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Associations of chemical tracers and faecal indicator bacteria in a tropical urban catchment

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ABSTRACT

Surface water contamination by human faecal wastes is a widespread hazard for human health. Faecal indicator bacteria (FIB) are the most widely used indicators to assess surface water quality but are less-human-specific and have the potential to survive longer and/or occur naturally in tropical areas. In this study, 13 wastewater chemicals (chloride, boron, orthosphophate, detergents as methylene blue active substances, cholesterol, cholestanol, coprostanol, diethylhexyl phthalate, caffeine, acetaminophen, ibuprofen, sucralose and saccharin) were investigated in order to evaluate tracers for human faecal and sewage contamination in tropical urban catchments. Surface water samples were collected at an hourly interval from sampling locations with distinct major land uses: high-density residential, low-density residential, commercial and industrial. Measured concentrations were analysed to investigate the association among indicators and tracers for each land-use category. Better correlations were found between different indicators and tracers in each land-use dataset than in the dataset for all land uses, which shows that land use is an important determinant of drain water quality. Data were further segregated based on the hourly FIB concentrations. There were better correlations between FIB and chemical tracers when FIB concentrations were higher. Therefore, sampling programs must be designed carefully to take the time of sampling and land use into account in order to effectively assess human faecal and sewage contamination in urban catchments. FIB is recommended as the first tier in assessment of surface water quality impairment and chemical tracers as the second tier. Acetaminophen and coprostanol are recommended as chemical tracers for high-density residential areas, while chloride, coprostanol and caffeine are recommended for low-density residential areas.

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1. Introduction

Human faecal contamination poses a widespread hazard for human health (Arnone and Walling, 2007). In a study conducted in highly urbanised tropical Singapore, Ekklesia et al. (2015) found that leaking sanitary sewers can pose a particularly prevalent source of such contamination. However, pathogenic microorganisms usually appear intermittently at low concentrations and therefore are difficult to cultivate and identify (Savichtcheva and Okabe, 2006). Thus, indicators of bacteriological surface water quality have traditionally been used for routine examination of surface water. Consensus regarding suitable faecal indicators has evolved since the early 1970s when water quality managers relied almost exclusively on indicator bacteria including the coliform group, faecal streptococci, *Escherichia coli* (*E. coli*) and enterococci (Cabelli, 1977; Cohen and Shuval, 1973). The aforementioned faecal indicator bacteria (FIB) are known to be less source-specific (USEPA, 1986) and influenced by a variety of environmental factors, including the potential to survive longer and/or occur naturally in tropical areas. Indeed, other studies have questioned the use of such indicators in tropical environments (Hazen, 1988; Muñiz et al., 1989; Rivera et al., 1988). There is therefore a need to search for alternative means to detect bacteriological contamination. Thus, it is worthwhile to evaluate the use of wastewater chemicals as tracers of human sewage. Some possibilities include inorganic chemicals (such as boron, chloride and orthophosphate), natural organics (such as faecal sterols and stanols) and artificial organics (such as chemicals in laundry detergents, plasticisers, caffeine, pharmaceuticals and artificial sweeteners). The presence of trace organics in surface waters depends on usage patterns, for example, pharmaceutical prescription practices. Chemicals selected as tracers should be used or produced regularly in order for the tracers to occur in sufficiently high concentrations. This implies that consumer habits should not change and that use of the compound should not be phased out within a few years.

The preceding discussion distinguishes "indicators" from "tracers". By "indicators" we mean the traditional measures of water quality that have long been taken as evidence of bacteriological contamination by wastewater. FIB are chief examples of such indicators. By "tracers" we mean chemicals thought to be associated with sewage, but whose connection to sources of contamination are not yet sufficiently understood that their simple presence can be taken as unambiguous evidence of contamination by sewage.

Previous studies investigating tracers for bacteriological contamination have been motivated by the desire to develop laboratory analysis methods (Al-Odaini et al., 2010), to study the tracers' environmental fate and transport (Buerge et al., 2009; Isobe et al., 2004; Tixier et al., 2003), to assess the occurrence of certain tracers in various environments (Buerge et al., 2009, 2003; Buser et al., 1999; Nguyen et al., 2011; Wu et al., 2008; Xu et al., 2011) and to understand their correlation with conventional indicator bacteria (Isobe et al., 2002; Leeming and Nichols, 1996; Wu et al., 2008). Al-Odaini et al. (2010) developed a laboratory analysis methodology that suits Malaysian surface water samples. Their study detected several chemical compounds including diclofenac and acetaminophen in river water samples. Isobe et al. (2004) detected coprostanol in river water samples from the Mekong Delta, Vietnam and several rivers in West Malaysia. The presence of coprostanol in an aerobic environment is considered to be an indication of recent faecal input to the waters (Isobe et al., 2004) since coprostanol degrades rapidly under aerobic conditions. The reported half-life of coprostanol is generally less than 10 days at 20 °C (Isobe et al., 2002). Tixier et al. (2003) found that ibuprofen is not eliminated by photo-transformation but undergoes significant degradation in the human body, wastewater treatment plants and natural environments. Buerge et al. (2009) found that hydrolysis, photolysis and biodegradation of saccharin play only a minor role in its environmental fate. In addition, saccharin is an anionic compound at pH values typical of natural waters and therefore is expected to be quite mobile in the subsurface. These properties of saccharin are consistent with the requirements for a good tracer. Buerge et al. (2003) reported that caffeine concentrations in lakes varied from 6 ng/L (in sparsely populated areas) to 164 ng/L (in densely populated areas), while caffeine in rivers were found to have concentrations of up to 250 ng/L Buser et al. (1999) reported that ibuprofen was detectable in surface waters at up to 8 ng/L in lakes and rivers in Switzerland and in the North Sea. Wu et al. (2008) reported that samples collected in the Marina Reservoir catchment in Singapore had ibuprofen concentrations from 37 ng/L (reservoir samples) up to 195 ng/L (drain samples). Buerge et al. (2009) detected sucralose in domestic wastewaters and natural waters, while saccharin was found at lower levels in treated wastewater. Nguyen et al. (2011) and Xu et al. (2011) detected perfluorochemicals such as perfluorooctane sulfonate ranging from 1 to 156 ng/L and emerging organic contaminants including hormones and pharmaceuticals in the ng/L range in surface waters in Singapore and discussed the possibility that these compounds may have originated from leaking sanitary sewers. Leeming and Nichols (1996) reported coprostanol is better correlated with enterococci than faecal coliform in Derwent estuary, Tasmania, Australia. In contrast, Isobe et al. (2002) reported that coprostanol is better correlated with *E. coli* than total coliform and faecal streptococci and better correlated with total coliform than faecal streptococci in the Mekong Delta, Vietnam and West Malaysia. The contrasting behaviour of coprostanol with enterococci/faecal streptococci and *E. coli*/faecal coliform may be caused by the different climates in which the sampling locations are located: temperate for Tasmania, Australia and tropical for West Malaysia and Mekong Delta, Vietnam. Although Wu et al. (2008) obtained good correlation between caffeine and faecal coliform, the correlation was derived from three samples only and may be an artefact of the small number of samples rather than representative of actual conditions. Despite the wide variety of the studies mentioned here, no single chemical or group of chemicals has emerged from this body of work as a clear substitute for FIB. Indeed, a comprehensive study by Hyer (2007) recommended a "weight-of-evidence" approach that considered nine traditional water quality parameters, including FIB, as easy-to-measure indicators as well as thirteen chemical species as more-difficult-to-measure "confirmatory" tracers.

The literature provides only limited guidance on the use of chemical tracers as alternatives to FIB. Good tracers depend on local usage and cultural practices, and much of the world has yet to be comprehensively sampled. Although some of the studies described above occurred in a tropical setting, including Southeast Asia, the number of studies is relatively small and data on chemical tracers and even indicator bacteria are generally lacking for tropical climates. The suitability of different tracers can be anticipated to depend on land use, but this variable has not been systematically addressed in prior work. A related issue is that many studies have simply shown the presence of tracers and have not explored links of these chemicals to sources. Further, the simple presence of chemicals does not necessarily indicate suitability as a potential tracer: concentrations need to be sufficient for consistent detection and quantification. Finally, both indicator and chemical concentrations can be expected to vary with time, but most studies have consisted of grab samples without systematic attention to time variation.

This study sought to address some of the gaps in prior studies. The setting is a tropical climate in Southeast Asia and the study was intensive in both time and space. Samples were collected at the outlets from thirteen nearly homogenous catchment areas which allowed evaluation of the effects of land use. Samples were collected at an hourly interval. Indicator bacteria were analysed for all hourly samples, while chemicals were analysed for selected samples based on the indicator bacteria analysis results. In addition, this study also identified tracers that occurred at sufficiently high concentrations to be quantifiable by reasonably facile methods and with considerable specificity. Hence, the objectives of this study were to (i) identify potential tracers that are practical for routine monitoring and (ii) investigate correlations between FIB and potential tracers in terms of land use and FIB concentrations. Overall, we sought to evaluate the degree of agreement between chemical tracers and FIB and thereby whether chemical tracers can provide evidence of human faecal and sewage contamination.

2. Materials and methods

2.1. Site description

This study was conducted in Singapore which has a tropical climate with annual rainfall of approximately 2200 mm, no distinct wet or dry season and daily mean temperature varying over a narrow range between 26.0 °C and 27.7 °C during the year (NEA, 2014). Singapore is an urbanised island city-state with an extensive network of concrete-lined storm drains for the conveyance of runoff. An entirely separate network of sewer lines conveys wastewater to wastewater treatment plants. Thirteen urban sub-catchments with specific land uses (Fig. 1) were sampled. The sub-catchments were delineated using ArcGIS Desktop 10 (Esri, Redlands, CA, USA). The land-use proportions were determined using GIS land-use data provided by the Public Utilities Board (PUB), Singapore. Six of the sub-catchments are high-density residential areas consisting of high-rise apartment buildings, five are low-density residential areas consisting of single-family housing units, one is in a commercial area and one is in an industrial area. Table 1 summarises the characteristics of the thirteen sub-catchments. Fig. S1 shows the drain network of each sub-catchment. In addition, raw sewage samples from five manholes in both high- and low-density residential areas and non-urban water samples from two streams in a nature reserve were collected. The latter was collected to represent background conditions not influenced by development.

2.2. Sampling campaign

Hourly sampling over 12 h, starting at 08:00 and ending at 19:00, was conducted from January 2011 to July 2012. Details about sampling at each sampling site can be found in Table S1. The sampling was only conducted during dry-weather conditions to eliminate surface runoff as a potential source of contamination and allow clearer identification of sewage

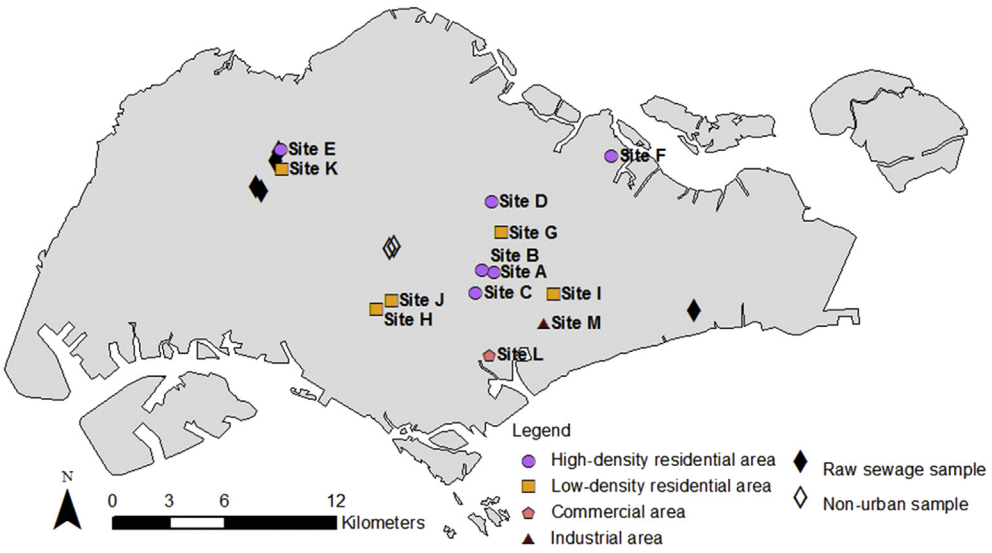

Fig. 1 – Sampling points of urban sub-catchments, raw sewage and non-urban samples in Singapore.

Table 1 – Sub-catchment land-use patterns. Bold type indicates predominant land use.

Sampling site	Total area (ha)	High-density residential area	Low-density residential area	Commercial area	Industrial area	Road	Other
Site A	24	**54%**	0%	23%	0%	15%	8%
Site B	30	**77%**	0%	5%	0%	17%	1%
Site C	30	**53%**	0%	30%	0%	13%	3%
Site D	30	**69%**	0%	18%	0%	12%	1%
Site E	37	**84%**	0%	4%	0%	11%	2%
Site F	91	**64%**	0%	8%	0%	16%	12%
Site G	29	0%	**78%**	0%	0%	20%	2%
Site H	21	0%	**80%**	1%	0%	18%	1%
Site I	8	0%	**86%**	0%	0%	14%	0%
Site J	7	12%	**65%**	0%	0%	22%	2%
Site K	7	0%	**76%**	0%	0%	24%	0%
Site L	15	0%	0%	**65%**	0%	31%	4%
Site M	7	2%	0%	10%	**63%**	19%	7%

contributions. If rainfall occurred during the sampling period, samples compromised by surface runoff were discarded and not analysed for chemical tracers. Samples were collected in two ways: (i) manual grab sampling and (ii) sampling with auto-samplers. When grab sampling was conducted, care was taken so as not to disturb the bottom sediments. All manually collected samples were kept in ice coolers from the time of collection until delivery to the laboratory. Sterile water was used as field blank samples when manual sampling was conducted and analysed for FIB to confirm that there was negligible cross contamination during sample collection, storage and transportation. When sampling with auto-samplers, two Avalanche® refrigerated auto-samplers (Teledyne Isco, Lincoln, NE, USA), were used at each site to collect hourly samples over 12 h. The samples collected by the auto-samplers were stored in the field in a refrigerated compartment at 4 °C. A HOBO® U10 temperature data logger (Onset Computer Corporation, Cape Cod, MA, USA) was installed in the refrigerated compartment to monitor temperature and confirm that the refrigeration system was in good working order.

2.3. *Faecal indicator bacteria analysis*

Samples for FIB analysis were delivered to the Nanyang Technological University Environment Lab and refrigerated at 4 °C. The samples were analysed for three faecal indicator bacteria (total coliform (TC), *E. coli* and enterococci) not more than 38 h after collection using the most-probable-number (MPN) method. The samples were vigorously shaken before analysis and typically each sample was analysed with three dilution ratios (1, 100 and 10,000). 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 labelled 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 41 °C ± 0.5 °C (for enterococci trays) for 24–28 h 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 error (Harris, 2007; Peters et al., 1974) due to dilution and the smallest range of 95% confidence interval of the MPN value was selected.

2.4. *Chemical tracers and analysis*

Samples for chemical analysis were sent to an accredited commercial laboratory, where they were preserved according to standard protocols. The volume of each hourly sample sent for chemical analysis was 3.75 L. Due to cost considerations, not all samples collected in this study could be selected for chemical analysis. With the hourly sampling, it is reasonable to assume that samples with high FIB concentrations are associated with fresh sewage and hence will show the chemical tracers in significantly higher concentrations. Therefore, samples to be analysed for chemical tracers were chosen based on the bacteria count: i.e., only samples with either very high or very low FIB counts were analysed for chemical tracers. Thus, a total of 230 samples out of 614 hourly dry-weather samples (refer to Table S1) were selected for chemical analysis. In addition, five raw sewage samples and two non-urban background water samples were also analysed for comparison purposes.

A set of 18 chemicals was selected owing to their likely presence in sanitary sewage. However, two chemicals (bromide and triclosan) were omitted after the first sampling campaign in January 2011 showed these chemicals were almost never detected. Further, three chemicals (di-n-butyl phthalate, diclofenac and acesulfame-k) were omitted from the suite of possible tracers during data analysis because they were also rarely detected. Therefore, only 13 chemicals as described below are discussed in this paper. They include inorganic parameters (chloride, boron and orthophosphate), detergents (as methylene blue active substances or MBAS), faecal sterols (cholesterol, cholestanol and coprostanol), a plasticiser (diethylhexyl phthalate or DEHP), a stimulant (caffeine), pharmaceutical compounds (acetaminophen and ibuprofen) and artificial sweeteners (sucralose and saccharin). Chloride is typically elevated in sewage while boron and orthophosphate are closely associated with detergents and household cleaning products whose residues are common in

domestic sewage (Hyer, 2007). Together with faecal coliform bacteria and surfactants, boron was identified by Hyer (2007) as the most sensitive tracer in the identification of minor (low-volume) sewage sources. Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant in commercial detergents (Rapaport and Eckhoff, 1990) and is detected by the MBAS analysis. Cholesterol is a natural metabolic product and is microbially reduced into coprostanol (5β-cholestan-3β-ol) and related isomers within the intestines of humans and other higher animals (Seurinck et al., 2005). Leeming et al. (1996) found on average ten times more coprostanol on a dry-weight basis in human faeces than in the faeces of animals that had similar faecal sterol profiles to humans. Microorganisms within the environment metabolise cholesterol to generate cholestanol (Hagedorn and Weisberg, 2009). DEHP is used as a plasticiser in polyvinylchloride (PVC) (Wams, 1987) and is emitted to the environment from plastic pipe and other products. Caffeine is contained in beverages (coffee, tea and caffeinated soft drinks), foods (chocolate products) and drug products (Wu et al., 2008). According to Buerge et al. (2003), caffeine is considered a suitable tracer because caffeine consumption is assured to continue at comparable amounts into the future. Pharmaceuticals are relatively water-soluble and non-volatile (Benotti and Brownawell, 2007) and can reach detectable concentrations in surface water if widely used and if the compounds show mobility and persistence in the aquatic environment (Buser et al., 1999). According to Wilkison et al. (2002), acetaminophen is one of the most frequently detected over-the-counter pain medications in stream samples. Artificial sweeteners are used in beverages, food and other consumer products such as toothpaste. Most of the artificial sweeteners consumed are excreted and no industrial or agricultural use of artificial sweeteners is known.

Chloride (limit of detection or LOD = 0.1 mg/L) was measured with the ion chromatography or IC (SM 4110B) method, orthophosphate (LOD = 3 μg/L) with flow injection analysis (FIA) using the conditions set in SM 4500-P (G), boron (LOD = 1.5 μg/L) with inductively coupled plasma (ICP) according to SM 3120B and detergent (LOD = 50 μg/L) with the methylene-blue active substances (MBAS) method (Eaton et al., 2005). Cholesterol (LOD = 0.1 μg/L), cholestanol (LOD = 0.1 μg/L), coprostanol (LOD = 0.05 μg/L) and DEHP (LOD = 2 μg/L) were analysed as total concentrations with gas chromatography-mass spectrometry (GCMS) using USEPA Method 8270C. Caffeine, acetaminophen, ibuprofen, sucralose and saccharin (all with LOD = 0.01 μg/L) were extracted using solid-phase extraction (SPE) under acidic conditions. The extracts were concentrated and analysed by liquid chromatography-tandem mass spectrometry (LCMSMS) following USEPA Method 1694. Caffeine and acetaminophen were run in the positive electrospray ionization (ESI+) mode, while the rest were run in the negative electrospray ionization (ESI-) mode. Artificial sweeteners were analysed using a variation of the method proposed by Scheurer et al. (2009).

Duplicate runs were conducted to assess the precision of the analytical process. The average coefficient of variation of the duplicates was less than or equal to 11%. Lab blanks were analysed for every run to ensure no contamination had occurred. All blanks were below detection. In the data analysis to follow, samples with non-detect values were recorded as half of the respective LOD.

2.5. Statistical analyses

All concentration data were log-transformed. Box-and-whisker plots were created with Matlab (The MathWorks, Inc., Natick, MA, USA) and used to illustrate the distribution of the data. The Wilcoxon-Mann-Whitney and Kruskal–Wallis tests, which are non-parametric tests, were conducted in Matlab to test for statistical differences between chemical concentrations in different land uses at the 5% significance level. Spearman correlation analysis was conducted in Matlab to find associations among indicators and tracers at the 5% significance level. Log-transformed FIB concentrations were normalised based on minimum (min) and maximum (max) log-transformed FIB concentrations for each 12-hr set of samples according to Ekklesia et al. (2015):

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

Normalising FIB concentrations accentuates the "shape" of the 12-hr FIB concentration data and allows the identification of temporal patterns, if any. The normalised FIB concentrations were sorted into three groups: (i) normalised *E. coli* or enterococci (Ent) concentrations $\geq$ 0.70 designated as the "high" dataset, presumably indicative of fresh sewage, (ii) 0.30 < normalised *E. coli* or enterococci concentrations < 0.70 designated as the "moderate" dataset and (iii) normalised *E. coli* and enterococci concentrations $\leq$ 0.30 designated as the "low" dataset. Spearman correlation analysis was carried out on the "high", "moderate" and "low" FIB datasets with the chemical data.

3. Results and discussion

3.1. Overview of chemical data

Concentrations of the chemical tracers in each land use are plotted as box plots in Fig. 2. Concentrations measured in raw sewage samples and non-urban samples are also included in the plots for comparison. Both non-urban samples had chemical concentrations below LOD, except for chloride (7.2 and 2.0 mg/L), boron (0.0088 and 0.0028 mg/L), orthophosphate (0.004 and 0.005 mg/L), cholesterol (0.1 μg/L and below LOD) and caffeine (both 0.02 μg/L). Cholesterol and caffeine concentrations in non-urban samples are far below those in urban catchments (Fig. 2 (f) and (i)). The existence of chemicals that are used or produced by humans in urban drains, in contrast to non-urban water, provides an indication of inflow from leaking sewers into surface water drains, consistent with the findings by Ekklesia et al. (2015).

Based on the Wilcoxon-Mann-Whitney test, the concentrations of all chemical tracers, except cholesterol, are significantly ($p \leq 0.05$) different between high-density and low-density residential areas (p values for each chemical tracer can be found in Table S2). The median and geometric mean concentrations of most of the chemical tracers in high-

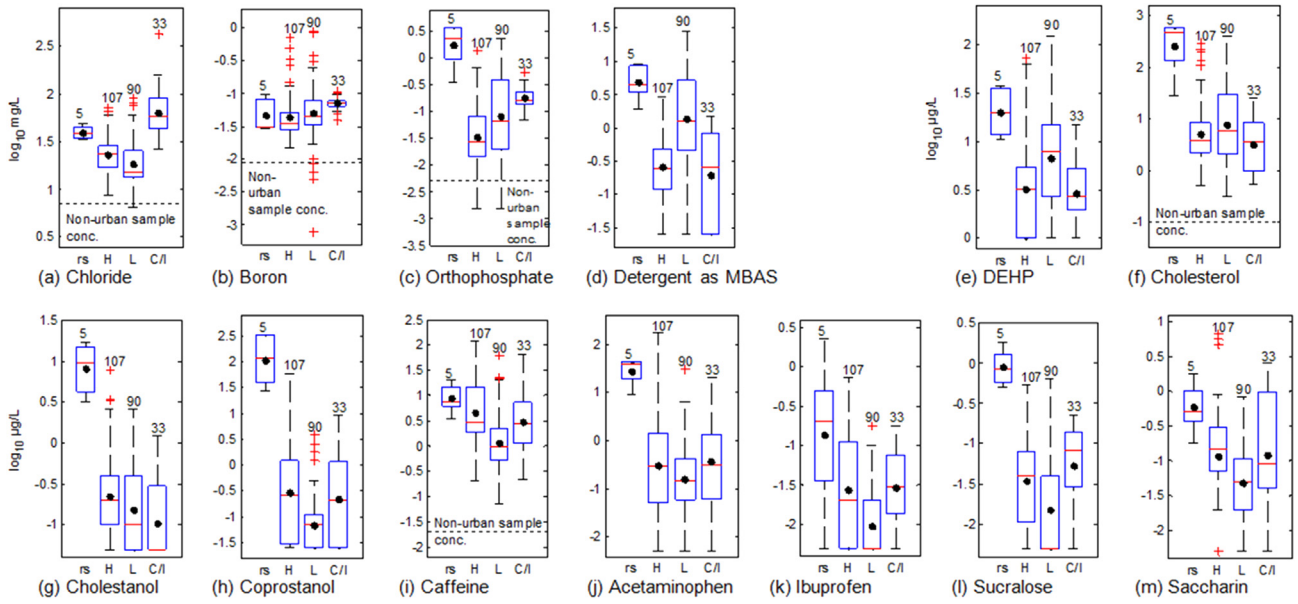

Fig. 2 – Box plots of 13 chemical parameters based on land use (H, L, C/I). rs = raw sewage, H = high-density residental areas, L = low-density residential areas, C/I = commercial/industrial areas, • = gomentric mean concentration, – = detected chemical concentartions of non-urban water samples, + = values beyond 1.5 times the interquartile range or 1.5 times the difference between 75th and 25th percentiles. The number above each box plot indicates the numbers of samples.

density residential areas are higher than in low-density residential areas, except for boron, orthophosphate, detergent as MBAS, DEHP and cholesterol. The higher median and geometric mean of most of the chemical tracers indicate that there is a higher degree of contamination in the high-density residential areas than in the low-density residential areas. This is in agreement with a study by Nanyang Technological University (2008) which reported the highest bacteria count was obtained from an area with 70% high-density residential land use. However, the study collected dry-weather samples from relatively downstream locations within Kranji Reservoir catchment, Singapore which received flow contributions from a variety of land uses. Therefore, land-use impacts on bacterial contamination could not be distinguished. Young and Thackston (1999) also reported that faecal bacteria densities were related to housing density. The higher median and geometric mean of boron, orthophosphate and detergent as MBAS in low-density residential areas (Fig. 2 (b), (c), and (d)) may be caused by the discharge of laundry water directly to drains, as evidenced by the foamy water that was regularly observed flowing in the drains in the low-density residential areas. Clothes washing is often carried out in backyards in low-density residential areas and on occasion discharged directly into surface drains instead of the sewer.

Due to a limited number of sites sampled for commercial and industrial areas, these land uses are combined into a single category for analysis. Most of the chemical concentrations in commercial/industrial areas were lower than raw sewage with the exception of chloride and boron concentrations (Fig. 2 (a) and (b)). This is presumed to be associated with the proximity of these areas to the sea and the resulting mixing of sea water in the surface drains. Based on the Kruskal–Wallis test, most of the chemical concentrations in commercial/industrial areas were significantly ($p \leq 0.05$) different from those in low-density residential areas, except for cholestanol and acetaminophen. However, only the concentrations of chloride, boron, orthophosphate and cholestanol in commercial/industrial areas were significantly ($p \leq 0.05$) different from those in high-density residential areas. This shows that contamination patterns in commercial/industrial areas differ more from low-density residential areas than from high-density residential areas.

3.2. *Correlation analysis based on land use*

Measured concentrations were analysed to find associations between the chemical tracers and FIB for each land-use category. The correlation coefficients are reported in Table 2. In the high-density residential dataset, *E. coli* is most closely correlated with these tracers: orthophosphate, detergent as MBAS, DEHP, cholesterol, cholestanol, coprostanol, acetaminophen, ibuprofen and sucralose. The correlation coefficients between *E. coli* and those tracers are higher in the high-density residential dataset with r_s = 0.41, 0.22, 0.30, 0.49, 0.35, 0.48, 0.69, 0.31 and 0.36 respectively, (see Table 2 – high-density residential) than in the combined dataset where r_s = 0.30, 0.21, 0.27, 0.39, 0.22, 0.40, 0.43, 0.21 and 0.14 respectively (see Table 2 – all). Enterococci is better correlated with DEHP, cholesterol, acetaminophen, ibuprofen and sucralose in the high-density residential dataset with r_s = 0.25, 0.26, 0.33, 0.29 and 0.47 respectively (see Table 2 – high-density residential) than in the combined dataset where r_s = *insig.*, *insig.*, 0.26, 0.22 and 0.40 (*insig.* indicates correlations are insignificant) respectively (see Table 2 – all). Unlike low-density

Table 2 – Correlations between chemical tracers and FIB distinguished by land use. Numerical values shown in bold type indicate correlations significant at $p \leq 0.05$. N values are the number of pairs of samples used in the correlation analysis.

	All N = 230			High-density residential areas N = 107			Low-density residential areas N = 90			Commercial/ industrial areas N = 33		
	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent
Chloride	**0.22**	0.11	**0.31**	0.07	0.10	**0.25**	**0.53**	**0.29**	0.20	-0.01	0.11	-0.29
Boron	**0.15**	0.08	0.12	-0.05	0.08	0.07	**0.54**	0.18	0.12	0.17	-0.14	0.26
Orthophosphate	**0.29**	**0.30**	0.06	0.15	**0.41**	-0.04	**0.57**	**0.25**	0.09	0.18	**0.35**	0.01
Detergent as MBAS	**0.20**	**0.21**	**-0.19**	**0.28**	**0.22**	0.00	**0.66**	**0.26**	0.11	-0.08	0.32	**-0.45**
DEHP	**0.22**	**0.27**	0.05	0.13	**0.30**	**0.25**	**0.56**	**0.40**	0.20	0.01	-0.12	-0.08
Cholesterol	**0.42**	**0.39**	0.08	**0.43**	**0.49**	**0.26**	**0.60**	**0.31**	0.16	0.33	**0.43**	-0.21
Cholestanol	**0.21**	**0.22**	0.06	**0.23**	**0.35**	0.13	**0.32**	**0.23**	0.13	-0.17	-0.24	-0.29
Coprostanol	**0.28**	**0.40**	**0.17**	**0.30**	**0.48**	0.11	**0.29**	**0.34**	**0.23**	**0.40**	**0.65**	-0.21
Caffeine	**0.17**	0.10	**0.31**	0.12	0.04	**0.22**	0.07	**0.22**	0.18	**0.45**	0.19	**0.58**
Acetaminophen	**0.44**	**0.43**	**0.26**	**0.64**	**0.69**	**0.33**	0.05	-0.02	0.06	**0.39**	**0.47**	0.16
Ibuprofen	0.10	**0.21**	**0.22**	0.18	**0.31**	**0.29**	**-0.30**	-0.16	-0.07	0.26	**0.67**	-0.26
Sucralose	**0.31**	**0.14**	**0.40**	**0.51**	**0.36**	**0.47**	-0.12	-0.11	0.08	0.29	0.09	**0.46**
Saccharin	0.04	**0.16**	0.02	-0.17	0.16	-0.06	**0.24**	-0.06	-0.09	0.27	**0.59**	-0.10

Correlation: 1.00 0.90 0.80 0.70 0.60 0.50 0.40 0.30 0.20 0.10 0.00 (High … Low)

residential areas, laundry water is rarely discharged into surface drains in high-density residential areas, it is instead discharged directly into sanitary sewers. Thus, the correlation of *E. coli* with orthophosphate in drains in high-density residential areas may suggest sewer leakage as the contamination source, apparently with insufficient or minimal soil adsorption to attenuate orthophosphate during subsurface transport from the underground sewers into the surface drains. Insufficient soil adsorption is plausible if soapy water has seeped through the soils for long periods and therefore the available sorption sites have been exhausted. Walter et al. (1996) reported this phenomenon in Cape Cod, MA, USA where continuous orthophosphate loading from the disposal of secondarily treated sewage had filled available sorption sites and therefore orthophosphate was mobile and detected in a downstream waterbody. Adsorption could also be minimal when water from leaking sewers travels to the surface drains through preferential flow pathways such as subsurface voids as suggested by Ekklesia et al. (2015). In general for high-density residential areas, *E. coli* is better correlated with the tracers than is enterococci—chloride, caffeine and sucralose being exceptions. The correlations are insignificant between cholestanol/coprostanol and enterococci.

In the low-density residential dataset, TC is better correlated with chloride, boron, orthophosphate, detergent as MBAS, DEHP, cholesterol, cholestanol and coprostanol with $r_s = 0.53, 0.54, 0.57, 0.66, 0.56, 0.60, 0.32$ and 0.29 respectively (see Table 2 – low-density residential) than in the combined dataset where $r_s = 0.22, 0.15, 0.29, 0.20, 0.22, 0.42, 0.21$ and 0.28 respectively (see Table 2 – all). Similarly to the combined dataset and the high-density residential dataset, *E. coli* is generally better correlated with chemical tracers than is enterococci. *E. coli* is better correlated with chloride, detergent as MBAS, DEHP, cholestanol and caffeine in the low-density residential dataset with $r_s = 0.29, 0.26, 0.40, 0.34$ and 0.22 respectively (see Table 2 – low-density residential) than in the combined dataset where $r_s =$ *insig.*, 0.21, 0.27, 0.22 and *insig.* respectively (see Table 2 – all). More than half of the samples from low-density residential areas were non-detectable for ibuprofen (60 out of 90) and sucralose (48 out of 90). This suggests that ibuprofen and sucralose are not suitable tracers for low-density residential areas.

For the commercial/industrial dataset, *E. coli* is better correlated with orthophosphate, cholesterol, coprostanol, acetaminophen, ibuprofen and saccharin with $r_s = 0.35, 0.43, 0.65, 0.47, 0.67$ and 0.59 respectively (see Table 2 – commercial/industrial) than in the combined dataset where $r_s = 0.30, 0.39, 0.40, 0.43, 0.21$ and 0.16 respectively (see Table 2 – all). Similarly to residential areas, *E. coli* shows stronger correlation with more tracers than does enterococci, which shows a strong positive correlation with only caffeine and sucralose. Chloride, boron, DEHP and cholestanol are not significantly correlated with FIB.

In general, the correlations for specific land uses show different characteristics from the correlations for the combined dataset: there are better correlations among different indicators and tracers in each land-use dataset than in the combined dataset (Table 2). Further, FIB correlate best with different parameters depending on the land use. These results show that land use affects drain water quality and that analysis based on land use enables better data interpretation. Generally there is a greater number of significant ($p \leq 0.05$) correlations between chemical tracers and FIB in the high-density residential areas than in the low-density residential areas. Correlations between *E. coli* and orthophosphate, cholesterol, cholestanol, coprostanol, acetaminophen, ibuprofen and sucralose are higher in the high-density residential areas with $r_s = 0.41, 0.49, 0.35, 0.48, 0.69, 0.31$ and 0.36 respectively (see Table 2 – high-density residential) than in the low-density residential areas where $r_s = 0.25, 0.31, 0.23, 0.34,$ *insig.*, *insig.* and *insig.* respectively (see Table 2 – low-density residential).

Some of these tracers are indicative of recent sewage contamination and their correlation with FIB corroborates an association with human sewage. Coprostanol is hydrophobic, and therefore readily incorporated into bottom sediments, and is also biodegradable under aerobic conditions (Isobe et al., 2002). In this study, drain water samples were

sampled during dry weather with care so as not to disturb the bottom sediments. Hence, the presence of coprostanol in the water samples indicates recent human faecal contamination. Sidhu et al. (2013) reported frequent detection of acetaminophen and caffeine in stormwater runoff in Australia. They pointed out that caffeine and acetaminophen are biodegradable (and hence labile) indicators of the presence of sewage in environmental water samples. Therefore, the presence of caffeine and acetaminophen also suggests recent contamination by raw sewage. Although Wu et al. (2008) have attributed elevated caffeine levels in Singapore to sewer leakage, the use of caffeine, as well as saccharin, in Singapore must be interpreted cautiously as these compounds may be discharged directly into surface drains from food courts which are more common found in the high-density residential and commercial areas. The presence of the other tracers is believed to be clearly associated with leakage from sanitary sewers as discussed by Ekklesia et al. (2015).

3.3. *Chemical data segregation based on FIB*

In this section, we further analyse the sampling results with samples from residential areas segregated based on the concentrations of *E. coli* and/or enterococci (described in Section 2.5). Commercial/industrial areas are excluded from this analysis due to insufficient data. Fig. 3 depicts the distribution of the segregated chemical data based on land use and normalised *E. coli* or enterococci values. Land use and concentration magnitude are captured in the letter-number codes below each graph panel. For example, H1 (or L1) refers to samples obtained from high-density (or low-density) residential areas where the normalised *E. coli* or enterococci values are greater or equal to 0.70. Fig. 3 shows that generally the geometric means of chemical concentrations are consistent with FIB concentrations and decrease from the "high" (H1 or L1) to the "low" (H3 or L3) dataset. In particular, the geometric means of cholesterol, cholestanol, coprostanol, acetaminophen and sucralose in high-density residential areas and of coprostanol and saccharin in low-density residential areas decrease from the "high" to the "low" dataset. This observation shows that generally higher normalised FIB concentrations are accompanied by higher chemical concentrations. Since the chemical parameters analysed in this study are associated with wastewater, high FIB concentrations are likely to have resulted from human faecal and sewage contamination presumably from leaking sewers.

The Spearman correlation coefficients between chemical parameters and FIB are presented in Table 3 for the dataset obtained from the high-density residential areas and in Table 4 for the dataset obtained from the low-density residential areas. For high-density residential areas, there is a generally greater number of significant ($p \leq 0.05$) correlations between FIB and chemical tracers in the "high" dataset than in the "moderate" and "low" datasets. *E. coli* is more highly correlated with coprostanol in the "high" dataset ($r_s = 0.61$, Table 3) than in the "moderate" ($r_s = 0.52$, Table 3) and "low" ($r_s = insig.$, Table 3) datasets. In the "high" dataset, *E. coli* has a greater number of significant ($p \leq 0.05$) correlations with chemical tracers than does enterococci. TC is better correlated with cholestanol, coprostanol, acetaminophen and ibuprofen in the "high" dataset with $r_s = 0.29$, 0.33, 0.67 and 0.26 respectively (see Table 3 – "high" dataset) than in the high-density residential dataset where $r_s = 0.23$, 0.30, 0.64 and *insig.* respectively (see Table 3 – high-density residential). *E.*

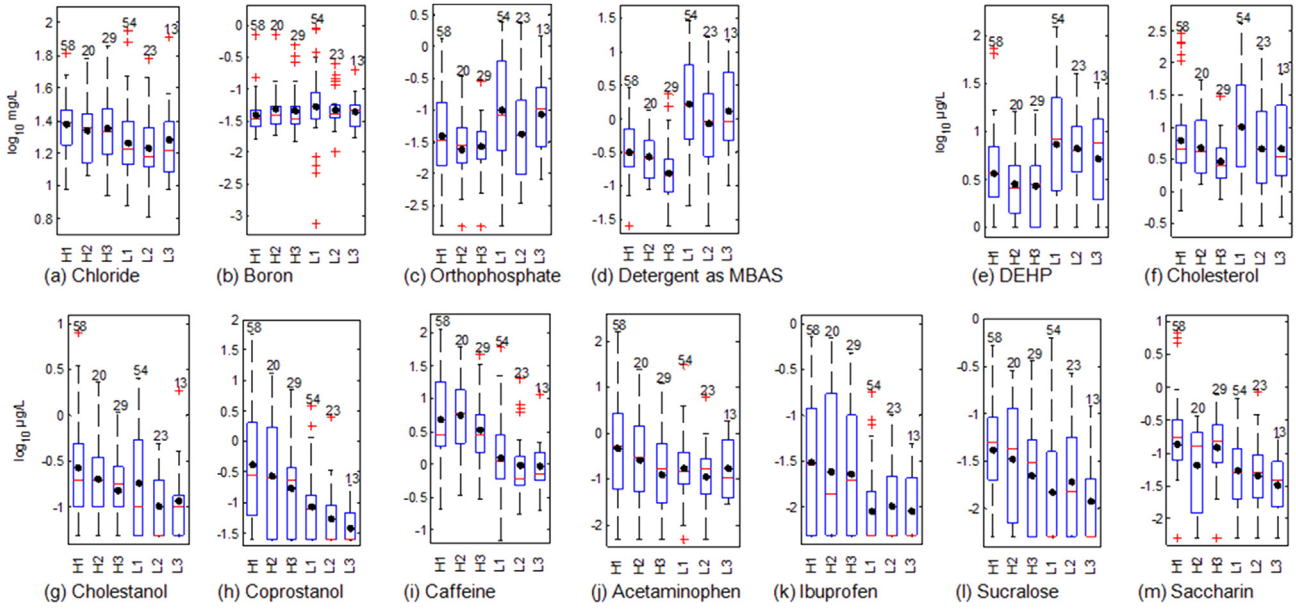

Fig. 3 – Box plots of 13 chemical parameters based on land use (H and L) and concentrations groups (1, 2, or 3). H = high-density residential areas, L = low-density residentials areas, 1 = the "high" dateset (normalised values of *E. coli* or enterococci concentration ≥ 0.70), 2 = the "moderate" dataset (0.30 < normalised values of *E. coli* or enterococci concentrations < 0.70), 3 = the "low" dataset (normalised values of *E. coli* and enterococci concentrations ≤ 0.30), • = geometric mean concentration, + = values beyond 1.5 times the interquartile range or 1.5 times the differences between 75th and 25th percentiles. The number above each box plot indicates the number of samples.

Table 3 – Correlation matrix between chemical tracers and FIB for high-density residential areas. Numerical values shown in bold type indicate correlations significant at $p \leq 0.05$. N values are the number of pairs of samples used in the correlation analysis.

	High-density residential areas N = 107			"High" dataset *E. coli* or enterococci ≥ 0.70 N = 58			"Moderate" dataset 0.30 < *E. coli* or enterococci < 0.70 N = 20			"Low" dataset *E. coli* and enterococci ≤ 0.30 N = 29		
	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent
Chloride	0.07	0.10	**0.25**	-0.01	0.12	0.26	0.25	0.16	0.36	-0.06	-0.03	0.35
Boron	-0.05	0.08	0.07	-0.05	0.21	0.12	-0.01	0.29	0.17	-0.02	-0.04	0.32
Orthophosphate	0.15	**0.41**	-0.04	0.15	**0.50**	-0.05	0.23	0.37	-0.06	0.16	0.21	-0.21
Detergent as MBAS	**0.28**	**0.22**	0.00	0.06	-0.01	**-0.42**	0.14	0.04	0.09	0.18	0.08	-0.20
DEHP	0.13	**0.30**	**0.25**	0.25	**0.34**	0.23	-0.21	**0.47**	-0.06	-0.03	0.00	**0.45**
Cholesterol	**0.43**	**0.49**	**0.26**	**0.43**	**0.59**	0.18	0.43	0.36	0.10	0.07	0.01	0.02
Cholestanol	**0.23**	**0.35**	0.13	**0.29**	**0.50**	0.11	0.12	0.10	-0.09	-0.11	-0.09	-0.10
Coprostanol	**0.30**	**0.48**	0.11	**0.33**	**0.61**	0.03	0.32	**0.52**	0.05	-0.04	0.15	0.05
Caffeine	0.12	0.04	**0.22**	0.18	0.16	0.22	-0.07	-0.14	0.29	-0.06	-0.27	0.10
Acetaminophen	**0.64**	**0.69**	**0.33**	**0.67**	**0.76**	**0.29**	0.34	**0.67**	0.30	**0.66**	**0.71**	0.06
Ibuprofen	0.18	**0.31**	**0.29**	**0.26**	**0.43**	**0.29**	0.01	**0.58**	0.17	0.11	0.03	**0.48**
Sucralose	**0.51**	**0.36**	**0.47**	**0.48**	**0.41**	**0.37**	**0.53**	0.11	**0.67**	0.36	0.29	0.34
Saccharin	-0.17	0.16	-0.06	-0.06	**0.30**	-0.09	**-0.58**	0.19	-0.14	-0.24	-0.25	-0.01

Correlation: 1.00 0.90 0.80 0.70 0.60 0.50 0.40 0.30 0.20 0.10 0.00 (High … Low)

coli is also better correlated with most of the chemical tracers (orthophosphate, DEHP, cholesterol, cholestanol, coprostanol, acetaminophen, ibuprofen, sucralose and saccharin) in the "high" dataset with $r_s = 0.50, 0.34, 0.59, 0.50, 0.61, 0.76, 0.43, 0.41$ and 0.30 respectively (see Table 3 – "high" dataset) than in the high-density residential dataset where $r_s = 0.41, 0.30, 0.49, 0.35, 0.48, 0.69, 0.31, 0.36$ and *insig*. respectively (see Table 3 – high-density residential).

For low-density residential areas, *E. coli* has significant ($p \leq 0.05$) correlations with chloride, cholesterol, cholestanol, coprostanol and caffeine, while enterococci shows significant ($p \leq 0.05$) correlation with cholesterol in the "high" dataset. Significant correlations between the FIB and these chemicals are not found in the "moderate" and "low" datasets (Table 4). Similarly to the high-density residential areas, *E. coli* has better correlation with other tracers than enterococci in the "high" dataset. This indicates that *E. coli* shows a stronger response to human faecal and sewage contamination compared to enterococci. The correlations of ibuprofen or sucralose with other parameters, either positive or negative, in the segregated datasets may be invalid because neither of these compounds was detected in about half or more of the samples analysed. TC is better correlated with chloride, boron, DEHP, cholesterol, cholestanol and coprostanol in the "high" dataset with $r_s = 0.61, 0.56, 0.57, 0.64, 0.40$ and 0.36 respectively (see Table 4 – "high" dataset) than the low-density dataset where $r_s = 0.53, 0.54, 0.56, 0.60, 0.32$ and 0.29 respectively (see Table 4 – low-density residential). *E. coli* is also better correlated with chloride, DEHP, cholestanol and caffeine in the "high" dataset with $r_s = 0.38, 0.44, 0.31$ and 0.41 respectively

Table 4 – Correlation matrix between chemical tracers and FIB for low-density residential areas. Numerical values shown in bold type indicate correlations significant at $p \leq 0.05$. N values are the number of pairs of samples used in the correlation analysis.

	Low-density residential areas N = 90			"High" dataset *E. coli* or enterococci ≥ 0.70 N = 54			"Moderate" dataset 0.30 < *E. coli* or enterococci < 0.70 N = 23			"Low" dataset *E. coli* and enterococci ≤ 0.30 N = 13		
	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent	TC	*E. coli*	Ent
Chloride	**0.53**	**0.29**	0.20	**0.61**	**0.38**	0.20	0.38	0.22	0.36	**0.65**	0.49	0.12
Boron	**0.54**	0.18	0.12	**0.56**	0.23	0.16	**0.67**	0.08	-0.09	0.00	-0.08	0.06
Orthophosphate	**0.57**	**0.25**	0.09	**0.56**	0.24	0.19	0.36	0.13	-0.16	**0.80**	0.37	-0.19
Detergent as MBAS	**0.66**	**0.26**	0.11	**0.63**	0.20	0.19	**0.72**	0.37	-0.02	**0.68**	0.29	-0.34
DEHP	**0.56**	**0.40**	0.20	**0.57**	**0.44**	0.23	**0.65**	**0.50**	0.16	0.55	**0.62**	0.02
Cholesterol	**0.60**	**0.31**	0.16	**0.64**	**0.28**	**0.29**	0.33	0.04	-0.36	0.45	0.42	-0.44
Cholestanol	**0.32**	**0.23**	0.13	**0.40**	**0.31**	0.17	0.00	-0.10	-0.21	-0.03	-0.31	-0.10
Coprostanol	**0.29**	**0.34**	**0.23**	**0.36**	**0.31**	0.20	-0.30	-0.01	-0.08	0.30	0.01	-0.30
Caffeine	0.07	**0.22**	0.18	0.20	**0.41**	0.15	**-0.51**	-0.07	0.16	0.02	**-0.61**	0.34
Acetaminophen	0.05	-0.02	0.06	0.02	-0.08	0.00	0.14	0.04	0.07	-0.24	-0.32	0.43
Ibuprofen	**-0.30**	-0.16	-0.07	-0.26	-0.21	-0.16	**-0.46**	-0.08	0.10	-0.31	-0.01	0.11
Sucralose	-0.12	-0.11	0.08	-0.06	-0.18	-0.01	-0.01	-0.17	0.17	-0.47	0.50	**0.59**
Saccharin	**0.24**	-0.06	-0.09	0.20	-0.12	-0.01	0.18	-0.14	-0.40	0.33	-0.01	-0.49

Correlation: 1.00 0.90 0.80 0.70 0.60 0.50 0.40 0.30 0.20 0.10 0.00 (High … Low)

(see Table 4 – "high" dataset) compared to the low-density residential dataset where r_s = 0.29, 0.40, 0.23 and 0.22, respectively (see Table 4 – low-density residential). Lastly, enterococci is better correlated with cholesterol in the "high" dataset with $r_s = 0.29$ (see Table 4 – "high" dataset) compared to the low-density residential dataset where r_s = *insig*. (see Table 4 – low-density residential).

Generally, for residential land use, there is a greater number of significant ($p \leq 0.05$) correlations in the "high" dataset than in the "low" dataset. The correlation coefficients between chemical tracers and FIB are also higher in the "high" dataset than in the "moderate" and "low" datasets. This implies that there is better agreement between chemical parameters and FIB when FIB concentrations are high. The chemical parameters analysed in this study were selected because they are associated with sanitary wastewater. Consistently high FIB concentrations are most likely caused by wastewater, presumably leaked from sewers and associated building connections, while lower FIB concentrations are likely urban background concentrations. Studies reported natural occurrence, die-off, re-growth of FIB and the ability for FIB to survive longer in tropical climates (Hazen, 1988; Muñiz et al., 1989; Rivera et al., 1988), resulting in background FIB concentrations that are likely to be higher in tropical areas. The different strengths of the correlations found in the different datasets in this study also imply that any sampling program must be designed carefully in order to effectively assess human faecal and sewage contamination of surface waters.

3.4. *Chemicals as sewage tracers*

The analysis of the segregated data shows that when FIB concentrations are high, there is a greater number of significant ($p \leq 0.05$) correlations between chemicals and FIB and there are stronger correlations between chemicals and FIB. Based on this, we recommend that FIB can be used as a first tier and chemical tracers as a second, confirmatory, tier in assessment of surface water quality impairment due to human faecal and sewage contamination. In this way, chemical tracers can be used to protect against the false positives that are known to occur in tropical areas. The different indicators and tracers can also provide different lines of evidence about bacterial contamination. However, there is no one chemical tracer that is far superior to the others for all land uses. The analysis of the data for high- and low-density residential land use shows that different chemical tracers correlate more or less strongly with FIB for different land uses. Thus, as discussed below, certain chemical tracers are better than others for certain land uses.

Based on the correlation analyses in Section 3.3, acetaminophen is the tracer most highly correlated with *E. coli* ($r_s = 0.76$) in high-density residential areas. Correlation analysis of the "high" datasets for high-density residential land use (Table 3) also reveals that *E. coli* is moderately correlated with orthophosphate (r_s = 0.50), cholesterol (r_s = 0.59), cholestanol ($r_s = 0.50$), coprostanol ($r_s = 0.61$), sucralose ($r_s = 0.43$) and saccharin (r_s = 0.41). In Singapore, saccharin (and caffeine) must be used cautiously in the high-density residential areas (as well as commercial areas) as these tracers are often discharged directly from open-air areas in food courts. Because of the consistent correlations, for high-density residential land use, the recommended tracers are acetaminophen and coprostanol.

The strong correlation between acetaminophen and *E. coli* disappears going from high-density to low-density residential areas and correlations of other tracers with *E. coli* are generally weaker in low-density residential than in high-density residential areas. Detergent as MBAS must be used cautiously in the low-density residential areas as it can be discharged directly from backyard laundry washing. Ibuprofen and sucralose are not good tracers for low-density residential areas because they are below the LOD for more than half of the samples in the "high" dataset. For low-density residential areas (Table 4), *E. coli* is moderately correlated with chloride ($r_s = 0.38$), cholestanol ($r_s = 0.31$), coprostanol ($r_s = 0.31$) and caffeine ($r_s = 0.41$) in the "high" dataset. Given the generally weaker correlations within low-density residential areas, a larger suite of tracers is needed. Therefore, for low-density residential areas, the recommended tracers are chloride, coprostanol and caffeine.

4. Conclusions

This study supports the following conclusions:

1. The study design facilitated detection of recent human faecal and sewage contamination by (i) including labile chemical tracers (coprostanol, caffeine and acetaminophen) and (ii) sampling frequently (hourly) relative to the short response time of concrete-lined drains from urban catchment areas.
2. Land use affects drain water quality as shown by better correlations among different indicators and tracers in each land-use dataset than in the combined dataset for all land uses.
3. Concentrations of all chemical tracers, except cholesterol, were found to be significantly different between high-density and low-density residential areas. Median and geometric mean concentrations of most of the chemical tracers in high-density residential areas are higher than in low-density residential areas. This shows that generally high-density residential areas have a higher degree of contamination than low-density residential areas.
4. Data segregation based on hourly FIB concentrations showed that there is better association between chemical tracers and FIB with relatively high concentrations than with relatively moderate or low concentrations or with all FIB concentrations. Sampling programs must be designed carefully to take the time of sampling and also land use into account in order to effectively assess human faecal and sewage contamination of surface waters.
5. FIB are recommended as the first tier in assessment of surface water quality impairment and chemical tracers as the second tier. FIB generally indicate the likely presence of human faecal contamination but nonetheless are subject to false positives in tropical areas. Confirmation by high chemical concentrations thus guards against false positives. However, there is no one chemical parameter that is

superior for all land uses. Acetaminophen and coprostanol are recommended as confirmatory tracers for high-density residential areas. A larger suite of tracers is needed for low-density residential areas, where correlations between chemical tracers and *E. coli* are generally weaker. A confirmation tracer suite of chloride, coprostanol and caffeine is recommended for low-density residential areas.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.02.037.

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