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Microbial Contamination in Urban Tropical Lentic Waterbodies and Ponds along an Urbanization Gradient

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ABSTRACT

This study aimed to investigate the seasonal variation of microbial quality in urban waterbodies along urbanization gradients. Bimonthly samples were collected from 14 recreational lakes and flood mitigation ponds in the Klang Valley, Malaysia, between May and October 2017. Samples were analysed for the presence and abundance of *Escherichia coli*, *Clostridium perfringens*, faecal coliforms (FC), faecal streptococci (FS), enterococci and total coliforms as indicator organisms, using standard methods. All studied lakes contained indicator bacteria that exceeded the National Lake Water Quality Standards (NLWQS) to varying degrees. The mean of the FC/FS ratios in all lakes exceeded four, indicating that the faecal contamination might have originated from human sources. *Escherichia coli*, *C. perfringens* and faecal coliform concentrations were negatively correlated with temperature ($P < 0.01$) and positively correlated with turbidity and suspended solids ($P < 0.05$). Non-parametric test results revealed that only the density of *C. perfringens* varied significantly according to season and urbanization impacts ($P < 0.05$). The Secchi depth transparency and dissolved oxygen (DO) levels explain the largest variation in bacterial communities. This study showed that contamination of faecal bacteria in the waterbodies varied spatially and temporally along urbanization gradients. Water quality monitoring and improvements are needed before the waterbodies can be used for direct body-contact recreation and as alternative water sources for drinking purposes.

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INTRODUCTION

Small waterbodies, including flood mitigation ponds and ex-mining pools, are important alternative water sources during drought. Due to their urban positioning, much of the area around these ponds has been converted to recreation parks worldwide, thus the waterbodies are used for primary or secondary recreational purposes. Converting ex-mining pools to water storage supply and recreational facilities has raised concerns about the impact of water quality on humans, in particular, the heavy-metals content (Kusin et al., 2016). Additionally, the presence of pathogenic bacteria such as *E. coli* and faecal enterococci can cause various water-borne diseases on contact with humans. The type of disease is dependent on the species involved. Examples are diarrhoea (*E. coli*), leptospirosis (*Leptospira* spp.), Cryptosporidiosis (*Cryptosporidium* spp.) and cholera (*Vibrio cholerae*) (Hipsey & Brookes, 2013; World Health Organization [WHO], 2003). All these water-borne diseases induce abdominal pain, diarrhoea and vomiting (WHO, 2003). Despite the increasing number of studies on water quality undertaken in developing countries, studies involving microbiological analyses in countries such as Malaysia are limited to biochemical oxygen demand (BOD), chemical oxygen demand (COD), and faecal coliform and total coliform studies (Department of Environment [DOE], 2016; Said et al., 2012a, 2012b). In developed countries, such as the United States and those in Europe, pathogenic bacteria, protozoa and viruses are widely monitored as water quality parameters. However, such microbiological assessment is not commonly undertaken in developing countries due to limited expertise, a lack of facilities and budget restraints on the complex and costly analytical testing procedures required for microbial detection. The faecal contamination of urban waterbodies remains a problem for many of the developing countries in Asia and Africa, due to lack of sanitation, and this leads to continued faeces-related diseases such as cholera and diarrhoea (Demanou & Brummett, 2003; Henny & Meutia, 2014; Komarulzaman et al., 2017).

A new standard for lake water was introduced by the National Hydraulic Research Institute of Malaysia in 2015 and approved for application in Malaysia in 2017. In addition to BOD, COD and total coliforms, the National Lake Water Quality Criteria and Standards (NLWQS) recommended the use of *Escherichia coli*, enterococci and *Clostridium perfringens* as bacterial indicators for the ambient water quality of stagnant water (National Hydraulic Research Institute of Malaysia [NAHRIM], 2015; Sharip & Suratman, 2017). All these faecal bacterial indicators are present in human faeces, in the order *C. perfringens* < enterococci < *E. coli* (Edberg et al., 2000). Their survival rates in the environment, are in the order *E. coli* (weeks to a month) < enterococci (weeks) < *C. perfringens* (months to years) (Edberg et al., 2000). Little is known about the occurrence of most of these faecal bacterial indicators in the waterbodies in Malaysia, making informed decision-making for effective management difficult.

Few microbial studies have been carried out in waterbodies that are used for public consumption, in particular, in those that meet recreational needs. This includes the occurrence of protozoa, namely, *Giardia* and *Cryptosporidium* oocysts, which have been studied in rivers and recreational lakes in the Malaysian Peninsular (Jali & Ithoi, 2009; Lee et al., 2014; Onichandran et al., 2013). These studies frequently detect *Giardia* rather than *Cryptosporidium* in rivers, with more oocysts found in the downstream part of the river followed by midstream and upstream sections (Jali & Ithoi, 2009; Lee et al., 2014). In another study, conducted in two recreational waterbodies, *Cryptosporidium* spp., *Giardia* spp., *Ascaris* spp., *Acanthamoeba* spp. and hookworm were detected in both recreational lakes, while *Schistosoma* spp. were found in only one lake (Onichandran et al., 2013). A study carried out by Hamzah and Hattasrul (2007) in Chini Lake in Malaysia, in 2005, reported faecal contamination, namely, total coliforms and *E. coli*, in the range 10^2 to 10^5 in some parts of the lake, indicating that the lake was unsuitable for recreational use. The occurrence of faecal bacteria in water in temperate countries has been associated with physical factors such as temperature, solar radiation and salinity that influence their survival rates (Hughes, 2003; Sadowsky & Whitman, 2011; Whitman et al., 2004). For example, the higher the solar irradiance and salinity levels, the lower the survival rates of faecal coliforms in the water (Sadowsky & Whitman, 2011; Whitman et al., 2004). Elevated levels of *E. coli* in lake water were detected during the wet season or after rainfall events due to surface run-off while higher levels of *E. coli* in the near-shore water was linked to resuspension of beach sand driven by onshore winds (Isobe et al., 2004; Whitman et al., 2006). The urbanization process could also shape the microbial density: higher densities of *E. coli* were found at sites near residential and commercial areas compared to other land uses in the upper Blackstone River watershed, USA (Wu et al., 2011).

The present study aimed at investigating the occurrence of six faecal indicator bacteria (*C. perfringens*, total coliforms, faecal coliforms, faecal streptococci, *E. coli* and enterococci) in urban tropical lentic waterbodies and how their abundance correlates with environmental factors and the level of urbanization. This study also aimed to provide baseline data on the faecal indicator bacteria occurrence, in line with the application of the national lake water quality standard throughout the country to support effective management of lakes and ponds.

MATERIALS AND METHODS

Study Site

This work focused on 14 ponds of varying sizes, types and shapes, as shown in Figure 1 and Table 1. The waterbodies included five recreational lakes and nine flood-retention ponds that had recently been identified as potential sites for conjunctive water supply systems for greater Kuala Lumpur. Two ponds (L4 and L7) received discharges from nearby wastewater treatment facilities.

Field Measurement

In situ field measurements were carried out in triplicates on all ponds and lakes. In situ measurements of total depth, dissolved oxygen (DO), pH, temperature, salinity and conductivity were recorded with an YSI multi-parameter probe (YSI Incorporated, Yellow Springs, OH) and Secchi depths (SD) were measured using a Secchi disk. All parameters in the multi-parameter probe were calibrated using standard procedures prior to sampling. Salinity was included to detect levels of chlorination in the water. Surface water samples were collected in 1-litre amber bottles, stored in cooler boxes at 4°C and sent to an accredited laboratory for microbial analysis using established and validated methods. Total coliform, faecal coliform, *E. coli*, enterococci and faecal streptococci were analysed using membrane filtration as described in the standard methods for the examination of water and wastewater (American Public Health Association [APHA], 2012). Total coliform was measured using the membrane filtration method, enterococci and faecal streptococci using membrane filter techniques and *E. coli* and faecal coliform using a thermotolerant membrane filter procedure. *C. perfringens* was analysed using the membrane filtration method (United Kingdom Environment Agency [UKEA], 2010). Chromocult Coliform Agar was used to grow *E. coli*, faecal coliforms and total coliforms at 35°C, over an incubation periods of two days. The media and incubation periods for growing faecal streptococci and enterococci and for growing *C. perfringens* were Kenner Faecal Agar (48 hours) and Tryptose Sulfite

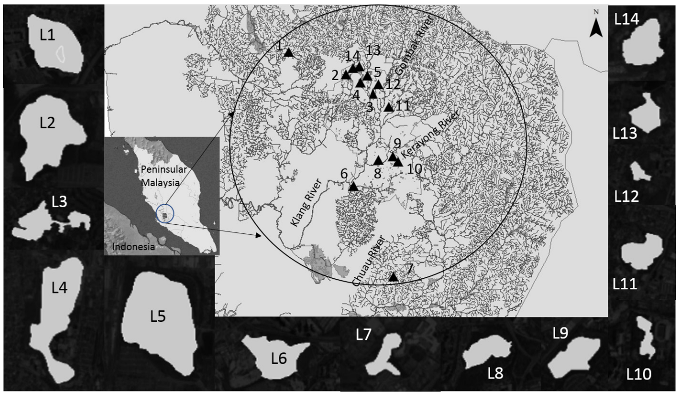


Figure 1. Map of the study urban lakes. Numbers refers to lake

Table 1
Physical characteristics of the studied lakes

Lake	Latitude and Longitude	Surface Area (km^2)	Types of Ponds	Ranging Depth
L1	101°31.009'E, 3°15.000'N	0.28	Water Recreational	0.3 – 1.7
L2	101°38.013'E, 3°13.008'N	0.42	Water Recreational	0.3 – 2.1
L3	101°42.006'E, 3°10.011'N	0.14	Water Recreational	0.6 – 3.7
L4	101°40.000'E, 3°12.013'N	0.40	Retention pond	1.4 – 5.0
L5	101°40.010'E, 3°13.006'N	0.65	Retention pond	1.7 – 29.0
L6	101°39.005'E, 3°3.0137'N	0.21	Retention pond	0.5 - 2.5
L7	101°42.007'E, 2°56.004'N	0.08	Retention pond	1.3 – 1.9
L8	101°41.009'E, 3°6.002'N	0.12	Retention pond	2.3 – 16.3
L9	101°42.013'E, 3°6.008'N	0.15	Retention pond	5.5 – 16.3
L10	101°43.003'E, 3°5.016'N	0.05	Water Recreational	0.6 – 2.2
L11	101°43.002'E, 3°12.004'N	0.07	Water Recreational	0.3 - 0.7
L12	101°43.003'E 3°11.003'N	0.02	Retention pond	0.9 – 1.0
L13	101°39.015'E, 3°14.003'N	0.09	Retention pond	0.3 – 0.4
L14	101°39.006'E, 3°13.016'N	0.16	Retention pond	0.3 – 2.1

Cycloserine Agar (24 hours) respectively. All bacterial colonies were calculated based on CFU calculations.

Additional water samples were taken in separate bottles: 100-ml bottles for the analysis of total phosphorus (TP), 500-ml bottles for the analysis of ammoniacal nitrogen, nitrate, iron, manganese and chemical oxygen demand (COD) and 1-litre bottles for the analysis of biochemical oxygen demand (BOD) and chlorophyll *a* (Chl *a*), in accordance with the standard method (APHA, 2012). *In situ* measurements of total depth, dissolved oxygen (DO), pH, temperature, salinity and conductivity were recorded with an YSI multi-parameter probe (YSI Incorporated, Yellow Springs, OH) and Secchi depths (SD) were measured using a Secchi disk. Total suspended solids (TSS) concentrations were measured using a portable suspended solids meter (Lovibond, The Tintometer Ltd, Amesbury, UK). All measurements and sample collections were performed twice: during the dry months (16th May – 8th June) and the wet months (1st -15th October), at no fewer than two locations in each lake and pond.

Data Analysis

Clustering and non-metric multidimensional scaling (NMDS), based on the Bray-Curtis dissimilarity matrix (Clarke, 1993), were performed to visualize multivariate patterns among the indicator bacteria within the 14 lakes and ponds under study. The waterbodies included five recreational lakes and nine flood-retention ponds that had recently been identified as potential sites for conjunctive water supply systems for greater Kuala Lumpur. Principal component analysis (PCA) was initially applied to reduce the number of environmental variables and to summarize the pattern of correlations between total depth (maximum water depth), Secchi depth, temperature, pH, DO, turbidity, conductivity, BOD, COD, ammoniacal nitrogen, nitrate, ammonium, total suspended solids, surface area and shoreline length. Principal coordinates analysis (PCoA) was performed to correlate environmental variables with the ordination axes and to extract meaning from the axes produced in NMDS (Anderson & Willis, 2003). NMDS, PCA and PCoA calculations were based on lake averages.

Correlations between bacterial parameters and environmental variables were generated to assess the linear relationships between the parameters. Non-parametric multivariate multiple regression (DISTLM) was employed to model the relationship between the microbial parameters and the 15 environmental variables. Each variable was initially examined in a marginal test (excluding other variables) and then subjected to a forward selection procedure (Bayesian information criterion, BIC) with sequential tests. Significance was performed using 9,999 permutations of the residuals under the reduced model.

Following the scale described by Pawlikiewicz and Jurasz (2017), an urban impact assessment (UIA) was included as a factor in the analysis, in order to evaluate the impact of urbanization within a 1-km radius of the lake. The three-scale categories used were: 1 – high-density development and completely urbanized; 2 – mid-density development with significant urbanization and 3 – low-density urbanization area. Seasonal differences were also analysed using non-parametric Wilcoxon signed-rank tests and these were based on two categories: dry (May to September) and wet (October to December). Wilcoxon signed-rank tests were also used to determine any significant differences in microbial parameters between the ponds used for recreation and the flood-retention ponds. The linear relationships between individual variables were based on the Spearman correlation. Microbial data and other environmental variables were log transformed to improve normality prior to multivariate analysis. Correlation and non-parametric Wilcoxon signed-rank tests were performed using SPSS 16.0 (SPSS Inc.) and multivariate methods (NMDS, PCA, PCoA and DISTLM) were conducted using PRIMER 6 and PERMANOVA+ (Plymouth Marine Laboratory, Plymouth, UK).

RESULTS

Water Quality and Bacterial Indicator Ratios

The temperature in the study lakes ranged between 26.7°C and 32.6°C, while the pH ranged between 7.35 and 8.51; all of which are within the NLWQS limits. Dissolved oxygen (DO) concentrations ranged between 4.91 and 10.8 mg/L, while TSS ranged between <0.1 and 366 mg/L. Three lakes (L6, L7 and L10) had DO values of less than 6.5 mg/L and lakes L9 and L13 had DO values exceeding 9.5 mg/L. The BOD and COD in these lakes were high. All BOD values exceeded the standard limit of 3 mg/L, with values ranging between 5.7 and 21.5 mg/L. The COD concentration ranged between 8.1 and 131.6 mg/L and only one lake (L7) had a COD level that complied with the NLWQS (<10 mg/L). The highest BOD and COD values were recorded in L14, followed by L6 and L13. Ammoniacal nitrogen was high in all lakes, ranging between 0.08 and 6.5 mg/L. High BOD and ammoniacal nitrogen levels have been associated with faecal contamination. Secchi depth transparencies were in the range <0.1 m to 2.1 m, while Chl *a* was in the range 1.7 to 86 µg/L. Based on biological productivity, one lake was categorized as oligotrophic, two lakes were mesotrophic, and the remainder were categorized as hypereutrophic. Nine waterbodies failed to meet the transparency criteria due to their low transparency (<0.6 m) and only two recreational lakes met the Chl *a* limit (10 µg/L). The TP values ranged between 0.05 and 1.1 mg/L and all of the samples exceeded the criteria of 0.01 mg/L and 0.035 mg/L for primary and secondary body contact.

Mean *E. coli* and *C. perfringens* contents were in the range 0.5 to 79,133 cfu100 ml^{-1} and 6.8 to 3,333 cfu100 ml^{-1}, respectively (Table 2). The highest *E. coli* and *C. perfringens* values were recorded in lake L6. Only L3 and L12 had *E. coli* of less than 100 cfu100 ml^{-1}. The faecal coliform and total coliform values in all lakes were in the ranges 700 to 60,900 cfu100 ml^{-1} and 2,425 to 205,000 cfu100 ml^{-1} respectively. The faecal coliform values in the samples taken from all the lakes exceeded the recommended 150 cfu100 ml^{-1} and the total coliform values in two lakes (L3 and L10) were below the recommended values for recreational purposes of 5000 cfu100 ml^{-1}. Enterococci and faecal streptococci in all samples were <1 cfu100 ml^{-1}.

An NMDS plot shows that the bacterial communities in the studied lakes are grouped into four distinct clusters for samples with more than 95% similarity (Figure 2). The hypereutrophic lakes (L9 and L13) form one cluster with 97.9% similarity, the cluster L11 and L7 has 96.7% similarity, the cluster L4 and L14 has 96.6% similarity and the cluster

L6 and L1 has 96.5% similarity. The cluster L3 and L12 with 93.1% similarity, has low *E. coli* and *C. perfringens content.* Cluster (L4, L5, L7, L11, L14) with 85% similarity contains high faecal and total coliforms. No distinct pattern was observed in the microbial quality of the clustered lakes in terms of trophic levels and lake use.

According to Gannon and Busse (1989), an FC/FS ratio of more than four is listed as originating from a human source, while a FC/FS ratio of <0.7 originates from animal sources. In this study, all samples had a FC/FS ratio of more than four, indicating that faecal contamination in the water originated from in human sources. The NLWQS recommended levels for *E. coli*, enterococci and faecal coliforms are 100 cfu100 ml^{-1}, 33 cfu100 ml^{-1} and 150 cfu100 ml^{-1}, respectively. This yield an EC/FC (*E. coli*/faecal coliforms) ratio of 0.67. The mean EC/FC ratio in all lakes was in the range 0 to 1.34; only two ponds (L14 and L15) were well above the ratio of 0.67 set by NLWQS, while the others were within the recommended values.

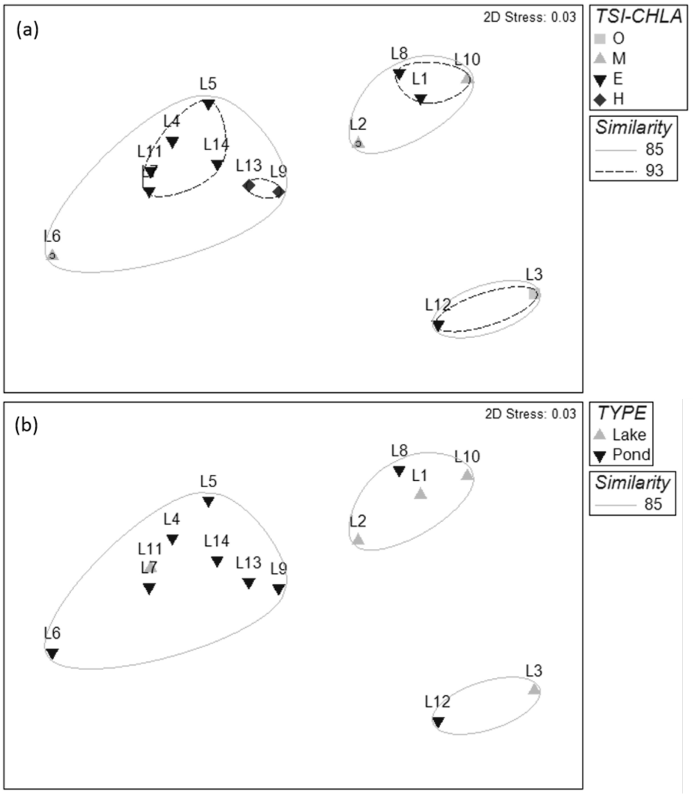


Figure 2. NMDS plots of microbial quality in urban lakes with (a) trophic levels and (b) lake type. O – oligotrophy; M – mesotrophy; E – eutrophy; H – Hyper-eutrophy

Table 2

Mean values (±SE) of microbiological parameters in the studied lakes

Lake	Clostridium perfringens	*Escherichia coli*	faecal coliform	Total Coliform	Enterococci	Trophic Status
			cfu 100 ml^{-1}			
L1	7±4	116±50	1200±491	5920±3331	<1	Eutrophic
L2	17±11	147±77	1725±880	25350±13561	<1	Mesotrophic
L3	34±34	0.5±0	700±700	3250±1350	<1	Oligotrophic
L4	40±47	7050±5013	15725±6848	96350±54689	<1	Eutrophic
L5	8±6	1500±463	14467±6018	89350±41884	<1	Eutrophic
L6	3333±1241	79133±72940	60867±56575	60167±52934	<1	Mesotrophic
L7	276±148	3367±953	20117±15055	204667±189431	<1	Eutrophic
L8	6±3	45±22	2680±1845	10080±5784	<1	Eutrophic
L9	94±56	550±289	2540±498	39400±20611	<1	Hyper-eutrophic
L10	6±3	110±64	825±284	2425±487	<1	mesotrophic
L11	79±39	3067±593	50833±29311	228333±13146	<1	Eutrophic
L12	45±32	0.5±0	850±177	31200±14707	<1	Eutrophic
L13	63±30	1175±683	3425±856	42700±11066	<1	Hyper-eutrophic
L14	37.3±13	2964±1876	6900±1804	59267±32879	<1	Eutrophic
NLWQS	absent	100 (600)	150 (1000)	5000	33	

Note. Values in NLWQS refers to the Category A (Primary body contact) of the standard, items in bracket represents Category B (Secondary body contact) of NLWQS

Relationship between Bacterial Quality, Water Quality and Urbanization Impacts

The correlations between bacterial parameters and the environmental variables are shown in Table 3. All *E. coli*, *C. perfringens* and faecal coliforms were negatively correlated with temperature ($P < 0.01$) and positively correlated with turbidity ($P < 0.05$). In terms of nutrients, *E. coli*, faecal and total coliforms were positively correlated with TP. All four bacterial parameters were positively correlated with TSS ($P < 0.05$) and negatively correlated with Secchi depth transparency ($P < 0.05$). No correlation was observed between microbial quality and surface area or shoreline length. Dissolved oxygen (DO) and pH levels were significantly correlated with temperature (r = 0.736 and r = 0.701). NMDS test results indicated that only *C. perfringens* significantly differed between seasons ($P = 0.039$) and according to urbanization impact ($P = 0.033$) (Figure 3a & 3b). Other than L9, where the mean values were higher than in the dry season, *C. perfringens* was not detected in any of the lakes during the wet season. There was little variation in the mean *E. coli* concentration between the dry and wet seasons, but *E. coli* values were higher in some waterbodies during the wet season (L5, L14).

DISTLM showed that seven parameters explained the variation within the bacterial community. Secchi depth, DO and TP varied significantly between lakes. The results of DISTLM tests (Table 4) revealed a positive match between the environmental variables and FIB. Secchi depth explained the largest contribution (~49.4%) of the variation in the microbial composition in all datasets. This was followed by DO and TP concentrations, contributing a further 19.1% and 6.3% of the variance, respectively. Taken together, other parameters, such as TSS, manganese, chlorophyll-*a* and salinity, explained about 16% of the variance. Figure 4 illustrates the PCoA ordering of the bacterial values and environmental data.

Table 3

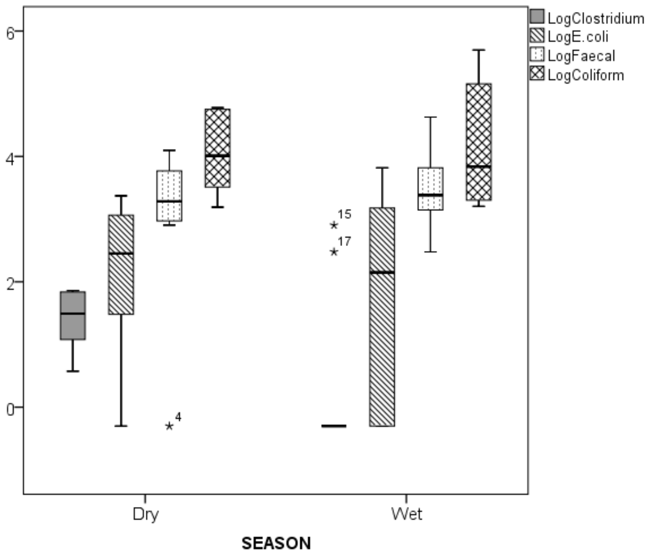
Correlation between bacterial concentrations and physico-chemical parameters

	Clostridium perfringens	*Escherichia coli*	faecal coliform	Total Coliform
Total depth	-0.209	-0.024	0.046	-0.088
SD	-0.659*	-0.641*	-0.718**	-0.711**
Temperature	-0.402	-0.719**	-0.635*	-0.503
pH	0.002	-0.385	-0.297	-0.275
DO	-0.178	-0.530	-0.367	-0.200

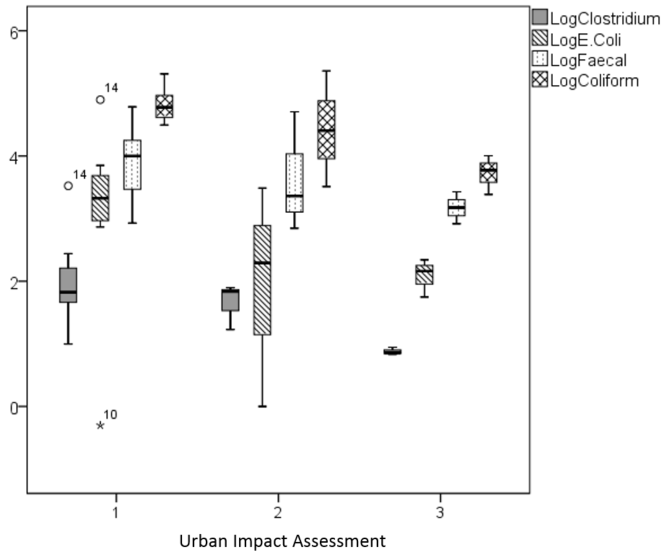
Table 3 *(Continue)*

	Clostridium perfringens	*Escherichia* coli	faecal coliform	Total Coliform
Conductivity	0.458	0.407	0.310	0.123
BOD	0.525	0.349	0.327	0.407
COD	0.284	0.209	0.055	-0.051
TP	0.301	0.587*	0.653*	0.560*
AN	0.279	0.481	0.420	0.363
NO_3	0.339	-0.088	-0.175	-0.356
Chl *a*	0.196	0.248	0.336	0.477
Turbidity	0.653*	0.552*	0.560*	0.578**
TSS	0.764**	0.577*	0.621*	0.632*
Surface area	-0.099	0.244	0.200	0.112
Shore length	0.046	0.231	0.108	0.068
C. perfringens	1	0.582*	0.530	0.574*
E. Coli	0.582*	1	0.895**	0.815**
faecal coliform	0.530	0.895**	1	0.908**
Total coliform	0.574*	0.815**	0.908**	1

Note. * = $P < 0.05$, ** = $P < 0.01$



3(a)



3(b)

Figure 3(a) & 3(b). Boxplot of microbial quality with seasons and urban impacts

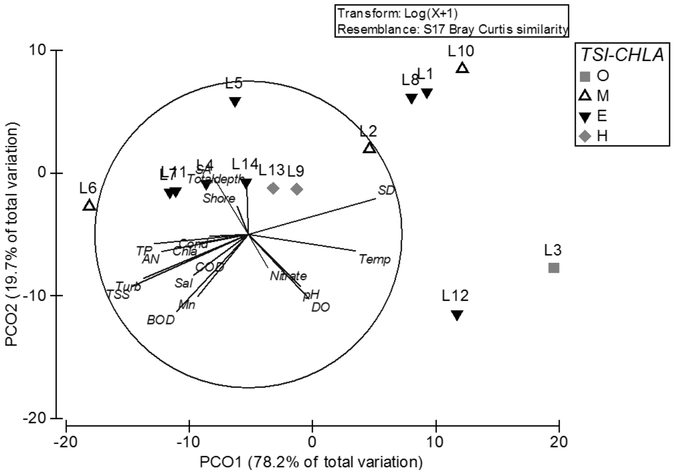


Figure 4. PCoA plots of microbial quality with other environmental variables. O – oligotrophy; M – mesotrophy; E – eutrophy; H – Hyper-eutrophy

Table 4

Results of the DISTLM analysis of the dataset of the fourteen lakes

Sequential Tests					
Variable	BIC	Pseudo-F	P	% Var	% Cum.
SD	65.375	11.73	0.001**	49.43	49.43
DO	61.368	6.6828	0.002**	19.11	68.54
TSS	60.273	3.0566	0.062	7.36	75.91
Sal	59.887	2.1712	0.147	4.68	80.59
TP	57.058	3.8226	0.043*	6.28	86.87
Manganese	56.363	1.8823	0.202	2.78	89.65

Note. BIC Bayesian Information Criterion, %Var percentage of variance in species data explained, % Cum cumulative percentage of variance explained *= $P < 0.05$, **= $P < 0.01$

DISCUSSION

This study shows that urban lakes and ponds in the Klang Valley have high bacterial loads. *C. perfringens*, *E. coli*, total coliform and faecal coliform presence in varying concentrations was observed in all lakes, and most lakes exceeded the national lake water quality standard for recreational purposes. Only enterococci and faecal streptococci were not detected in all samples throughout the study period. The bacterial indicator ratio indicated that most FIB was human in origin. *Escherichia coli* is known as a principal facultative aerobic bacterium found in the intestinal tracts of humans and animals, and the content in faeces varies among host species. The *E. coli* content per weight in human faeces and those of domestic animals ranges between 10^7 and 10^9 and between 10^4 and 10^6, respectively (Tenaillon et al., 2010). The contamination of *C. perfringens,* enterococci and *E. coli* in the environment may occur through sewage effluent discharges, manure and other animal waste run-off and the waste water from slaughterhouses (Balière et al., 2016; Jang et al., 2017; Mueller-Spitz et al., 2010). However, a few studies have reported the presence of *E. coli* in sand beaches and sediment (Alm et al., 2003; Fujioka et al., 1998) and total and faecal coliform bacteria have been found in pristine streams and in groundwater samples (Hazen, 1988). The content of enterococci per weight in mammal faeces, namely, humans and dogs, and in bird faeces, are usually between 10^4 and 10^6 and 10^4 and 10^8 bacteria and between 10^2 and 10^6 bacteria respectively (Boehm & Sassoubre, 2014).

Seasonal variations in bacterial quality have been reported in a few published studies. For example, elevated levels of coliforms and protozoans have been observed at weekends and on public holidays compared to weekdays, due to the larger number of people present in households and their subsequent waste discharges

(Jali & Ithoi, 2009). Similarly, preliminary observations in urbanized wetland inlets indicate a highly variable content of *E. coli* and coliforms, with higher levels of *E. coli* during dry periods and higher concentrations of total coliforms and COD during wet periods (Sharip et al., 2017). In this study, significant seasonal differences in bacterial quality were observed for *C. perfringens*. Seasonal trends for *E. coli*, faecal coliforms and total coliforms varied according to the lake, indicating that recent inputs of human faeces could be related to localize effects in the catchment area, such as those associated with on-site sewage facilities and pollution run-off. Sewage overflow or backflow from independent septic tanks that drain into urban storm water has been linked to *E. coli* detection in other lakes, such as Lake Michigan in the US and Putrajaya Lake in Malaysia (Bower et al., 2005; Sharip et al., 2016). Failing sewage pipes, resulting from cracks caused by invasive tree roots and shifting soils, have been reported as the main cause of *E. coli* leaks into the storm water run-off of the Dickinson Bayou watershed (Morrison et al., 2017). Leaky sewage lines, the illegal renovation of toilets or the inadequate construction of sewage facilities/septic tanks, such as those at construction sites and in old housing areas, are known issues in developing countries that could contribute to the discharge or run-off of water contaminated with human faeces into storm water drains and waterbodies.

In terms of urbanization impact, variation in the bacterial parameters of the surrounding developments was only significant for *C. perfringens*. The lowest *C. perfringens* values were found in lakes located close to low levels of housing development. The values of *E. coli* and coliforms were fairly low in low-impact development areas. The presence of higher numbers of bacterial pathogens in highly populated areas suggests a larger contribution of human faeces, and possibly those of small domestic mammals and birds, to the bacterial contamination found in this study. This was dependent on the waterbody. Lakes found within highly populated areas are generally poor in water quality and have higher FIB content, due to human-related waste. For example, both L4 and L7 received discharges from nearby sewage treatment ponds that flowed into rivers and drains respectively before entering the ponds, while L14 received storm water drain discharges from ongoing construction sites nearby that intermittently caused pollution via an inadequate sewage treatment system. However, the faecal indicator bacteria of birds and mammals may affect some lakes. For example, L7 and L9 have an abundance of birds and otters that have become important elements in the urban habitat and in microbial contamination.

In this study, temperature, DO and TP explain the largest variation in the bacterial parameters. Elevated levels of *E. coli* are negatively correlated with temperature and this is consistent with the published findings (Sampson et al., 2006). *Escherichia coli* bacteria have been reported to survive for a longer period in cooler water (Sampson et al., 2006). This result is contrary to the findings of other studies on the temperature dependence of the survival of *E. coli* (Staley et al., 2014). Surface water temperature is known to be a function of solar radiation: high solar irradiation, together with low cloud cover, increases the water column temperature and decreases bacterial density (Whitman et al., 2004). Despite the fact that the intestines of mammals have the optimal temperature for *E. coli*, around 36ºC-40ºC, the death rate for the bacteria is higher in warm temperatures (Jang et al., 2017). Dissolved oxygen (DO) and pH were both positively correlated with temperature. Higher temperatures can promote photosynthetic activities by algae and the release of DO. Dissolved oxygen (DO) also contributed to 20% of the variance in the bacterial parameters. High FIB values lead to the increased consumption of DO in water and a reduction in DO levels (Sadowsky & Whitman, 2011). Some studies have shown that the best pH levels for *E. coli* are between 6 and 8, though elevated levels of *E. coli* were observed at lower pH values. High pH values or an acidic environment reduce the survival of *E. coli* (Sampson et al., 2006).

Clostridium perfringens, faecal coliforms and total coliforms were positively correlated with suspended solids and turbidity, indicating a linear relationship with the suspended sediments. The linear relationship suggests turbidity could be a proxy for water quality and a possible warning of pollution. FIB have a tendency to adsorb on suspended sediments or particles – the higher the particulate content the greater the increase in bacterial content. Additionally, turbid water reduces solar radiation and increases the survival of bacteria; reducing the bacteria die-off rate leads to a rise in FIB levels. The distribution of faecal pollution, namely, *C. perfringens* spores, was found to be associated with suspended sediments in the near-shore waters of Lake Michigan (Mueller-Spitz et al., 2010).

The NLWQS recommended levels of *E. coli* and enterococci are 100 cfu100 ml^{-1} and 33 cfu100 ml^{-1}, respectively. The level for *E. coli* was based on the values set in the Putrajaya Lake Water Quality Standard (Majizat et al., 2016), which provided a good indicator for monitoring fresh faecal contamination in the lake due to the short survival time. The criteria for enterococci were based on United States Environmental Protection Agency criteria from established work on the relationship between enterococci density and the gastrointestinal illness rate among bathers on freshwater beaches in the US (United States Environmental Protection Agency [USEPA], 2012). The use of both *E. coli* and enterococci in the water standards ensures the protection of human health through the detection and the control of FIB. Additionally, *C. perfringens* spores are some of the best available parameters for indicating potential remote pollution due to their ability to resist environmental stress. In this study, *C. perfringens* concentrations were observed in all lakes and ranged from 5 to 330 cfu100 ml^{-1}. The highest values of *C. perfringens* were found in L6, which is the furthest downstream of the Klang river basin lakes, indicating possible long-term depositions. Byamukama et al. (2005) showed that the combination of *E. coli* and *C. perfringens* spores in current use formed a good basis for detecting both recent and remote faecal pollution events in environments with low physical turnover, such as lakes and ponds. Our work has shown variation in the *E. coli* and *C. perfringens* concentrations in different lakes, with significant correlation of the two species. Both bacteria were strongly associated with pathogenic and faecal contamination (Rodrigues & Cunha, 2017) and selecting both parameters can provide a useful indicator of faecal pollution in waterbodies (Byamukama et al., 2005). Monitoring both *E. coli* and *C. perfringens,* will enable a better understanding of the short-term and long-term impacts of faecal contamination in waterbodies and microbial persistence in the environment (Abia et al., 2015). This study found that faecal coliforms were highly correlated with *E. coli* and that total coliforms (supported by the rationale of the NLWQS recommendation of faecal coliforms as an optional monitoring parameter), were more useful for purposes of categorization (Sharip & Suratman, 2017). The use of *E. coli*, *C. perfringens* and enterococci was intended to increase public protection by ensuring that other biological indicators could be tested should one parameter fail to be detected. These FIBs can also be used as surrogate indicators of other pathogenic microorganisms, such as *Cryptosporidium* and *Giardia* (Brookes et al., 2005). The use of multiple bacterial indicators will increase reliability when assessing the risk of microbial contamination in waterbodies used for recreation and drinking water.

CONCLUSIONS

Overall, all urban ponds and lakes had significant microbial contamination, possibly of human origin. Among the microbial parameters, only *C. perfringens* significantly differed between seasons and according to urbanization impact. *Escherichia coli* values were significantly higher in a few waterbodies during the wet season. No correlation was observed between microbial quality and surface area or shoreline length. Temperature, dissolved oxygen (DO), total phosphorus (TP) and transparency are some of the parameters that are associated with the bacterial parameters. Further work is needed to control faecal pollution in ponds and to improve lake water quality before the lakes can be used for direct body-contact recreational purposes. In addition, the proposal to extract water stored in these waterbodies for use as an alternative water supply must consider the spatial and temporal variation in the bacterial content and the water quality of the waterbodies, to ensure cost-effective treatment technologies.

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