from wastewater treatment plants were induced from the fact that night soil is mixed with influent with no installation of disinfection process.

Figure 4 shows the effects of sunlight on mortality rates in wastewater by Chick's law as functions of initial enumeration count and enumeration count at time t. Mortality rate of pathogen indicator microorganisms was estimated as 1.22–1.59 day^{-1}, and these values were higher than intrinsic mortality rate and smaller than those by settling.

Table 2 Mortality rate of indicator microorganisms in various water samples measured at 20°C (day^{-1})

	Sediment of an urban stream	Surface water of an urban stream	Effluent from a wastewater treatment plant
Total coliform	0.2127	0.2507	0.2187
Fecal coliform	0.2265	0.2577	0.2259
E. coli	0.2218	0.2755	0.2127
Fecal streptococci	0.1933	0.2441	0.2333

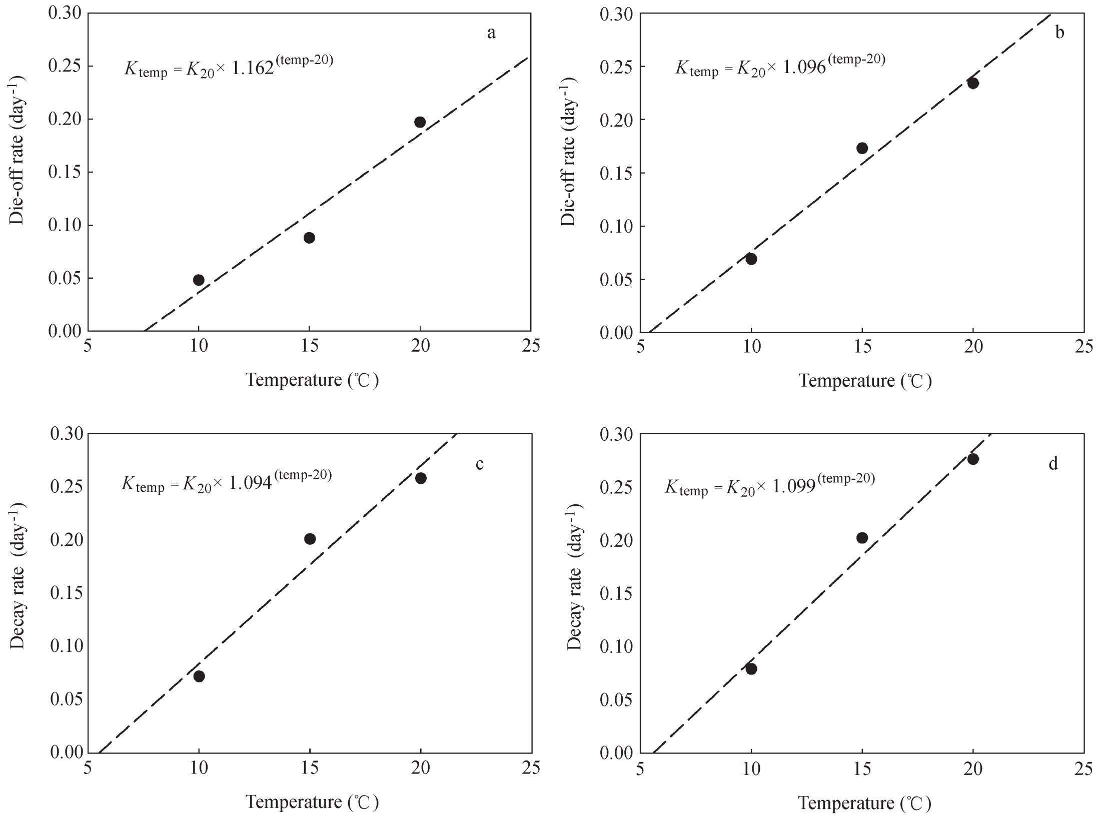

Fig. 3 Determination of empirical equations for estimating mortality rate considering temperature effects. (a) total coliform; (b) fecal coliform; (c) *E. coli*; (d) fecal streptococci.

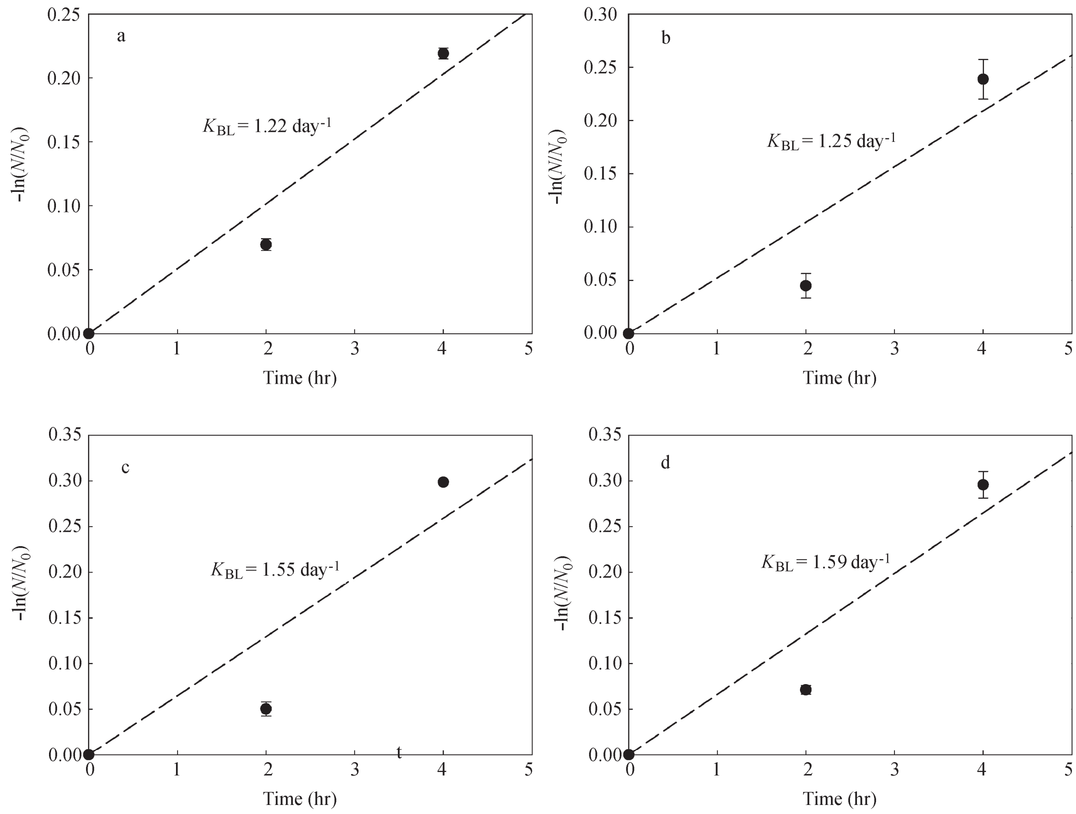

Fig. 4 Effects of sunlight on mortality rate (k_{BL}) of wastewater by Chick's law. (a) total coliform; (b) fecal coliform; (c) *E. coli*; (d) fecal streptococci.

These values are smaller compared to 2.3 day^{-1} under 25.5°C, 23.7 cal/cm^2 sunlight estimated by Yukselen et al. (2003), which due to suspended solid exhibit direct decay of indicator microorganisms.

3 Conclusions

In this research, pathogen indicator microorganism levels discharged from point and non-point pollutant sources were measured. Mortality rates of the indicator microorganisms were reported as functions of affecting factors including settling time, temperature, and sunlight. Results of this research can be used as input data for water quality modeling or can be used as design factors for attenuation facilities.

Mortality rates of indicator microorganisms in domestic sewage, estimated by assuming first order kinetics at 20°C, were determined as follows 0.197 day^{-1} for total coliform day, 0.234 day^{-1} for fecal coliform, 0.258 day^{-1} for *E. coli*, and 0.276 day^{-1} for fecal streptococci. Sensitivity of mortality rates of total coliform against temperature was estimated to be $K_{\text{temp}} = K_{20} \times 1.162^{(\text{temp-20})}$ for the range of 10–20°C. Mortality rates due to sunlight were measured to be 1.22–1.59 day^{-1}, while mortality rates due to settling for 40 min were estimated to be 9.2×10^3 –20.0×10^3 day^{-1}.

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