Temporal variation of faecal indicator bacteria in tropical urban storm drains

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5 **Temporal Variation of FIB in Urban Storm Drains**

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Temporal Variation of Faecal Indicator Bacteria in Tropical Urban Storm Drains

Abstract (314 words)

Human faecal contamination poses a widespread hazard for human health. In urban areas, sewer leakage may be an important cause of faecal pollution to surface water. Faecal indicator bacteria (FIB) are the most widely used indicators to monitor surface water quality. However, assessing whether a water body is meeting water quality criteria is made difficult by the high variability of FIB concentrations over time. In this study, the variation of FIB concentration in surface water from tropical urban catchments is investigated. Eleven urban sub-catchments were sampled hourly over 24-hr and samples analysed for FIB. It was found that FIB show a diurnal pattern that is characterised by daytime FIB concentrations that are significantly higher than nighttime FIB concentrations. This observed diurnal variation of FIB closely follows that of sewer flows and contrasts with observations in rural streams where FIB concentrations are known to be low in the daytime and high during the night. Field tracer tests provide qualitative evidence of sewage exfiltration and transport to drains via preferential flow paths. The diurnal FIB variation and field tracer tests indicate the likelihood of surface water contamination due to leaking sewers. The results further suggest that contamination of surface-water drains is likely a widespread problem in tropical urban areas due to extensive drainage networks and the persistence of FIB under tropical conditions. Because of FIB variation over time, the time at which samples are collected is important in being able to capture the daily maximum and

minimum FIB concentrations. The Kruskal-Wallis test shows that hourly sampling from 04:00 to 07:00 and from 12:00 to 15:00 results in significantly different FIB concentration (minimum and maximum, respectively). Furthermore, the Wilcoxon-Mann-Whitney test shows that sampling at 12:00 and 14:00 results in significantly higher FIB concentrations, while sampling at 05:00 and 04:00 or 05:00 and 06:00 results in significantly lower FIB concentrations, than sampling at other hours of the day.

Keywords

faecal indicator bacteria; urban storm drain; diurnal variation

1. Introduction

Human faecal contamination poses a widespread hazard for human health (Arnone and Walling, 2007). For surface waters in urban catchments, leaking sewers can be an important cause of faecal pollution of the drainage system arising as a result of ageing or damaged sewer lines (Held et al., 2006; Whitlock et al., 2002). Sewage from leaking sewers may exfiltrate through unsaturated soil into groundwater and is finally discharged into surface waters such as at shorelines (Boehm et al., 2003; Boehm et al., 2004) or storm drains (Sauer et al., 2011; Sercu et al., 2009; Sercu et al., 2011b).

The presence and concentration of faecal indicator bacteria (FIB) are often used to assess whether a water body meets water quality standards. FIB are non-pathogenic organisms that are believed to be associated with pathogenic organisms but are more easily measured. However, the assessment of water quality for bacterial contamination is made difficult by the high variability of indicator bacterial concentrations over time. Many water quality monitoring programs focus primarily on low-frequency dry-weather sampling (Traister and Anisfeld, 2006). Leecaster and Weisberg (2001) showed that sampling frequency affects the number of high FIB events that are detected. Traister and Anisfeld (2006) suggested the importance of sampling programs that capture a fuller range of stream conditions. Besides sampling frequency, sample collection time can dramatically influence the concentration of indicator bacteria detected (Boehm et al., 2002) and hence different sample collection times could result in different management decisions (Enns et al., 2012). Boehm et al. (2002) and Kwasi et al. (1999)

recommended that ideally samples should be collected in the early morning before sunlight has had a chance to reduce FIB concentrations. However, it is important to note that those studies sampled surface waters at beaches that are exposed to sunlight. Therefore, when the surface water is not exposed to sunlight (such as in covered drains or open drains that are shaded by trees or buildings), the suggestion to sample in the early morning period may not be applicable. Some studies have observed a diurnal cycle of FIB at beaches (Boehm et al., 2002; Enns et al., 2012) and streams (Desai and Rifai, 2013; Traister and Anisfeld, 2006) caused by sunlight-induced die-off, with higher concentrations in the night and lower in the afternoon. However, Desai and Rifai (2010) point out the complexity of this phenomenon and its dependence on flow, turbidity, total suspended solids, temperature, location and land use in the catchment.

Sercu et al. (2009) sampled storm drains during dry weather in Santa Barbara, CA, USA and reported the exclusive occurrence of high human-specific *Bacteroides* marker with high FIB concentrations. Assuming similar behaviour, high FIB concentrations in covered and well shaded storm drains should indicate human faecal contamination such as from leaking sewers. To the best of the authors' knowledge, this possibility has not been investigated in tropical, urban storm drains. Two studies conducted in Singapore detected perfluorochemicals such as perfluorooctane sulfonate in 1-156 ng/L (Nguyen et al., 2011) and emerging organic contaminants including pharmaceuticals in the ng/L range (Xu et al., 2011) in surface drains and attributed the presence of these contaminants to non-point sources which include leaking sewer lines. Therefore, the objectives of this study were to investigate: (i) how FIB concentrations in tropical urban drainages vary on a daily time scale where there is only a slight

variation between daytime and nighttime temperatures and where there is little effect of sunlight because the drainages are mostly covered or well shaded, (ii) if leaking sanitary sewers are a major influence on FIB concentrations in urban storm drains and (iii) how water quality monitoring practice should be adapted in order to take into account diurnal variations in FIB concentrations.

2. Materials and Methods

2.1. Site description

This study was conducted at eleven urban sub-catchments in Singapore (Figure 1). The climate in Singapore is tropical with annual rainfall of approximately 2,200 mm with no distinct wet or dry seasons and daily mean temperature varying over a narrow range between 26.0 °C and 27.7 °C through the twelve months of the year (NEA, 2014). Table 1 provides land-use information for the eleven sampling sites; land use is predominantly residential. The sub-catchments were delineated using ArcGIS Desktop 10 (Esri, Redlands, CA, USA) and land-use proportion was determined using GIS land-use data provided by the Public Utilities Board (PUB), Singapore. Six of the sub-catchments are mainly high-density residential areas consisting of high-rise apartment buildings while the other five sub-catchments are mainly low-density residential areas consisting of single-family housing units.

2.2. Sampling campaign

Fourteen sets of 24-hr FIB samples were collected at the eleven sampling sites during dry weather (baseflow) between January and July 2012 (inclusive). Sampling during dry-weather conditions eliminates surface runoff as a potential source of contamination. The sampling dates are summarised in Table S1. Sampling was conducted three times at site A and twice at site H on different days to see if diurnal patterns were repeatable. The patterns at Site H were repeated very closely between the two sampling series. Two of the three sampling series at Site A showed similar (typical) patterns; one showed atypical fluctuations. Sampling mostly started at 20:00, with the exception of site A, site D and site I which started at 08:00 instead. Most of the 24-hr sets of samples were collected using two Avalanche® refrigerated auto-samplers (Teledyne Isco, Lincoln, NE, USA). The samples collected by the auto-samplers were stored in the field in a refrigerated compartment at 4 °C for not more than 22 hours after the start of sampling. A HOBO® U10 temperature data logger (Onset Computer Corporation, Cape Cod, MA, USA) was installed in the refrigeration compartment to ensure that the refrigeration system was in good working order. Each auto-sampler drew samples from the drainage channels via a dedicated sampling line. In operating the auto-sampler, precaution was usually taken to prevent contamination from water left in the sampler tubing from the prior sample by pre-rinsing the sampling lines before each sampling. Carryover was tested in the field on six separate occasions by alternately drawing drain water followed by sterile water between rinsing cycles. Both single and double rinse cycles were tested and the results showed that overall carryover between sample bottles was less than 5% (Table S2) and thus carryover from

one sample to the next was considered to be insignificant. In certain cases (samples from 08:00 to 19:00 at site D, site E and site K) for logistical reasons manual grab sampling was used to complement sampling with the auto-sampler. Independent tests comparing results from manual grab samples and samples collected concurrently with the auto-sampler showed that the log-transformed results differed by less than 5%. Care was taken during manual grab sampling so as not to disturb the bottom sediments. During grab sampling, samples were collected in sterile 532-mL Whirl-Pak® bags (Nasco, Fort Atkinson, WI, USA) and mixed in a plastic container which was first rinsed with drain water, to form a 5 L composite. Field blank samples consisting of sterile water were also collected in the Whirl-Pak® bags to ascertain if there was any cross-contamination during sample handling. The collected samples were kept in ice coolers from the time of collection until delivery to the environment laboratory at Nanyang Technological University. Out of the 336 (14 sites x 24 samples per site) samples to be collected, 3 samples were missed due to sampler operation issues. In addition, 11 other samples were collected during periods with light rains. Although the light rains did not result in runoff, the samples were rejected nevertheless. These 14 samples are not included in the analysis presented in this paper.

2.3. Faecal indicator bacteria analysis

Samples were analysed for three FIB (total coliform (TC), *Escherichia coli* (*E. coli*) and enterococci) using the most-probable-number (MPN) method not more than 38 hr after collection. All samples were vigorously shaken before analysis. Each sample was analysed with

three dilution ratios which were usually 1, 100 and 10,000. More contaminated TC and *E. coli* samples from site F were analysed with dilution ratios of 10, 1,000 and 100,000. For each dilution, a 100-mL sample was first prepared in a washed and autoclaved glass bottle and then one aliquot of Colilert® or Enterolert® (IDEXX Laboratories, Westbrook, ME, USA) reagent was added. The bottle was shaken until the reagent dissolved. The mixture was then poured into a labeled Quanti-Tray®/2000 (IDEXX Laboratories, Westbrook, ME, USA). The trays were sealed and incubated at 35 °C ± 0.5 °C (for the TC and *E. coli* trays) or at 41 °C ± 0.5 °C (for enterococci trays) for 24-28 hr and the results were read following the manufacturer's instructions. When samples with different dilution ratios yielded readings that were not all fertile or all sterile, the reading with the least propagation of errors (Harris, 2007; Peters et al., 1974) was selected. Twelve out of 322 samples exceeded the upper range for TC (>24,196,000 MPN/100mL after 10,000-fold dilution) and are reported at the highest reading (24,196,000 MPN/100mL).

2.4. Tracer test

A tracer test was conducted in the vicinity of Site A (Figure 3) which is located close to a wet market (a type of outdoor market for meat and produce commonly found in Southeast Asia), an open food court, a school and several blocks of high-rise residential apartments. The tracer test was conducted by injecting 200-ppt (part per thousand) brine solution into inspection chambers or ICs (shallow manholes that intercept sewage and grey water from residential dwellings directing the wastewater into public sewers) as shown in Figure 3. Specific

conductance was monitored at the upstream drain at 10-min intervals with a YSI 30 conductivity meter (YSI, Inc., Yellow Springs, OH, USA).

2.5. Statistical analyses

FIB concentrations were log-transformed and then normalised based on minimum (min) and maximum (max) log-transformed FIB concentrations for each 24-hr set of samples according to:

$$\text{normalised conc} = \frac{(\log_{10} \text{conc} - \log_{10} \text{min conc})}{(\log_{10} \text{max conc} - \log_{10} \text{min conc})}$$

Normalising the data accentuates the “shape” of the 24-hr FIB concentration time series and allows the identification of a diurnal pattern, if any. The minimum and maximum FIB concentrations for each 24-hr set of samples are summarised in Table S1. Statistical analyses were conducted on the normalised dataset. Box-and-whisker plots were created using Matlab (The MathWorks, Inc., Natick, MA, USA) and used to illustrate the distribution of the data.

The root-mean-square error (RMSE) was used as a measure of the deviation of a time series from its average value. The autocorrelation function, represented by a set of coefficients (r_k), was used to detect the time interval over which a correlation exists within the time series. r_k is commonly graphed as a function of k in a correlogram, where k is the lag expressed as a number of time intervals. Any test of the significance of r_k will only give a realistic result when the autocorrelation coefficients are calculated for the lag up to but not larger than a quarter of

the number of observations (Berthouex and Brown, 2002; McKillup and Dyar, 2010). The 95% confidence interval of the autocorrelation coefficient is given by $r_k \pm 1.96/\sqrt{N - k + 3}$, where r_k estimates the population autocorrelation coefficient (ρ_k) and N is the total number of observations in the time series (McKillup and Dyar, 2010). Any autocorrelation coefficient that lies outside the confidence interval of $r_k = 0$ in a correlogram is significant. RMSE and autocorrelation were calculated with Microsoft Excel (Microsoft, Redmond, WA, USA).

Wilcoxon-Mann-Whitney and Kruskal-Wallis tests, which are non-parametric tests, were conducted on the log-transformed data in Matlab to test for statistical differences between different groups of FIB concentrations at the 5% significance level. The results of the Wilcoxon-Mann-Whitney and Kruskal-Wallis tests are expressed as p values. Prior to the Wilcoxon-Mann-Whitney test, FIB concentrations were first segregated into two groups based on sampling time: daytime (samples collected from 08:00 to 19:00) and nighttime (samples collected from 20:00 to 07:00) datasets. Before the Kruskal-Wallis test was carried out, the 24-hr data were first segregated into four six-hour groups. Different ways of segregating the data were tested so as to find groups that differentiate between daytime and nighttime well. As stated in Section 2.2, sampling mostly started at 20:00, therefore 20:00 was taken as the start time of the first set of four six-hour groups (20:00 – 01:00, 02:00 – 07:00, 08:00 – 13:00 and 14:00 – 19:00). The second set of four six-hour groups consisted of 21:00 – 02:00, 03:00 – 08:00, 09:00 – 14:00 and 15:00 – 20:00 groups of data while the third set was 01:00 – 06:00, 07:00 – 12:00, 13:00 – 18:00 and 19:00 – 24:00. When the Kruskal-Wallis tests showed that differences between the groups were significant ($p \leq 0.05$), the Tukey-Kramer test was conducted to identify the groups of

samples that gave significantly different results. The Tukey-Kramer test is a multiple comparison test which shrinks the error rate of individual comparison to keep the simultaneous family error rate at 5% in order to achieve valid statistical comparisons (Berthouex and Brown, 2002). A repeat analysis with the Kruskal-Wallis and Tukey Kramer tests was carried out using six four-hour groups. Similar to the six-hour groupings, three combinations of four-hour groupings were tested.

3. Results and discussion

3.1. Diurnal variation of FIB concentration

The normalised 24-hr FIB concentration time series are grouped based on sampling time and then plotted versus time of day in box-and-whisker plots in Figure 2. The arithmetic mean and median for TC and *E. coli* show distinct diurnal trends: the concentrations are high in the daytime, peaking at around 10:00 – 14:00, and low in the nighttime, with a minimum at around 04:00 – 06:00. This trend is less significant for enterococci (explained further in Section 3.2). The diurnal pattern of FIB concentrations in the present study is different from other studies (Boehm et al., 2002; Desai and Rifai, 2013; Enns et al., 2012; Traister and Anisfeld, 2006) in which observed patterns were marked by high FIB concentrations in the nighttime due to re-growth and low concentrations in the daytime, due to sunlight-induced die-off. The difference in diurnal variations between the present study and these published studies which were conducted in temperate or subtropical areas may be attributed to the lesser influence of

sunlight in this study since the sampling sites are either covered (typical of drainages in highly urbanised catchments) or shaded by buildings and trees. However, if the effect of sunlight were the only difference, no variation would be expected in drainages that are covered or sheltered based on the studies by Desai and Rifai (2013) and Traister and Anisfeld (2006) which reported insignificant differences between daytime and nighttime FIB concentrations at well shaded sampling sites. The diurnal variations observed in the present study imply that other factors besides sunlight are playing a significant role.

Figure 2 shows that concentrations of all three FIB are high during the daytime and low during nighttime and that FIB spike strongly between 10:00 and 14:00 and again, but more weakly, in the evening at about 20:00. These spikes mimic the peaks in typical daily sewage flows (Chesner and Pai, 1981; Enfinger and Stevens, 2006) which is high during the day and low during the night (especially late at night and early in the morning), with a higher morning peak and lower evening peak (Butler and Graham, 1995). This agreement between sewage flow and FIB concentration, as well as the proximity of shallow sewer lines to storm drains at the sampling sites, leads to the hypothesis that the observed FIB concentrations arise primarily from human faecal and sewage contamination from leaking sewers and that inactivation by sunlight and re-growth during the night play inconsequential roles in governing diurnal variation of FIB concentrations in urban drainages. The continued decline in the nighttime concentrations that is consistent with the patterns shown in Enfinger and Stevens (2006) is presumably due to continually decreased sewer flows as more and more of the population goes to bed. With less flow in the sewers and inspection chambers, there is less leakage and infiltration of sewage into

the surface drains. The time when sewer flows are at a minimum is probably between 03:00 – 04:00 (see Figure 2 of Enfinger and Stevens (2006)). The time of lowest contamination level occurs later (05:00, see Figure 2), which is consistent with the travel time required from source to the point of measurement.

Field tracer tests were conducted to test the hypothesis that leakage from sanitary sewers is the main cause of the observed diurnal variations in FIB. Prior to the tracer tests, water samples were collected from the drains in the vicinity of two apartment blocks at Site A indicated in Figure 3 (a) and analysed for FIB. The results for *E. coli* sampled on 14 January 2013 showed that TL05 and TL06 were locations with high FIB concentrations (Figure 3 (a)). A repeat sampling at TL05 and TL06 was conducted on 17 January 2013, during which additional upstream sites, TL05-01 through -04 and TL06-01 through -04, were also tested (Figure 3 (b)). The data from these samples allowed the location for possible leakage to be narrowed to the row of inspection chambers immediately south of one of the apartment blocks. Tracer tests at these ICs were therefore completed to investigate the possible contamination of surface drains by sewage exfiltration.

Two tests were conducted. For the first test, the brine solution was injected into inspection chambers IC1 and IC2 (Figure 3 (c)) on 24 January 2013 at 11:15 to simulate an instantaneous pulse of sewage. The specific conductance was monitored at TL05-02. A spike in specific conductance was observed at 12:35, about 1 hr 20 min after the start of injection and a second spike was observed at 14:55, about 3 hr 40 min after the start of injection (Figure 4 (a)). The

two spikes may indicate there are different preferential pathways from the two emplaced sources, IC1 and IC2 (Figure 3 (c)). The tracer experiment was repeated on 4 February 2013 at 11:20. On this occasion, a slight increase in conductivity was observed at 12:20, about 1 hr after the start of brine injection, and a larger spike was observed at 13:50, about 2 hr 30 min after the start of brine injection (Figure 4 (b)). The spikes of specific conductance in Figure 4 occurred within a few hours after the start of brine injection and indicate the likelihood of (1) sewage leaking from the sewage infrastructure, and (2) leaked sewage traveling to nearby storm drains with travel times of about one to four hours.

The groundwater table at the study site is generally shallow (about 2 m below ground surface) and above the level of gravity sanitary sewer lines; thus groundwater would be expected to infiltrate sewers rather than sewage to exfiltrate from sewer lines. However, the connections between discharge pipes from buildings to the public sewers, including ICs, are shallow and generally above the groundwater table. Further, these connections are prone to damage by backfilling during construction and by differential ground settling after construction (Tan et al., 2009). Sewage from leaking sewer pipes would follow preferential pathways in the soil or in the bedding beneath sewer lines, and flow to discharge points created by weep holes, joints, and cracks in nearby drain lines. Lundy and Gogel (1988) have shown the importance of preferential pathways in sewer bedding in the transport of gasoline from leaking underground storage tanks. Doshi (2012) found through groundwater modelling that subsurface travel times would be short in the dense urban infrastructure of Singapore. Finally, leakage is most likely at the peak times of domestic water use and wastewater discharge when there would be flow at

building connections. The increase of leakage during times of peak wastewater flow and short travel times for leaked sewage to reach storms drains would cause the FIB concentration in surface drains proximate to leaking sewers to closely follow the time pattern of sewage flows. Thus, the demonstrated phenomenon of sewage exfiltration and flow to surface drains via preferential flow paths provides a consistent explanation for the observed diurnal spikes in FIB concentrations as well as the overall pattern and timing of FIB concentrations in drains.

3.2. Atypical trends in diurnal variation of FIB

Figure 5 plots 24-hr FIB concentrations observed at site A (17-18 January 2012), site D and site F. An examination of the FIB data from these locations reveals atypical behaviour, with high nighttime FIB concentrations in addition to high daytime FIB concentrations. Further analysis was undertaken to investigate the likely cause of this anomaly. The RMSE statistic was employed to quantify deviations of FIB concentrations from a typical diurnal pattern such as those observed in Figure S3 (a - i) which are characterised by high daytime and low nighttime FIB concentrations. The RMSE values for TC, *E. coli* and enterococci deviations in each 24-hr set of samples were summed and the results are shown in Table 2.

Table 2 shows that the total RMSE for temporal deviations has the highest value at site F. Site F is a high-density residential estate that was under development with several construction works in progress during the sampling program. Site F exhibits consistently elevated nighttime FIB concentrations in samples from 01:00 to 07:00 (Figure 5 (c)). The high nighttime FIB

concentrations originated from unknown sources (presumably from the construction sites) during the night. Site A (17-18 January 2012) and site D have the second highest total RMSE values. Both site A and site D are mature high-density housing estates and the high RMSE values can be attributed to the existence of wet markets located within these sub-catchments (wet markets are not present in other sub-catchments). The activities at wet markets, which start in the early hours of the morning, would have increased sanitary flow and thus the chance for leakage in the early morning. Besides, although the used water from wet markets is supposed to be channelled into the sewer system, it is likely that some of the used water overflows to the nearby surface drains. Depending on market operations, the impact of wet market activities on the measured FIB concentrations may not be consistent throughout the sampling campaign which creates an added degree of noise in the FIB field data in contrast to sewer flows which peak and trough more consistently each day. The variability introduced by wet markets would seem to explain the fact that two of the three sets of samples from site A (9-10 January 2012 and 18-19 January 2012) show typical diurnal variation while one set of samples from site A (17-18 January 2012) shows atypical diurnal variation (Table 2).

Site F, site J and site K are the sites with the three highest RMSE values for enterococci (Table 2). Despite the high RMSE values for enterococci, RMSE values for *E. coli* at site J and site K are low (Table 2). This shows that *E. coli* is more closely associated with human faecal and sewage contamination than enterococci, which is consistent with the observation in Figure 2 that *E. coli* shows a stronger diurnal variation than enterococci. This observation is in agreement with Duris et al. (2013) who reported that *E. coli* is better correlated than enterococci with pathogen

genes and caffeine, a common indicator for sewage contamination. Sauer et al. (2011) also showed that *E. coli* exhibited better correlation with the human *Bacteroides* genetic marker than enterococci.

3.3. Autocorrelation of typical FIB time series

Autocorrelation analysis was conducted on the normalised FIB data that are classified as showing typical diurnal variations (Table 2). A total of nine 24-hr sets of samples were analysed, giving rise to 216 (9 x 24) data points used in the autocorrelation analysis. The number of lags considered was 54 (216 / 4) lags and the results are plotted as correlograms in Figure 6. The correlograms peak at 24 and 48 hr for all FIB. The autocorrelation coefficients at 24- and 48-hr lag are positive and exceed the 95% confidence limits (significant at $p \leq 0.05$). The correlation at 24-hr lag confirms the existence of a typical diurnal cycle. Since the autocorrelation was analysed on normalised FIB concentrations constructed from data obtained from different sampling sites, the observed correlation shows concentrations monitored 24 hr apart possess similar trends, reaching high or low values at regular intervals within a 24-hr period. Although the correlations fluctuate over the range of the lags with peaks repeated every 24 hr, the correlations at 48 hr are about the same magnitude as at 24 hr. The regular repetition of the autocorrelation function without the presence of an appreciable overall trend indicates that the FIB variation is stationary. This implies that peaks in FIB concentrations occur regularly at approximately the same times during the day across all sites and sampling days.

3.4. Significance of sampling time on FIB concentration

The Wilcoxon-Mann-Whitney tests were conducted to test for statistical differences between daytime and nighttime FIB concentrations for the sets of samples that exhibit typical FIB behaviour (Table 2). Prior to the analysis, FIB concentrations were first segregated into daytime and nighttime datasets. The p values of all FIB are lower than 0.05 (p values for TC = 2×10^{-4} , *E. coli* = 3×10^{-5} and enterococci = 2×10^{-3}) indicating that the differences in concentration between daytime and nighttime samples are significant.

Since significant variations in FIB concentrations exist during the course of a day, it is important to ascertain the time at which samples should be taken if it is desired to ascertain the maximum FIB concentration in a given sampling day. Among the different ways of segregating the data into four six-hour groups (Section 2.5), the first method (20:00 – 01:00, 02:00 – 07:00, 08:00 – 13:00 and 14:00 – 19:00) resulted in the lowest p values for all three FIB. This implies that this particular choice of four six-hour groups is the most discriminating among the three methods of groupings tested. The Kruskal-Wallis test showed significant differences ($p \leq 0.05$) among the four groups for all three FIB, with the significantly different groups for the first four six-hour groups identified by the Tukey-Kramer test shown in Table 3 (a). The results show that TC and *E. coli* concentrations over two six-hour groups (08:00 – 13:00 and 14:00 – 19:00) are significantly different from those at 02:00 – 07:00. Enterococci concentrations for three six-hour groups (08:00 – 13:00, 14:00 – 19:00 and 20:00 – 01:00) are also significantly different from those collected at 02:00 – 07:00. Similar analysis using four-hour groupings (Section 2.5) showed that

the groupings 20:00 – 23:00, 00:00 – 03:00, 04:00 – 07:00, 08:00 – 11:00, 12:00 – 15:00 and 16:00 – 19:00 produced the lowest p values for all three FIB and is the most discriminating sampling scheme. The results are shown in Table 3 (b) indicating that TC and *E. coli* concentrations of samples collected at 08:00 – 11:00 are significantly different from samples collected at 04:00 – 07:00. In addition, the concentrations of TC and *E. coli* in samples collected at 12:00 – 15:00 are significantly different from samples collected at 00:00 – 03:00 and 04:00 – 07:00. Similarly, enterococci concentrations at 12:00 – 15:00 are significantly different from those collected at 04:00 – 07:00.

Based on the analysis of the six four-hour groups, sampling should be conducted hourly from 12:00 to 15:00 in order to obtain maximum concentration estimates and from 04:00 to 07:00 to obtain estimates of minimum FIB concentrations. However, it is impractical to sample two ranges of hourly sampling times (04:00 – 07:00 and 12:00 – 15:00) on a routine basis.

Therefore, the Wilcoxon-Mann-Whitney test was used to find the minimum number of samples to be collected during the ranges of sampling times identified so as to obtain FIB concentrations that are significantly different (at 5% level) from the FIB concentrations during the remaining hours of the day and which would be indicative of the maximum and minimum concentrations.

The Wilcoxon-Mann-Whitney test was thus conducted for sites showing typical diurnal behaviour (Table 2). The FIB concentrations were segregated into two groups based on the sampling time. The first group contained samples from the suggested four-hour sampling time (12:00 – 15:00 or 04:00 – 07:00), while the second group contained samples from the other sampling times throughout the day. An incremental procedure was applied to the first group in

426 order to arrive at the minimum number of sampling times required. Therefore, FIB
427 concentrations for hourly samples collected during the four-hour sampling time were
428 compared, one at a time, to the samples collected at the remaining hours of the day to see if
429 samples collected during a particular hour would result in significantly different FIB
430 concentrations.

431

432 The sample collected at 12:00 showed significantly higher *E. coli* ($p = 0.042$) concentrations, but
433 not TC ($p = 0.108$) and enterococci ($p = 0.090$) concentrations. Sampling at 13:00, 14:00 or
434 15:00 also failed to reveal significantly higher concentrations for all FIB. Therefore,
435 combinations of two sampling times at 12:00 and 13:00, at 12:00 and 14:00 and at 12:00 and
436 15:00 were tested to see if sampling at two different times would result in significantly higher
437 concentrations for all three FIB. It was found that sampling at 12:00 and 14:00 resulted in
438 significantly higher concentrations for all three FIB (p values for TC = 0.017, *E. coli* = 0.016 and
439 enterococci = 0.043) while sampling at 12:00 and 13:00 or at 12:00 and 15:00, did not. Thus, a
440 sampling program that collects samples at 12:00 and 14:00 is recommended as an efficient
441 sampling strategy to obtain the expected daily maximum FIB concentrations.

442

443 In the same way, samples collected at 05:00 showed significantly lower enterococci ($p = 0.048$)
444 concentrations, but not TC ($p = 0.143$) or *E. coli* ($p = 0.103$) concentrations and similarly
445 sampling at 04:00, 06:00 or 07:00 did not reveal significantly lower concentrations for all FIB.
446 Therefore, combinations of two sampling times at 05:00 and 04:00, at 05:00 and 06:00 and at
447 05:00 and 07:00 were tested to see if sampling at two different times would result in

significantly lower concentrations for all three FIB. It was found that sampling at 05:00 and 04:00 and at 05:00 and 06:00 resulted in significantly lower concentrations for all three FIB. Sampling at 05:00 and 04:00 resulted in lower p values (p for TC = 0.026, *E. coli* = 0.010 and enterococci = 0.010) than sampling at 05:00 and 06:00 (p for TC = 0.032, *E. coli* = 0.016 and enterococci = 0.014). Samples collected at 05:00 and 07:00 showed significantly lower enterococci (p = 0.015) concentrations, but not for TC (p = 0.110) and *E. coli* (p = 0.077). A sampling program can collect samples at either 05:00 and 04:00 or 05:00 and 06:00. When a sampling is for a routine monitoring program, sampling at 05:00 and 06:00, rather than sampling at 05:00 and 04:00, is recommended as an efficient sampling strategy to obtain the expected daily minimum FIB concentrations.

3.5. *Implications of this study in tropical urban settings*

The findings in this study have important implications for water quality in tropical urban settings. Singapore has what is arguably the most advanced water management system in the world (Tortajada et al., 2013). Wastewater is collected from all developed parts of the city by a sewer system that is separated from the drainage system. The Singapore government also invests heavily in leak detection and rehabilitation to maintain the sewer system. Despite this well maintained system, our study suggests there is sewer leakage into the drains sampled. Here, an important factor is the tropical setting. The intense tropical rainstorms that occur in Singapore require a drainage system that extends upstream to surface drains around nearly every building. With such an extensive drainage system, there is a potential for any sewage leak

to enter the drain network. Sercu et al. (2011b) reported a possibility of sewage exfiltration from leaking sanitary sewers to nearby shallower storm drains. Moreover, FIB are persistent in the warm tropical climate, particularly in drains that are shielded from the sun. Thus, even a small isolated leak has the potential to affect a long reach of drain. The finding that FIB affects drain water in a well-run system such as Singapore's implies that FIB contamination of drain water is likely to be an issue in most tropical cities, particularly in places where water infrastructure is in poorer condition and less well maintained and operated.

4. Conclusions

The following can be concluded from the work described in this study:

- During dry weather, FIB in most of the 11 drains sampled in this study show a typical diurnal pattern that is characterised by high daytime and low nighttime FIB concentrations.
- FIB diurnal variation is stationary: the high FIB concentrations occur at about the same time across different sampling sites and different sampling days.
- The observed diurnal variation of FIB closely follows that of sewer flows. The existence of sewage exfiltration and preferential flow paths from sewers to drains indicates the likelihood of surface water contamination due to leaking sanitary sewers.
- The time of day at which samples are collected from urban drains is important and grab sampling at random times is unlikely to be an effective sampling strategy. Rather, samples should be collected at 12:00 and 14:00 to capture high FIB concentrations representative

of daytime and/or at 05:00 and 06:00 to capture low FIB concentrations representative of nighttime.

- The finding that FIB affects drain water in a well-run system such as Singapore's implies that FIB contamination of drain water is likely to occur in other cities with less advanced or aging infrastructure. This problem is expected to be particularly acute in wet tropical areas, where the drainage system is very extensive and where attenuation of FIB in urban drains is less than in temperate and cold climates.

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Table 1. Sub-catchment land use patterns.

Sampling site	Total area (ha)	High-density residential	Low-density residential	Commercial	Road	Other
Site A	24	54%	0%	23%	15%	8%
Site B	30	77%	0%	5%	17%	1%
Site C	30	53%	0%	30%	13%	3%
Site D	30	69%	0%	18%	12%	1%
Site E	37	84%	0%	4%	11%	2%
Site F	91	64%	0%	8%	16%	12%
Site G	29	0%	78%	0%	20%	2%
Site H	21	0%	80%	1%	18%	1%
Site I	8	0%	86%	0%	14%	0%
Site J	7	12%	65%	0%	22%	2%
Site K	7	0%	76%	0%	24%	0%

Table 2. RMSE values for TC, *E. coli* and enterococci deviations from typical pattern. Bold type indicates sites with atypical diurnal pattern. * denotes three highest RMSE values for enterococci. ** “mixed” indicates typical diurnal behaviour for *E. coli* and TC but atypical for enterococci.

Sampling site	Sampling date	RMSE			Total RMSE	Type of diurnal variation
		TC	<i>E. coli</i>	Enterococci		
Site A	9-10 January 2012	0.27	0.26	0.27	0.81	typical
	17-18 January 2012	0.39	0.19	0.27	0.86	atypical
	18-19 January 2012	0.27	0.27	0.22	0.76	typical
Site B	16-17 July 2012	0.23	0.28	0.22	0.72	typical
Site C	12-13 June 2012	0.27	0.22	0.21	0.7	typical
Site D	27-28 June 2012	0.3	0.3	0.26	0.86	atypical
Site E	29-30 July 2012	0.2	0.27	0.25	0.72	typical
Site F	21-22 May 2012	0.39	0.33	0.39*	1.11	atypical
Site G	9-10 July 2012	0.2	0.23	0.23	0.67	typical
Site H	6-7 March 2012	0.16	0.2	0.25	0.61	typical
	19-20 March 2012	0.23	0.24	0.23	0.7	typical
Site I	20-21 June 2012	0.19	0.16	0.21	0.57	typical
Site J	23-24 July 2012	0.23	0.17	0.35*	0.75	mixed**
Site K	30-31 July 2012	0.23	0.2	0.30*	0.73	mixed**

Table 3. Tukey-Kramer tests results for (a) six-hour and (b) four-hour groups. Stippled shading indicates times of low FIB concentration and gray shading indicates times of high FIB concentration.

(a)

FIB		six-hour groups (20:00 - 01:00, 02:00 - 07:00, 08:00 - 13:00 and 14:00 - 19:00)															
TC		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								
<i>E. coli</i>		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								
Enterococci		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								

(b)

FIB		four-hour groups (20:00 - 23:00, 00:00 - 03:00, 04:00 - 07:00, 08:00 - 11:00, 12:00 - 15:00 and 16:00 - 19:00)															
TC		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								
<i>E. coli</i>		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								
Enterococci		20:00	21:00	22:00	23:00	00:00	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	09:00	10:00	11:00
		12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00								

Figure titles

Figure 1. Eleven sampling points of urban sub-catchments in Singapore.

Figure 2. Box plots of normalised FIB concentrations grouped based on sampling time: (a) TC, (b) *E. coli* and (c) enterococci. The arithmetic means of the normalised FIB concentrations are superimposed on the box plots with a black circle symbol (●) connected by trend lines. The number above each data point indicates the number of samples. The box indicates the 25th percentile, median and 75th percentile values. The maximum whisker length is 1.5 times the interquartile range. Values beyond the whiskers are symbolised with plus sign.

Figure 3. Field verification of sewer leakage at Site A, adapted from Diagne (2013) (GIS location data for sewer and main drain lines provided by PUB): (a) *E. coli* concentrations on 14 January 2013, (b) *E. coli* concentrations on 17 January 2013 and (c) location for the injection of tracer.

Figure 4. Tracer test results at Site A: (a) 24 January 2013 (adapted from Diagne (2013), monitoring ceased at 15:15 pm due to rain) and (b) 4 February 2013.

Figure 5. Plots of 24-hr FIB concentrations showing atypical diurnal variation and error bars. Shaded time intervals indicate nighttime samples (collected from 20:00 to 07:00). The errors were calculated using propagation of errors based on estimation of MPN (at the 95% confidence interval) and sample dilution.

Figure 6. Correlograms of typical diurnal FIB concentrations (Table 2): (a) TC, (b) *E. coli* and (c) enterococci. The dashed lines represent the 95% confidence limits of $r_k = 0$ (zero autocorrelation coefficient).

Figure 1

(a)

(b)

Figure 2

(c)

Figure 2

Figure 3

Figure 4

(a) Site A, 17-18 January 2012

(b) Site D, 27-28 June 2012

(c) Site F, 21-22 May 2012

Figure 5

Figure 6