Variation of microorganism concentrations in urban stormwater runoff with land use and seasons

Ariamalar Selvakumar and Michael Borst

ABSTRACT

Stormwater runoff samples were collected from outfalls draining small municipal separate storm sewer systems. The samples were collected from three different land use areas based on local designation (high-density residential, low-density residential and landscaped commercial). The concentrations of microorganisms in the stormwater runoff were found to be similar in magnitude to, but less variable than, those reported in the stormwater National Pollutant Discharge Elimination System (NPDES) database. Microorganism concentrations from high-density residential areas were higher than those associated with low-density residential and landscaped commercial areas. Since the outfalls were free of sanitary wastewater cross-connections, the major sources of microorganisms to the stormwater runoff were most likely from the feces of domestic animals and wildlife. Concentrations of microorganisms were significantly affected by the season during which the samples were collected. The lowest concentrations were observed during winter except for *Staphylococcus aureus*. The Pearson correlation coefficients among different indicators showed weak linear relationships and the relationships were statistically significant. However, the relationships between indicators and pathogens were poorly correlated and were not statistically significant, suggesting the use of indicators as evidence of the presence of pathogens is not appropriate. Further, the correlation between the concentration of the traditionally monitored indicators (total coliforms and fecal coliforms) and the suggested substitutes (enterococci and *E. coli*) is weak, but statistically significant, suggesting that historical time series will be only a qualitative indicator of impaired waters under the revised criteria for recreational water quality by the US EPA.

Key words | automatic sampling, indicator organisms, land use, outfall, pathogen, rain fall, season, stormwater runoff

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INTRODUCTION

The national biennial water quality surveys consistently show bacterial loadings with nutrients, sediments and toxic chemicals as a primary contributor to impaired waters. A leading source of this impairment is stormwater runoff from agricultural and urban areas affecting an estimated 13% of impaired rivers, 18% of impaired lake areas and 32% of impaired estuaries (US EPA 2002). While not all waters are surveyed nor have all waters been evaluated for impairments, leading to questions of statistical bias, the results emphasize the large potential environmental risks to recreational and source waters linked to stormwater runoff (US EPA 2000). These risks emphasize the need for broad-based stormwater controls. One survey in which 1000 km of the Southern California shoreline was sampled the day after a storm to assess the spatial influence of rainfall on regional water quality showed that the storm events have a dramatic regional effect on the beach water quality and urban runoff outlets are the primary sources of contaminants (Noble *et al.* 2003).

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As the US Environmental Protection Agency (US EPA) moves to more stringent water protection through the statutory authorities of the Clean Water Act and Safe Drinking Water Act, both the Agency and practitioners need a better understanding of specific pollutants such as stormwater microrganism concentrations to predict the effects on receiving water. The use of computer-based modeling is a likely management tool for evaluating management alternatives. These models are increasingly linking water quality to land use types and benefitting from remotely gathered data and GIS-based data handling. This approach requires, however, understanding the concentration and load from a given land area based on land use. Practitioners have long recognized the differences in pollutant concentration load generation in broad land uses such as agricultural or urban, but the understanding of finer division categorization is less well quantified, leading to aggregated modeling blocks and therefore less targeted management actions. This project evaluates the hypothesis that there are different concentrations in seasonal stormwater runoff from different land uses.

Published data anecdotally support this hypothesis with some limited research directly supporting the supposition. However, there is little examination for a wide range of indicator organisms. Although some of the data are from outfalls known to have sanitary wastewater cross-connections, others either did not report or test for cross-connections (Oliveri *et al.* 1977). Most data collected, however, analyzed the receiving waters, not stormwater, forcing inferential analysis of the stormwater loads and concentrations (Mallin 1998; Young & Thackston 1999; Mallin *et al.* 2000). This emphasis on receiving water monitoring is consistent with the need to measure the bacteria concentration in the regulated media and directly supports public health considerations. Practical stormwater management, however, will not attempt to mitigate the load after mixing with the receiving water but rather operate within the stormwater system.

Relationship with land use

Oliveri *et al.* (1977) reported *Pseudomonas aeruginosa* and *Staphylococcus aureus* at levels of 10^2 to 10^3 and 10 to 10^2, respectively, in stormwater runoff collected from residential areas in Baltimore, MD. A 1999 study to establish the source of unexpectedly high river and stream bacterial concentrations near Nashville, TN showed fecal coliforms, fecal streptococci and enterococci concentrations directly related to the housing density, population, development, imperviousness and apparent animal density. Surface runoff samples from more densely populated, sewered areas generally showed higher bacterial counts than runoff from less developed areas with septic tanks, suggesting a relationship may exist between land use and potential bacterial loading (Young & Thackston 1999). This study, however, was designed to investigate the in-receiving water load and not the runoff concentrations. Weiskel *et al.* (1996) reported that fecal coliform concentrations in stormwater runoff from impervious surfaces were related to the surrounding land use. The highest fecal coliform yields, from a high-density residential area, were significantly higher than those associated with nearby moderate-density residential areas, commercial areas and low-density residential areas. Glenne (1984) and Chang (1999) independently concluded that population density, development age and percent of residential development may better predict bacteria levels in urban stormwater runoff than factors such as rain fall intensity, antecedent dry period, etc.

Studies by Mallin (1998) and Mallin *et al.* (2000) in North Carolina showed that fecal coliform abundance in estuarine creeks significantly correlated with watershed population, and even more strongly correlated with the percentage of developed land within the watershed. However, watershed imperviousness, which consists of roofs, roads, driveways, sidewalks and parking lots, was the most important anthropogenic factor associated with fecal coliform abundance in the estuarine waters (Mallin *et al.* 2000). Based on their data set, a regression model $FC = 5.4$ (percent imperviousness) - 29 was developed, where FC = concentration of fecal coliforms in the estuarine.

Hydroqual (1996) evaluated fecal coliform concentrations in seven small subwatersheds with different imperviousnesses in the Kensico watershed, part of the New York City drinking water reservoir system. The data fails to detect fecal coliform concentration increases with increasing impervious cover, contradicting the results.

Samples collected from tributaries in five different land uses (forest, mixed, industrial, low-density residential and

wetlands) in New Jersey's Whippany River watershed showed fecal coliform and enterococci concentrations in the tributary sample from the various land uses varied by at most one order of magnitude (Killam Associates 1997). Enterococci densities were higher than fecal coliform densities in all the samples.

A total of 136 stream water samples collected in five important hydrologic systems in the United States were analyzed for microbiological indicators to test a monitoring concept in a nationally consistent program. Total coliforms were found in 99%, *Escherichia coli* (*E. coli*) in 97% and Clostridium perfringens in 73% of stream water samples. Examination of the relationship between bacterial concentrations and potential explanatory factors (mean annual basin temperatures, human population density, live stock density, stream flow and land use) showed land use to be the most significant factor (Francy *et al.* 2000).

Relationship with season

There is evidence in the literature that microorganism concentrations in the stormwater varies seasonally. The earlier-mentioned Nashville, TN study showed much higher summer fecal coliform counts than winter (Young & Thackston 1999). The results are consistent with the data collected during the Nationwide Urban Runoff Program (NURP) study where fecal coliform densities in urban runoff during the warmer months of the year were approximately 20 times greater than those found during cooler periods (US EPA 1983). Stormwater from city streets, a suburban business district storm drain and a wooded hillside adjacent to a city park showed that peak total coliform, fecal coliform and fecal streptococci densities occurred either in summer or autumn (Geldreich *et al.* 1968). Stormwater samples collected in the residential and light-commercial portion of the Mt. Washington section of Cincinnati throughout the year indicated that total coliform, fecal coliform and fecal streptococci densities were greatest in summer and lowest in winter (Evans *et al.* 1968).

A study in two northwest Arkansas streams found significant seasonal influences on fecal coliform and fecal streptococci concentrations, with the highest concentrations occurring in summer (Edwards *et al.* 1997). Another study in a lake called Upvan Lake of Thane City, India also found seasonal variation of bacterial population in the water body. Bacterial counts increased during the summer months and decreased during winter months. The high summer population could be attributed to less variable dilution in summer and the lowest winter concentration could be due to lower multiplication and poor growth of organisms following low temperatures in winter (Bagde & Rangari 1999).

OBJECTIVES

There is suggestive evidence linking the concentration of microorganisms in urban stormwater runoff with season and land use (directly or indirectly) based on the literature discussed above (Glenne 1984; Mallin 1998; Young & Thackston 1999; Francy *et al.* 2000). Although some of the studies are from outfalls known to have cross-connections (Oliveri *et al.* 1977), others either did not report or test for cross-connections. Other studies sampled receiving waters only (Mallin 1998; Francy *et al.* 2000). Therefore, the data are very limited for use in assessing the effects of land use and seasons on organism concentrations in stormwater runoff.

This study investigates if variations in concentrations of microorganisms by at least 1/3-log at the 95% level of confidence are potentially attributable to land use and seasons. Differences less than 1/3-log have little practical importance even if there is statistical significance as the sensitivity of the analyses procedure is less than these.

PRELIMINARY RESEARCH

Before beginning the evaluation, the monitoring plan development required data from several preliminary experiments. The first evaluated the potential statistical distribution of multiple analyses of a sample. These data are necessary to develop the sample collection and analysis strategy of the final experimental design. Recognizing the sample load and laboratory throughput limitations, a separate experiment evaluated the effects of not meeting the Standard Method 9060B recommended 24-h holding time before completing the sample membrane filtrations.

Experiments evaluated the sample collection technique and laboratory treatment of the composite sample to help ensure evaluation of a representative subsample which results in a valid event mean concentration (EMC). The final preliminary experiment evaluated the need to pre-treat the samples using chemical, mechanical or a combination of both before filtration to include particle-associated microorganisms.

Preliminary evaluation of the statistical population confirmed that the analytical data followed a log-normal distribution. Researchers have commonly identified this distribution for microbial organisms as well as many other pollutants in stormwater (US EPA 1983; APHA *et al.* 1998; Crowther *et al.* 2001). The distribution of results from a set of ten analyses of a common diluted sanitary sewage source (assuming that organisms in both sanitary sewage and stormwater runoff follow the same statistical distribution) was tested using the Shapiro–Wilkes test of normality, the preferred statistical test (SigmaSoft) (StatSoft 1998). In each case, the analysis confirmed the non-normal distribution. Similar analysis of the log-transformed data supported the presumed log-normal distribution. The measured concentrations were within the range reported in the literature for diluted sanitary sewage and the standard distribution showed that four analyses were necessary to detect a difference of 1/3-log or more at the 95% level of confidence. Difference less than 1/3-log have little practical importance even if there is statistical significance.

Routine analysis procedures for regulatory and non-regulatory samples allow up to 24-h refrigerated holding period as per Method 9060B (APHA *et al.* 1998). Given the number of analyses desired to meet the statistical confidence levels and the limited laboratory analytical capacity, knowing if extending the holding period affected the analytical results became critical. Incoming stormwater samples were split into subsamples, with the first subsample analyzed immediately and other subsamples analyzed after being held at 4 °C for one and two days. Traditional analysis of variance of log-transformed analytical data showed no significant variation attributable to the increased holding time at the 95% level of confidence for any organism evaluated (Selvakumar & Borst 2004). This is consistent with the expected results based on temperature dependance of first-order decay constants (Thomann & Mueller 1987; Canteras *et al.* 1995). Similar work in the Chattahoochee River sponsored by the EPA and the Water Environment Research Foundation reached similar conclusions on extending the sample pre-analysis holding time (Selvakumar & Borst 2004). Based on these results, all samples were analyzed as quickly as possible within 48 h following a defined analytical sequence developed based on the apparent most time-sensitive bacteria measurements.

Although multiple analyses of independent samples drawn from a flow-weighted composite sample appear to be an appropriate technique to estimate uncertainty in the measured sample concentration, it is not clear how this compares to installing multiple samplers to collect and analyze multiple independent samples. The selected flow-meters can only trigger two samplers, so this experiment installed two samplers analyzing multiple subsamples from each sampler to test the hypothesis that the results were different. The data showed no significant differences between the mean log concentrations among the samplers. Based on these results, a single composited sample was collected at each site.

To test the technique of using a stirrer–siphon technique, another experiment was performed, as the composited sample volume frequently exceeded the ability to hand mix the container contents. Beyond the difficulties linked to the sample weight, even partially filled samples showed apparent settling effects. A technique was developed using an electric stirrer to maintain a more homogenous sample while siphoning a series of subsamples for analysis (hence stirrer–siphon). Total suspended solids analysis was selected for this evaluation as a more direct evaluation of particle suspension and separation than bacteria concentrations. Using the hand shaking technique, the relative standard deviation of the total suspended solids concentration was 28%. Using the stirrer–siphon technique, the relative standard deviation was reduced to 14%. Based on these results all incoming samples were subsampled using the stirrer–siphon technique.

Particle-associated bacteria are a well documented phenomenon in combined sewage (Glover & Herbert 1973; Moffa *et al.* 1975; Perdek & Borst 2000) and drinking water (Ridgway & Olson 1982). In some cases, the literature shows the association can effectively mask large portions of the total bacterial load from analytical procedures.

Unfortunately, a standard procedure to separate the bacteria from the solids is not established. The literature includes examples of both chemical (Camper *et al.* 1985) and mechanical (Glover & Herbert 1973; Moffa *et al.* 1975) procedures. Preliminary evaluations of runoff samples in this project trying chemical, mechanical and a combination of both chemical and mechanical showed inconclusive, analysis-specific results (Borst & Selvakumar 2003). In some conditions, the procedures intended to release the bacteria may increase the relative number of hidden bacteria. Without both a clear technique and an obvious advantage to the pretreatment, the separation procedures were not included in the analytical techniques applied.

EXPERIMENTAL DESIGN

Site description

This project was completed in the 246-km^2 Navesink River watershed (Figure 1). Discharging into Sandy Hook Bay, the Navesink estuary supports 2300 acres of commercially important shell fishing beds (NJDEP 1999). The watershed drains a variety of land uses with variable population densities (Scro 1993). The US Geological Survey estimates the mean annual flow from the river is about 2.3 m^3/s. The watershed is entirely within Monmouth County, NJ. The part of the county studied has separate stormwater and sanitary sewer systems. Its land use/land cover has been divided into fourteen major categories generally paralleling the United States Geological Survey classifications, e.g. residential with various levels of imperviousness, landscaped commercial/industrial, unlandscaped commercial/industrial and agricultural. The land uses selected for the study included high-density residential areas (65% imperviousness), low-density residential areas (17% imperviousness) and landscaped commercial areas (approximately 15% of the total area is vegetated).

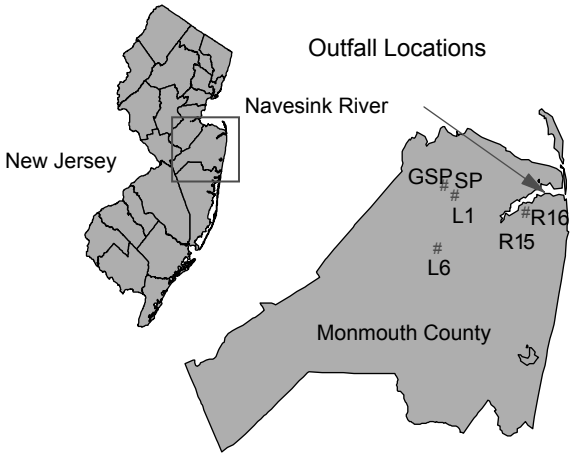

Figure 1 | New Jersey County map and location of stormwater outfalls selected for this study within Monmouth County.

Organism and indicator selection

Two human pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and five indicator organisms (total coliforms, fecal coliforms, fecal streptococci, enterococci and *Escherichia coli* (*E. coli*)) were selected for this study. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were selected based on their presence in abundance in stormwater reported in other studies (e.g. Milwaukee and Baltimore) (Oliveri *et al.* 1977) and their association with diseases transmitted through water contact (e.g. skin, ear and eye infections). Total coliforms, fecal coliforms, fecal streptococci, enterococci and *E. coli* are commonly used or proposed regulatory standards and/or bacterial indicators in water quality monitoring.

Experimental set-up

Preliminary sampling showed that the statistical probability distribution of multiple subsamples from each site followed a log-normal distribution. Using these results with the literature-reported expected concentrations and the concentrations measured during the preliminary experiments, *a priori* statistical analysis showed that two storm events per season from two outfalls in each land use were sufficient to establish concentration differences of 1/3-log at the 95% level of confidence and 80% statistical power. "Sewersheds" were selected such that they drain small areas, shown to be free of cross-connections, and within an urban watershed dominated by one land use. The outfalls were expected to be above the expected high-water depth to avoid back-flow and sample dilution, have safe, all-season public access and provide reasonable equipment security. It was difficult to find suitable locations that satisfy the criteria. Outfalls were visually monitored for two weeks generally following

established procedures (Pitt *et al.* 1993) before final selection to ensure no dry-weather flows suggesting potential inappropriate cross-connections or other sources such as car washing, irrigation of lawns, etc. Locations of the outfalls are shown in Figure 1 and the details are given in Table 1. The selected outfalls were near each other to reduce the variations introduced by geographic factors (e.g. antecedent dry period, rain duration and intensity, geology), recognizing that not all extraneous factors could be eliminated. The equinox and solstice dates were used to divide the year into four seasons. Rain events were defined as rainfall that produce enough runoff to allow collection of a sufficient sample for analytical needs, separated by at least 72 rain-free hours.

Sampling equipment

Area-velocity flowmeters (American Sigma, Loveland, CO) installed at each selected outfall recorded flow depth and velocity at 6-min intervals. Depth was measured using differential pressure (bubbler) or differential piezoresistive balanced bridge sensors. Twin 1 MHz piezoelectric crystals were used to measure Doppler-based velocity. Internal electronics combine the measured values using the storm sewer pipe internal geometry to compute an associated flow rate (American Sigma, Loveland, CO). Instrument systems included ancillary measurements of temperature, specific conductivity, dissolved oxygen and pH. Probes for these measurements were installed in 1-gallon plastic buckets positioned to collect the pipe discharge. Inter-rain event calibrations, following the manufacturer's outlined procedures, were completed before each rain event. Pre-calibrated automatic samplers (American Sigma, Loveland, CO) fitted with a single 10- or 20-L HDPE container were connected to each flowmeter. Pre-calibrated tipping bucket rain gages (Onset Corp., Bourne, MA; American Sigma, Loveland, CO; and Environmental Sensors, Inc., Escondido, CA) installed near the outfall and away from obstructions recorded local rainfall in 0.2 mm increments. All field instrumentation was battery operated and generated delimited ASCII data files.

Generally following EPA guidance (US EPA 1992) for collecting flow-weighted NPDES stormwater samples, the meters triggered the automatic samplers to collect samples when the flow water depth in the storm sewer initially reached 1 in (2.54 cm). Upon triggering, the automatic sampler's internal peristaltic pump purged the 9.5-mm diameter pre-cleaned vinyl sample line before the initial sample collection. The pump transferred 1-L stormwater aliquots for every 1350 L passed until the HDPE container is filled or the meter measured no flow for 3 h. The samplers notified collection teams by cellular modem that a sample was collected. The samples were recovered and immediately placed in a cooler with ice and transported to the EPA's laboratory for processing. The sampler collection bottles were throughly washed and acid/alcohol rinsed between storm events.

Sample analysis

Prior to subsample collection, the samples were continuously stirred in the original 10- and 20-L container using an

Table 1 | Characteristics of the outfalls selected for the study

Land use	Sewershed name	Longitude	Latitude	Drainage area (acres)	Outfall diameter (in)
High-density residential (65% imperviousness)	R15	74° 02′ 37.4″	40° 21′ 17.5″	6.04	15
	R16	74° 02′ 37.7″	40° 21′ 17.2″	10.2	24
Low-density residential (17% imperviousness)	L1	74° 09′ 48.9″	40° 22′ 32.0″	2.69	18
	L6	74° 11′ 32.5″	40° 18′ 45.4″	4.54	18
Landscaped commercial	GSP	74° 10′ 47.9″	40° 23′ 17.9″	0.35	15
	SP	74° 10′ 48.2″	40° 23′ 15.2″	5.73	36

electrically driven propeller (Stir Pak Mixer) for 3–5 min, while aliquots were extracted by a siphon from mid-water height for analysis. The samples were analyzed for two pathogens (*Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA)) and five indicator organisms (total coliforms (TC), fecal coliforms (FC), fecal streptococci (FS), enterococci (EN) and *E. coli* (EC)) following procedures listed in *Standard Methods* (APHA *et al.* 1998). For the purposes of these tests, the designated holding time began when the sample arrived at the laboratory. If the samples were not processed immediately, they were refrigerated at 4 °C. While no samples exceeded the holding time, in some cases the total elapsed time from first sample collection through filtration marginally exceeded the 24 h total. As outlined earlier, these samples are believed valid as independent experiments failed to detect changes in measured concentration with longer holding times when samples were maintained at 4 °C. Each sample was sequentially diluted with buffered distilled water using three dilution factors based on previous analyses of samples from the same source. Sequential dilutions generally used at least 10 mL aliquots and always used at least 5 mL. Samples from each dilution were analyzed in quadruplicate (four times). The dilution factors were selected to obtain the method-recommended colony count on at least one dilution set. All results were 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 of microorganism 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. Precision as the relative standard deviation of log-transformed data was set at no more than 70%.

Data analysis

The concentration from the analyses yielding plate counts in the method-specified range were used. If multiple dilutions produced counts in the desired range, all dilutions producing results within the range were used. If no dilution provided plates with the desired colony counts, then countable plates outside the range were used. The data analysis excluded non-quantitative data (e.g. too numerous to count or less than 1). The concentrations were $\log_{10}$-transformed prior to analysis and were tested using standard analyses of variance techniques at $\alpha = 0.05$ (i.e. 95% level of confidence). One-way Analysis of Variance (ANOVA) was performed to test the potentially statistically significant difference (e.g. rejection of the null hypothesis) between concentrations in stormwater from different land use areas and seasons on $\log_{10}$-transformed data. The statistical analysis was completed using Statistica '98 (Statsoft 1998).

RESULTS AND DISCUSSION

Samples from a total of 14 rainfall events were collected over 2 years. Precipitation amounts ranged from 1.8 mm to 51.8 mm and the rain intensity (cumulative rainfall/event duration) ranged from 0.9 mm/h to 15 mm/h. At least seven samples were collected from all outfalls, including at least one sample per season. Some samples were not collected because inlet tubes became clogged with vegetative material, ice or other debris or due to malfunctions in the flow-recording equipment. Although it was hoped to collect a composite sample from each outfall, samples primarily reflected the rising hydrograph limbs. The runoff had an average pH of 6.87 ± 0.02, conductivity of 0.12 ± 0.01 mS and a temperature of 17.47 ± 0.18 °C.

The microorganism concentrations found in urban stormwater runoff from the three different land uses are summarized in Table 2. The concentrations ranged for total coliforms ($4.2 \times 10^4 - 1.9 \times 10^5$ CFU/100 mL), fecal coliforms ($5.6 \times 10^3 - 2.2 \times 10^4$ CFU/100 mL), fecal streptococci ($3.5 \times 10^2 - 3.2 - 10^3$ CFU/100 mL), enterococci ($1.0 \times 10^3 - 6.6 \times 10^3$ CFU/100 mL), *E. coli* ($1.5 \times 10^3 - 8.5 \times 10^3$ CFU/100 mL), *Pseudomonas aeruginosa* ($3.4 \times 10^2 - 1.2 \times 10^3$ CFU/100 mL) and *Staphylococcus aureus* ($4.6 \times 10^3 - 1.8 \times 10^4$ CFU/100 mL). The concentrations in the stormwater runoff are similar to those reported in the stormwater NPDES database (Pitt *et al.* 2003). The concentrations are of the same order found by other investigators (Oliveri *et al.* 1977; Qureshi & Dutka 1979). The results further show that urban stormwater runoff is a major non-point source of human pathogens and indicators to receiving waters.

Table 2 | Concentrations of microorganisms in various land uses

	High-density residential (65% imperviousness) (R15 and R16 data)		Low-density residential (17% imperviousness) (L1 and L6 data)		Landscaped commercial (SP and GSP data)		
Organism	**Concentration (CFU/100 mL)**	***n***	**Concentration (CFU/100 mL)**	***n***	**Concentration (CFU/100 mL)**	***n***	**Are land use differences statistically significant?**
Total coliforms	$1.1 \times 10^5 - 1.9 \times 10^5$	147	$6.4 \times 10^4 - 1.3 \times 10^5$	74	$4.2 \times 10^4 - 7.9 \times 10^4$	112	Significant ($P < 0.001$)
Fecal coliforms	$1.2 \times 10^4 - 2.2 \times 10^4$	146	$5.6 \times 10^3 - 1.3 \times 10^4$	109	$7.2 \times 10^3 - 1.9 \times 10^4$	90	Significant ($P = 0.006$)
Fecal streptococci	$1.9 \times 10^3 - 3.0 \times 10^3$	105	$3.5 \times 10^2 - 7.1 \times 10^2$	103	$1.4 \times 10^3 - 3.2 \times 10^3$	79	Significant ($P < 0.001$)
Enterococci	$3.2 \times 10^3 - 5 \times 10^3$	72	$1.0 \times 10^3 - 2.2 \times 10^3$	80	$4 \times 10^3 - 6.6 \times 10^3$	73	Significant ($P < 0.001$)
E. coli	$2.0 \times 10^3 - 3.9 \times 10^3$	130	$1.5 \times 10^3 - 5.9 \times 10^3$	64	$3.1 \times 10^3 - 8.5 \times 10^3$	98	NS ($P = 0.13$)
Pseudomonas aeruginosa	$6.3 \times 10^2 - 1.2 \times 10^3$	95	$3.4 \times 10^2 - 6.5 \times 10^2$	69	$3.5 \times 10^2 - 6.5 \times 10^2$	104	Significant ($P < 0.001$)
Staphylococcus aureus	$1.1 \times 10^4 - 1.8 \times 10^4$	98	$6.5 \times 10^3 - 9.8 \times 10^3$	92	$3.2 \times 10^3 - 4.9 \times 10^3$	111	Significant ($P < 0.001$)

Notes: n = number of data
NS = not significant (P is set at ≤ 0.05)
Data were pooled from all four seasons
Concentrations are mean ± 95% level of confidence

Relationship between concentration of microorganisms and land uses

As shown in Table 2 and Figure 2, organism concentrations vary with land use in comparing three different land uses. Statistically significant differences were found between land uses for all microorganisms studied except for *E. coli*. The reason for this anomaly is unknown. Except for enterococci and *E. coli*, the highest geometric mean organism concentrations were from high-density residential land use areas. These concentrations were higher than those associated with low-density residential and landscaped commercial land uses. The same conclusion was made by other investigators (Bannerman *et al.* 1993; Weiskel *et al.* 1996; Mallin *et al.* 2000).

Microorganism concentrations from outfalls within a given land use also vary for all microorganisms in all three land uses as shown in Table 3. However, the mean concentrations vary by less than an order of magnitude, typically less than 1/2-log, suggesting that outfall selection is not critical to estimating the local runoff loading from a given land use.

Since the outfalls within the study area were free of cross-connections, the primary sources of microorganisms to the stormwater runoff are most likely from the feces of domestic animals and wildlife (Weiskel *et al.* 1996; Young & Thackston 1999). Another source may be invertebrates. The large number of pets and wildlife (e.g. rats, pigeons, racoons) in residential areas explain the large number of organism concentrations in runoff from residential areas. Pets and wildlife are very limited in commercial areas. It is often hypothesized that microrganisms and other pollutants accumulate on paved surfaces during dry periods, with surviving bacteria becoming entrained in the runoff generated during periodic storm events (Noble *et al.* 2003).

Researchers independently concluded that most of the fecal coliforms (sometimes 95%) found in urban stormwater were of nonhuman origin (Trial *et al.* 1993; Alderiso *et al.* 1996; Samadpour & Checkowitz 1998). For example, according to Lim & Oliveri (1982), dog feces were identified as the single greatest source, contributing fecal coliforms and fecal streptococci to highly urban Baltimore catchments. In the Puget Sound region, dogs and cats were implicated as the primary source of fecal coliforms in urban subwatersheds (Trial *et al.* 1993). Dogs and cats have also been suggested as the major fecal pollution sources in five estuarine watersheds in North Carolina (Mallin *et al.* 2000). Lim & Oliveri (1982) also noted that rats and pigeons can be a major source of bacteria in highly urban areas. Movement of these microorganisms to receiving waters can be reduced by a combination of behavioral and land management practices. These would include educational programs to reduce the amount of pet wastes deposited and left on the landscape, minimize the construction of impervious surfaces whereever possible, directing the runoff from existing impervious surfaces into pervious areas (such as constructed wetlands for passive treatment) and minimizing large open water sources to limit birds and geese inputs (Mallin *et al.* 2000).

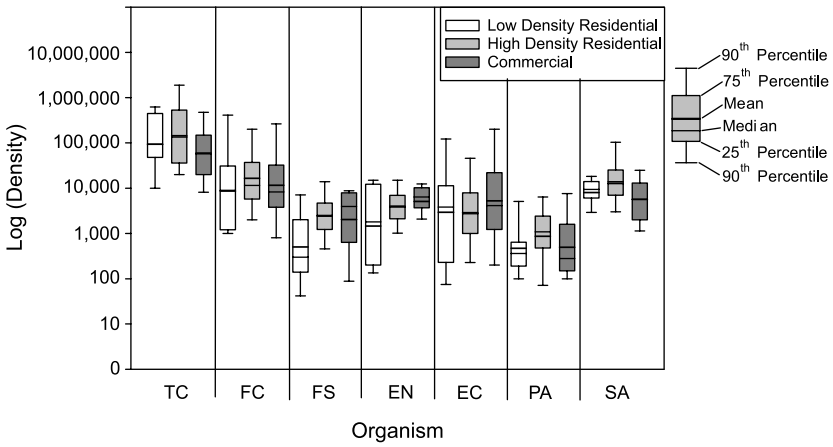

Figure 2 | Comparison of mean concentrations of microorganisms with land use. Notes: Data were pooled from all four seasons. TC: total coliforms; FC: fecal coliforms; FS: fecal streptococci; EN: enterococci; EC: *E. coli*; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*.

Table 3 | Concentration of microorganisms in various outfalls

Organism	Land use	Outfall	*n*	Concentration (CFU/100 mL)
Total coliform	High-density residential	R15	83	$7.9 \times 10^4 - 1.6 \times 10^5$
		R16	64	$1.2 \times 10^5 - 3.2 \times 10^5$
	Low-density residential	L1	33	$1.0 \times 10^5 - 2.4 \times 10^5$
		L6	41	$3.5 \times 10^4 - 1.1 \times 10^5$
	Landscaped commercial	GSP	45	$2.8 \times 10^4 - 5.4 \times 10^4$
		SP	66	$4.7 \times 10^4 - 1.2 \times 10^5$
Fecal coliform	High-density residential	R15	101	$7.9 \times 10^3 - 1.4 \times 10^4$
		R16	45	$2.5 \times 10^4 - 9.1 \times 10^4$
	Low-density residential	L1	61	$5.6 \times 10^3 - 1.9 \times 10^4$
		L6	48	$3.5 \times 10^3 - 1.2 \times 10^4$
	Landscaped commercial	GSP	50	$6.8 \times 10^3 - 2.8 \times 10^4$
		SP	40	$4.7 \times 10^3 - 1.9 \times 10^4$
Fecal streptococci	High-density residential	R15	69	$1.3 \times 10^3 - 2.3 \times 10^3$
		R16	36	$3.7 \times 10^3 - 6.2 \times 10^3$
	Low-density residential	L1	47	$3.5 \times 10^2 - 1.0 \times 10^3$
		L6	56	$2.8 \times 10^2 - 6.9 \times 10^2$
	Landscaped commercial	GSP	35	$2.9 \times 10^3 - 6.3 \times 10^3$
		SP	44	$6.8 \times 10^2 - 2.2 \times 10^3$
Enterococci	High-density residential	R15	44	$3.9 \times 10^3 - 6.8 \times 10^3$
		R16	28	$1.9 \times 10^3 - 4.0 \times 10^3$
	Low-density residential	L1	38	$7.9 \times 10^2 - 2.8 \times 10^3$
		L6	42	$8.5 \times 10^2 - 2.6 \times 10^3$
	Landscaped commercial	GSP	36	$3.6 \times 10^3 - 6.3 \times 10^3$
		SP	37	$3.5 \times 10^3 - 8.5 \times 10^3$
E. coli	High-density residential	R15	87	$1.3 \times 10^3 - 2.8 \times 10^3$
		R16	43	$3.5 \times 10^3 - 1.0 \times 10^4$
	Low-density residential	L1	27	$3.5 \times 10^3 - 2.8 \times 10^4$
		L6	37	$5.4 \times 10^2 - 2.8 \times 10^3$
	Landscaped commercial	GSP	45	$2.6 \times 10^3 - 1.2 \times 10^4$
		SP	53	$2.5 \times 10^3 - 9.5 \times 10^3$
Pseudomonas aeruginosa	High-density residential	R15	58	$7.9 \times 10^2 - 1.7 \times 10^3$
		R16	37	$3.1 \times 10^2 - 9.3 \times 10^2$
	Low-density residential	L1	34	$3.7 \times 10^2 - 8.5 \times 10^2$
		L6	35	$2.3 \times 10^2 - 6.8 \times 10^2$
	Landscaped commercial	GSP	52	$4.8 \times 10^2 - 1.0 \times 10^3$
		SP	52	$2.1 \times 10^2 - 5.1 \times 10^2$
Staphylococcus aureus	High-density residential	R15	56	$6.2 \times 10^3 - 1.1 \times 10^4$
		R16	42	$1.9 \times 10^4 - 4.0 \times 10^4$
	Low-density residential	L1	40	$6.6 \times 10^3 - 1.3 \times 10^4$
		L6	52	$5.6 \times 10^3 - 9.3 \times 10^3$
	Landscaped commercial	GSP	56	$2.1 \times 10^3 - 3.7 \times 10^3$
		SP	55	$4.4 \times 10^3 - 7.6 \times 10^3$

Notes: n = number of data
Concentrations are mean ± 95% level of confidence
Data were pooled from all four seasons
Differences within outfalls are statistically significant

Relationship between concentration of microorganisms and seasons

Concentrations of microorganisms significantly ($P < 0.05$) varied between seasons and generally the lowest concentrations were during winter (Table 4). The mean concentrations vary within about an order of magnitude. The organism concentrations during the summer were not significantly different from those observed during fall and spring. These findings are consistent with others, who also reported that organism concentrations were higher during warmer months than in cooler months (Geldreich *et al.* 1968; Evans *et al.* 1968; US EPA 1983; Edwards *et al.* 1997; Pianetti *et al.* 1998; Bagde & Rangari 1999; Young & Thackston 1999). The effects of season on concentrations of microorganisms are demonstrated in Figure 3.

The relationship between organism concentrations and season of sample collection has significant implications from the standpoint of stormwater treatment/storage. Since the results suggest that organism concentrations are higher during warmer months than during cooler months, any design for microorganism removal by structural BMPs must be based on concentrations during the warmer months than on the pooled data. Otherwise, the results can underestimate the concentrations during warmer months when recreational uses such as swimming and fishing occur. Also, if the concentration varies seasonally, and the receiving water temperature varies seasonally, and die-off kinetic rates vary with temperature (lower rates with lower temperatures) it may be possible to predict the downstream concentrations to alter disinfection operations at water treatment plants or issue warnings, advisories and closures based on prediced concentrations with few verification samples.

Change in concentrations of microorganisms over years

A data set consisting of the samples collected in both 2000 and 2001 allow for direct comparison of year to year concentrations. For 2000, data were collected only during summer and fall. For 2001, data were collected during all four seasons. The nested analysis of variance was used to compare 2000 and 2001 mean counts for all seasons. Year over year concentrations of microorganisms show no significant differences for the traditionally used indicators (total coliforms, fecal coliforms and fecal streptococci) as shown in Table 5. Alternate indicators (enterococci and *E. coli*) and pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) varied significantly between years. However, the differences in concentrations are minor.

Relationships among microorganisms

Analysis for pathogen organisms is not readily incorporated into routine monitoring as it is complex, costly and time-consuming. The microbial water quality monitoring relies only on indicators. In many instances, the presence of indicators suggests the occurrence of pathogenic organisms; however, the absence of indicators may not ensure the absence of pathogens (Geldreich 1996). Statistical tests were performed to determine the relationship among concentrations of various bacterial indicators and pathogens in stormwater runoff. If results show a strong relationship between indicators and pathogens, then it may only be necessary to monitor for indicators, which will potentially reduce sampling and analysis costs.

The relationships among the log-transformed concentrations of microbial indicators and pathogens were examined by Pearson correlation analysis (Table 6). Pearson correlation analysis measures the linear relationship between pairs of variables without regard to which variable is dependent or independent. The pairs of variables with positive correlation coefficients and P values below 0.05 tend to increase together. For the pairs with negative correlation coefficients and P values below 0.05, one variable tends to decrease while the other increases. For pairs with P values greater than 0.05, it is assumed there is no statistically significant relationship between the two variables. As shown in Table 6, the correlation coefficients among different indicators indicate a weak linear relationship, but are statistically significant. Correlation coefficients between indicators ranged from 0.425–0.84, and the strongest association was found between fecal streptococci and enterococci. This is likely because enterococci are a subset of fecal streptococci (i.e. not an independent variable).

The relationship between indicators and pathogens is poorly correlated and is statistically insignificant except for the relationship between *Pseudomonas aeruginosa* and

Table 4 | Seasonal concentration ranges of microorganisms

	Summer		Fall		Winter		Spring		
Organism	**Concentration (CFU/100 mL)**	***n***	**Concentration (CFU/100 mL)**	***n***	**Concentration (CFU/100 mL)**	***n***	**Concentration (CFU/100 mL)**	***n***	**Are seasonal differences statistically significant?**
Total coliforms	$1 \times 10^5 - 2.3 \times 10^5$	63	$7.2 \times 10^4 - 1.2 \times 10^5$	128	$1.4 \times 10^4 - 2.5 \times 10^4$	69	$2.4 \times 10^5 - 5.1 \times 10^5$	73	Significant ($P < 0.001$)
Fecal coliforms	$2.5 \times 10^4 - 8.2 \times 10^4$	95	$6.7 \times 10^3 - 1.1 \times 10^4$	111	$2.3 \times 10^3 - 4 \times 10^3$	87	$1.5 \times 10^4 - 2.8 \times 10^4$	56	Significant ($P < 0.001$)
Fecal streptococci	$8.5 \times 10^2 - 1.3 \times 10^3$	93	$2.5 \times 10^3 - 4.4 \times 10^3$	103	$1.5 \times 10^2 - 3.6 \times 10^2$	64	$3.8 \times 10^3 - 7.6 \times 10^3$	24	Significant ($P < 0.001$)
Enterococci	$1.4 \times 10^3 - 3.4 \times 10^3$	48	$4.7 \times 10^3 - 7 \times 10^3$	74	$5.8 \times 10^2 - 1.2 \times 10^3$	60	$7.8 \times 10^3 - 1.1 \times 10^4$	44	Significant ($P < 0.001$)
E. coli	$8.4 \times 10^3 - 3.3 \times 10^4$	69	$1.5 \times 10^3 - 2.4 \times 10^3$	91	$3.5 \times 10^2 - 1.1 \times 10^3$	59	$5.6 \times 10^3 - 1.1 \times 10^4$	68	Significant ($P < 0.001$)
Pseudomonas aeruginosa	$7.1 \times 10^2 - 1.3 \times 10^3$	60	$2.9 \times 10^2 - 4.7 \times 10^2$	103	$1.8 \times 10^2 - 3.5 \times 10^2$	66	$3.4 \times 10^3 - 6.1 \times 10^3$	40	Significant ($P < 0.001$)
Staphylococcus aureus	$5.5 \times 10^3 - 9 \times 10^3$	76	$6.2 \times 10^3 - 9.8 \times 10^3$	132	$1.2 \times 10^4 - 2.2 \times 10^4$	46	$5.2 \times 10^3 - 8.7 \times 10^3$	60	Significant ($P < 0.001$)

Notes: n = number of data
Data were pooled from all three land uses
Concentrations are mean ± 95% level of confidence

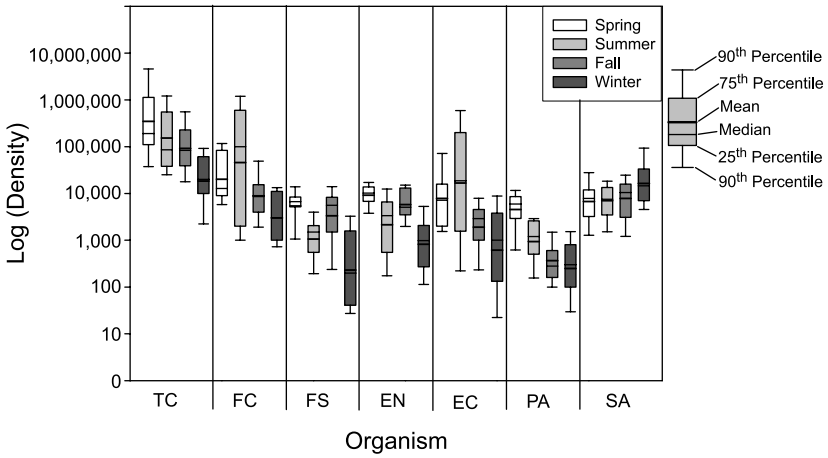

Figure 3 | Comparison of mean concentrations of microorganisms with season. Notes: Data were pooled from all four seasons. TC: total coliforms; FC: fecal coliforms; FS: fecal streptococci; EN: enterococci; EC: *E. coli*; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*.

total coliforms and *E. coli*, which are moderately correlated and statistically significant. Also, ratios of pathogens to indicators ranged over eight orders of magnitude (0.0001 to >3000), indicating that pathogen densities were very poorly related to the density of indicator organisms. This demonstrates why measurements of indicator organisms alone in stormwater runoff cannot be expected to give meaningful concentrations of pathogens sufficient to predict the microbiological water quality and therefore potential threat to humans. These findings are consistent with Pianetti *et al.* (1998), who concluded that clear and significant relationships amongst indicator bacteria and pathogens do not exist. It is also important to note that a wide variety of physical and chemical factors, such as sunlight, temperature, pH, etc., influence the survival capacity of organisms in aquatic environments. Based upon on these, the use of indicators as an indication of the presence of pathogens in stormwater runoff is considered inappropriate.

Table 5 | Results of nested analysis of variance for microorganisms from year to year

Organism	Year	Log_{10} (#/100 mL)	*n*	Are year to year variations statistically significant?
Total coliforms	2000	4.96 ± 0.11	97	Not significant
	2001	4.99 ± 0.11	235	
Fecal coliforms	2000	4.09 ± 0.14	74	Not significant
	2001	4.09 ± 0.12	271	
Fecal streptococci	2000	3.47 ± 0.12	72	Not significant
	2001	3.01 ± 0.11	215	
Enterococci	2000	3.81 ± 0.13	38	Significant
	2001	3.42 ± 0.09	187	
E. coli	2000	3.29 ± 0.15	61	Significant
	2001	3.61 ± 0.14	231	
Pseudomonas aeruginosa	2000	2.52 ± 0.10	76	Significant
	2001	2.87 ± 0.10	192	
Staphylococcus aureus	2000	3.83 ± 0.11	84	Significant
	2001	3.88 ± 0.07	217	

Note: n = number of data
Concentrations are mean ± 95% level of confidence
Differences are significant if $P < 0.05$

Table 6 | Relationships among organisms in stormwater runoff samples

Organism	FC	FS	EN	EC	PA	SA
Total coliforms	0.665 (S)	0.683 (S)	0.701 (S)	0.699 (S)	0.486 (S)	0.0455
Fecal coliforms		0.541 (S)	0.534 (S)	0.771 (S)	0.302	−0.286
Fecal streptococci			0.840 (S)	0.462 (S)	0.412	−0.0635
Enterococci				0.425 (S)	0.397	−0.275
E. coli					0.505 (S)	−0.203
Pseudomonas aeruginosa						−0.0831

Notes: Numbers are Pearson correlation coefficients
S- Significant; all other relationships are not significant at $P \leq 0.05$
FC - Fecal Coliforms; FS - Fecal Streptococci; EN - Enterococci; EC - *E. coli*
PA - *Pseudomonas aeruginosa*; SA - *Staphylococcus aureus*

CONCLUSIONS

Stormwater samples collected from storm sewers draining small municipal separate storm sewer systems shown to be free of sanitary-sewage cross-connections within an urban watershed dominated by a single land use were analyzed for pathogens (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and indicator organisms (total coliforms, fecal coliforms, fecal streptococci, enterococci and *E. coli*). The concentrations found in the stormwater runoff are similar to those reported in the stormwater NPDES database.

Other than *E. coli*, the microbial concentrations in stormwater runoff consistently vary within and between land uses. Generally, the concentrations in runoff from high-density residential areas are higher than the concentrations in other tested land uses. The higher concentrations in the more impervious areas, coupled with the increased runoff volume associated with these areas, will lead to higher receiving water indicator organisms and pathogen loadings. Microbial concentrations from outfalls within a given land use vary. The mean concentrations vary by less than an order of magnitude, typically less than 1/2-log, suggesting that the outfall selection (after screening) is not critical to estimate the local runoff loading from a given land use. Although concentrations vary among land uses, the relative differences are comparatively small with respect to the overall magnitude. These differences, coupled with other modeling uncertainties, do not warrant changing the existing aggregate approach to estimating the load from urban areas.

Seasonal concentrations in stormwater runoff vary within about an order of magnitude with winter concentrations generally lowest.

The correlation between the concentration of the traditionally monitored indicators and the alternate indicators is weak, but statistically significant, suggesting that historical time series will be only a qualitative indicator (not quantitative) of impaired waters under the revised criteria.

MANAGEMENT APPLICATIONS

Our research has several applications for the management of stormwater:

(a) The December 1999 Phase II stormwater regulations require NPDES permits for municipal separate storm sewer systems (MS4 s) serving urbanized areas with populations less than 100 000 (*Federal Register*, 1999). These regulations affect smaller communities with fewer resources than the Phase I stormwater communities. A likely outcome of these regulations is an increased national investment in best management practices (BMPs) for microorganism control. If the concentrations from certain land uses are higher, stormwater BMPs placed in these areas could have a greater impact on lowering the overall loads to targeted receiving waters.

(b) Section 303(d) of the Clean Water Act requires that States develop Total Maximum Daily Loads (TMDLs) for surface waters failing to meet water quality standards. TMDLs establish the pollutant-specific maximum allowable loadings including stormwater as a non-point source. Understanding the non-point-source allocations and the prospective management alternatives to control that load is paramount in developing and reaching TMDLs. Non-point-source load estimation requires both flow volume and pollutant concentration in runoff. Relatively simple, well-established models can provide good estimates of flow volume. However, estimation of concentrations is complicated by a lack of data and high variability in available data. This study gives a range of values that can be used for load calculations that can be coupled with in-stream die-off models to predict the load at a given downstream location. However, it is recommended that the aggregate approach be used for estimating loads from urban areas.

(c) Under EPA's Beach Environmental Assessment, Closure and Health (BEACH) program, the Agency is attempting to significantly reduce the infection risk from recreational water exposure (US EPA 1999). The sample collection and processing times introduce a delay in determining that a suspect recreational water is unsafe. Computer modeling is a potential tool to help local monitoring agencies evaluate water bacterial concentrations and potentially draw preliminary conclusions before the verification data is available. A variety of model approaches are candidates, but deterministic models (e.g. CORMIX, PLUMES, SWMM and HSPF) will require stormwater load estimates. The results from this study can be used to develop concentration ranges for such models.

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