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Microbiological Study in Coastal Water of Port Dickson, Malaysia

(Kajian Mikrobiologi Air di Pesisiran Pantai Port Dickson, Malaysia)

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ABSTRACT

The microbial composition in coastal water of the Port Dickson beach in Negeri Sembilan, Malaysia was analyzed using several microbial indicators for the purpose of selecting the best indicator for marine water pollution. The indicators studied were total coliform (TC), fecal coliform (FC), fecal streptococci (FS) and coliphage. Five locations were selected along the Port Dickson beaches and samplings were carried out in 1998 and 2001. The results showed an increase in the number of total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) between these two sampling by 98.12%, 86.12% and 99%, respectively. The numbers of TC, FC and FS exceeded the recommended limit for recreational seawater based on U.S. EPA 1986 standard. There was a positive correlation between TC, FC and FS and negative to coliphages.

Keywords: Coliphages; fecal coliform; fecal indicator; fecal streptococci; total coliform

ABSTRAK

Komposisi mikrob dalam air pesisiran pantai Port Dickson, Negeri Sembilan, Malaysia telah dianalisis dengan menggunakan beberapa penanda mikrob yang bertujuan untuk memilih penunjuk terbaik untuk pencemaran air laut. Penunjuk yang dikaji adalah koliform jumlah (TC), koliform tinja (FC), streptokokus tinja (FS) dan kolifaj. Lima lokasi dipilih di sepanjang pantai Port Dickson dan persampelan dijalankan pada tahun 1998 dan 2001. Keputusan menunjukkan peningkatan dalam bilangan koliform jumlah, koliform tinja dan streptokokus tinja di antara dua persampelan dengan nilai masing-masing 98.12%, 86.12% dan 99%. Nilai TC, FC dan FS adalah melebihi had yang dicadangkan untuk air laut rekreasi berdasarkan piawaian U.S. EPA 1986. Keputusan menunjukkan terdapat korelasi positif antara TC, FC dan FS dan korelasi negatif terhadap kolifaj.

Kata kunci: Kolifaj; koliform jumlah; koliform tinja; penunjuk tinja; streptokokus tinja

INTRODUCTION

In Malaysia, the coastline of the Straits of Malacca and the South China Sea are public lands under the administration of local authorities. Unfortunately, many of these fine beaches are not monitored by the local authorities to protect them from pollution due to rapid development. With increasing coastal populations, the discharge of wastewater, both the treated or untreated sewage has increased tremendously. In the case of Port Dickson beaches, there are eighty two (82) wastewater pipe lines discharge wastewater including sewage from hotels and houses directly into the sea (Kadaruddin 1997). These discharges lead to degradation of the marine water quality causing significant negative impacts on marine ecosystem in general and mangrove plants in particular. Herbicide, oil spill and water pollution can cause great damage to mangroves which are susceptible to pollution (Saintilan & Williams, 1999). Surveys conducted by the Malaysian Department of Environment found that majority of the coastal waters were polluted by suspended solids, *E. coli,* and oil and grease (DOE 2006).

Indicator bacteria, including total coliform (TC), fecal coliform (FC) and fecal streptococci (FS), have been used and accepted in water quality studies to assess the level of fecal contamination in water bodies (Garrido-Perez et al. 2008). The presence of these organisms has also been used to estimate the potential human health risks of other pathogenic organisms of fecal origin. On the other hand, these indicator bacteria do not provide information on the presence of enteric viruses in the aquatic environment; therefore, somatic coliphages have been proposed as an alternative pollution indicator for the presence of enteric viruses. Coliphages are viruses that infect and replicate in coliform bacteria and are not pathogenic to humans. Mose-Livina et al. (2005) showed that the numbers of enterococci and somatic coliphages predict the numbers of enteroviruses more accurately than total coliform or fecal coliform in bathing waters in Spain. Brezina and Baldini (2008) proposed *E. coli* ATCC 13706 somatic coliphages as a complimentary tool to diagnose the level of fecal contamination in estuarine waters with low pollution levels.

Therefore, the objective of this study was to evaluate the level of fecal pollution at Port Dickson beaches using several microbial indicators i.e total coliform (TC), fecal coliform (FC), fecal streptococci (FS) and coliphages to identify the best indicator for fecal pollution.

MATERIALS AND METHODS

SITES AND SAMPLING

Four sampling stations were located along the 54 km Port Dickson beaches; they were Saujana beach (A), Kemang Indah beach (B), Teluk Kemang public beach (C) and Blue Lagoon beach (D) (Figure 1). The distance between each station was between 200-300 m. Water samples were collected five times at each station between from October 2000 to March 2001. Water samples were collected at one meter from the wash zone of the beach (the zone that is in contact with the outer fringes of the water between low and high tide) at a depth of 20 cm using 1 L sterile Scott bottles. All the water samples were collected at the same locations starting from 9 am to 12 noon. The samples were stored in ice box during transportation and analyzed within 5 h of collection.

Measurement of physical parameters The physical parameters such as pH, water temperature, turbidity and dissolved oxygen (DO) were measured *in situ*. Temperature and DO were measured using YSL temperature and dissolved oxygen meter (Yellow Springs Instrument Co. Inc.), while pH and turbidity were measured using pH and turbidity meter (ELE International Eastman Way, England).

Microbiological analysis The analyses of total coliform (TC), fecal coliform (FC) and fecal streptococci (FS) were carried out by using membrane filtration procedure as described in APHA (1992) and Hamzah et al. (1997). A specific strain of *E. coli* C (ATCC No. 13706) was used as the host for the coliphages. The coliphages were enumerated using the double-agar-layer technique according to APHA (1992). The plaque formations were counted and expressed as plaque forming units (pfu) per 100 mL of water sample.

Data analysis The statistical analysis was performed using Minitab software. Correlations tests were used when analyzing the data between fecal indicators while ANOVA and Turkey's tests were used to compare the

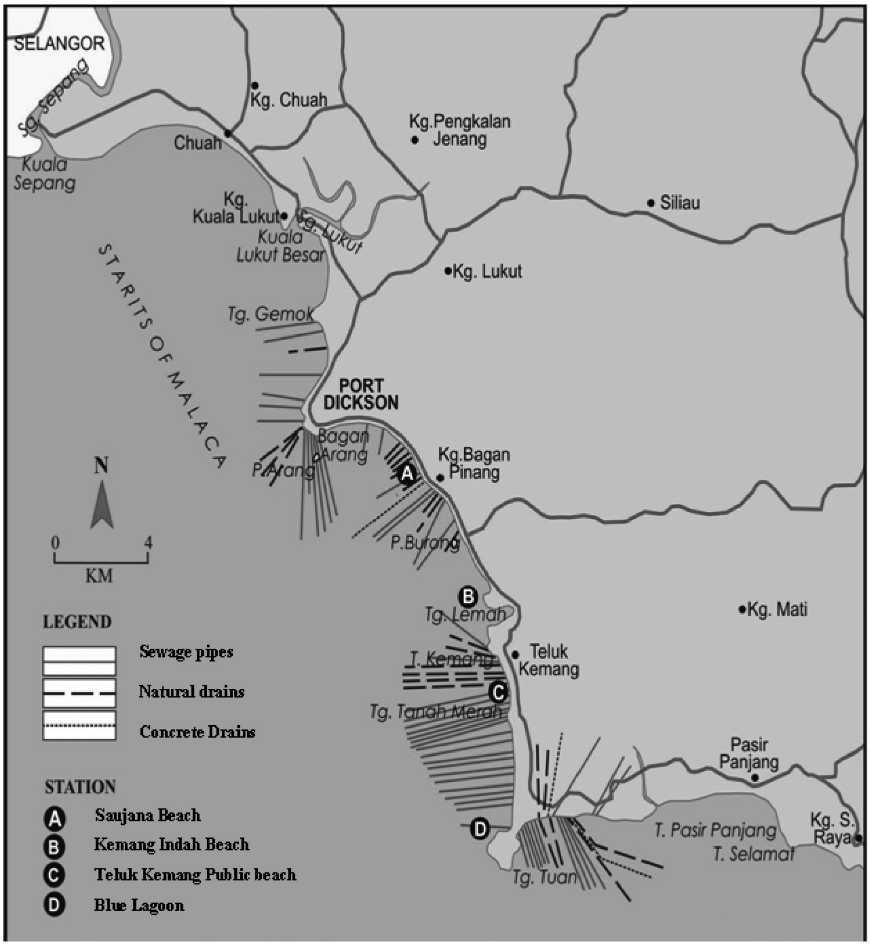


FIGURE 1. Sampling location and sewage pipes that contain contaminants along Port Dickson beaches

results between sites. All the tests were performed at a 95% confidence level.

RESULTS

PHYSICAL PARAMETERS

The water temperature from the four sites i.e Saujana (A), Kemang Indah (B), Teluk Kemang (C) and Blue Lagoon (D), were from 18.3 to 33.5°C (Table 1). The variation in water temperature was mainly a due to prevailing weather condition which was reflected in the statistical analysis which showed that there was no significant difference between locations (p≥0.05).

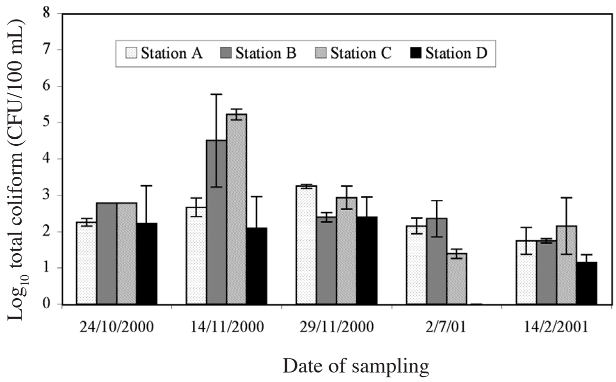
The lowest dissolved oxygen (DO) value recorded was 3.70 mg/L at Kemang Indah beach (B) while the highest was 8.14 mg/L at Teluk Kemang (C) beach (Table 1). Generally, the dissolved oxygen will be affected by water temperature, tides and water depths. There was no significant difference in pH for all sampling sites; it ranged between 7.59 to 7.79. The standard pH for seawater is 6.5-8.5 (DOE 2006) and the values obtained from these beaches were within the recommended standard. The standard value for turbidity for seawater is 25 NTU (U.S. EPA 1986). The turbidity values varied greatly from 6.05 to 32.95 NTU, but in most occasions (99.99%) the values were below the recommended standards. Turbidity is the indirect measurement of particulates suspended in water, which can originate from storm water runoff and/or marine sediment resuspension by near shore tides.

Bacteriological parameters The number of total coliform at all station was in the range of 2.5×10^2 to 1.5×10^6 cfu/100 mL (Figure 2a). The number of fecal coliform (FC) or *E. coli*, (Figure 2b) was between from 1.5×10^2 to 2×10^4 cfu/100 mL. The number of *E. coli* was generally stable within the two year period, but it exceeded the 100 MPN/100 mL limit for recreational use with body contact set by the Department of Environmental Malaysia (DOE 2006). Fecal streptococci were included as an additional bacteriological parameter for marine water. The numbers of fecal streptococci at all stations were high, ranging from 1.5×10^3 to 2.5×10^6 cfu/100 mL (Figure 2c), which was above 35 cfu/100 mL limit for recreational used in marine water (U.S. EPA 1986). The levels of coliphage were low in the range of 4-35 pfu/ 100 mL (Table 2). Only the Kemang Indah beach (B) showed a higher count of coliphage at an average of 34.8 pfu/100 mL.

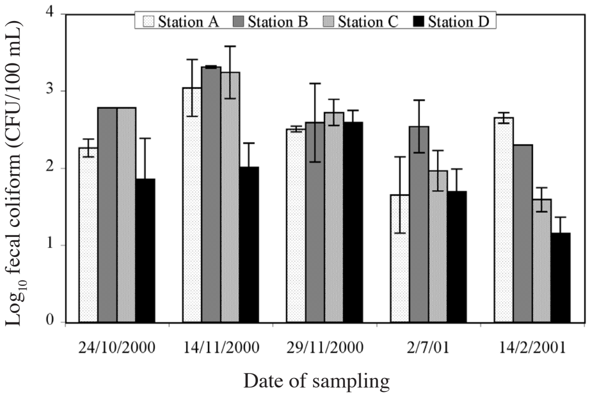
TABLE 1. The average value of water temperature, pH, dissolved oxygen and turbidity at four sampling stations, Saujana (A), Kemang Indah (B), Public Teluk Kemang (C) and Blue Lagoon (D) beaches

Date of sampling	Parameters	Beaches			
		A	B	C	D
24/10/2000	Temperature (^{0}C)	21.00 ± 0.99	23.30 ± 0.28	33.50 ± 0.14	28.90 ± 0.99
	pH	7.65 ± 0.03	7.71 ± 0.01	7.79 ± 0.07	7.78 ±0.02
	DO (mg/L)	7.77 ± 0.49	6.87 ± 0.61	6.22 ± 0.38	7.69 ± 0.69
	Turbidity (NTU)	10.25 ± 1.20	16.35 ± 0.21	10.85 ± 0.49	9.05 ± 0.21
14/11/2000	Temperature (^{0}C)	24.35 ± 0.21	28.30 ± 0.14	31.55 ± 0.21	32.40 ±0.14
	pH	7.76 ± 0.14	7.77 ± 0.02	7.79 ± 0.01	7.75 ± 0.07
	DO (mg/L)	7.17 ± 1.33	6.87 ±0.61	6.22 ± 0.38	7.69 ± 0.69
	Turbidity (NTU)	13.20 ± 0.99	20.35 ± 0.92	8.05 ± 0.92	11.95 ± 0.49
29/11/2000	Temperature (^{0}C)	18.90 ± 0.85	24.45 ± 0.49	27.05 ± 2.19	27.35 ±0.07
	pH	7.59 ± 0.04	7.64 ± 0.01	7.68 ± 0.14	7.80 ±0.02
	DO (mg/L)	5.54 ± 0.38	5.70 ± 0.32	6.47 ± 0.34	6.86 ±0.88
	Turbidity (NTU)	15.85 ± 1.77	32.95 ± 0.78	12.10 ± 0.42	6.05 ± 0.21
7/2/2001	Temperature (^{0}C)	29.70 ± 2.26	28.70 ± 0.71	25.95 ± 0.78	24.95 ±6.01
	pH	7.61 ± 0.02	7.72 ± 0.03	7.70 ± 0.01	7.66 ± 0.04
	DO (mg/L)	6.61 ± 0.31	6.68 ± 0.64	8.14 ± 0.47	6.01 ±0.87
	Turbidity (NTU)	19.95 ± 0.78	20.10 ± 0.99	13.90 ± 0.28	8.25 ± 0.21
14/2/2001	Temperature (^{0}C)	20.90 ± 0.57	23.35 ± 0.35	27.65 ± 0.78	31.85 ± 2.33
	pH	7.61 ± 0.04	7.76 ± 0.02	7.65 ±0.04	7.71 ± 0.01
	DO (mg/L)	6.06 ± 0.59	3.70 ± 0.75	5.12 ± 0.31	6.66 ± 0.45
	Turbidity (NTU)	17.90 ± 0.42	16.70 ± 0.28	13.05 ± 0.35	9.05 ± 0.49
28/3/2001	Temperature (^{0}C)	21.85 ± 0.64	23.55 ± 0.07	33.25 ± 0.07	28.90 ± 0.85
	pH	7.65 ± 0.03	7.71 ± 0.01	7.79 ± 0.07	7.78 ± 0.02
	DO (mg/L)	6.69 ± 1.04	7.53 ± 1.40	6.92 ± 0.6	7.28 ± 1.19
	Turbidity (NTU)	14.05 ± 0.64	19.85 ± 1.91	12.00 ± 0.71	8.00 ± 0.71

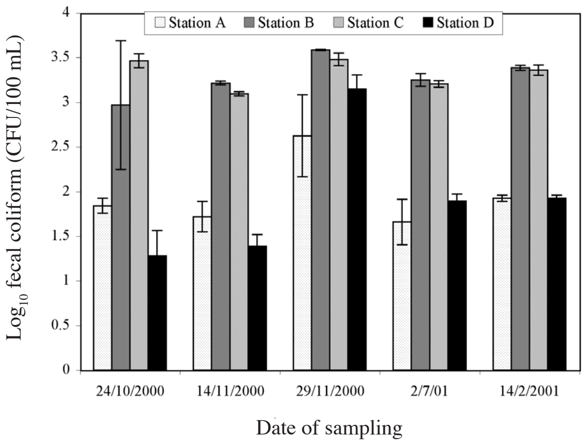
DO: dissolved oxygen; Beaches: A: Saujana; B: Kemang Indah; C: Teluk Kemang; D: Blue Lagoon; Standard turbidity 25 NTU (U.S. EPA 1986).



(a)



(b)



(c)

FIGURE 2. The number of (a) total coliform (TC), (b) fecal coliform (FC) and (c) fecal streptoccocci (FS) in phase 2 sampling. Sampling was done six times at Saujana beach (A), Kemang Indah (B), Teluk Kemang public beach (C) and Blue Lagoon (D)

TABLE 2. The average number of coliphages. Sampling was done five times at Saujana beach (A), Kemang Indah (B), Teluk Kemang public beach (C) and Blue Lagoon (D)

No. of sampling	Location / number of coliphage (PFU/100 mL)			
	A	B	C	D
14/11/ 2000	62	28	11	3
29/11/2000	9	9	3	1
7/2/2001	16	22	11	6
14/2/2001	14	23	11	10
28/3/2001	4	92	11	0
Average	21.0	34.8	9.4	4.0

PFU: plaque forming unit
Location: A: Saujana; B: Kemang Indah; C: Teluk Kemang; D: Blue Lagoon

DISCUSSION

The physical parameters were measured to determine water quality of coastal water which would influence the microbial growth in the marine water. Water temperature can cause bacterial mortality and rate of its multiplication. According to Solic and Krstulovic (1992) increase in temperature and salinity was more detrimental to fecal coliform survival in the presence of sunlight. The range of temperatures recorded in this study was the normal ambient temperatures for a tropical climate.

pH has a direct impact on the recreational users of water only at very low or very high values. Under these circumstances, pH may have effects on the skin and eyes (WHO 2003). According to Hernendez-Delgado and Toranzos (1995) the optimum pH for coliform growth is pH 6-7, while for coliphages the optimum is pH 7.0-7.7. The pH recorded in this study was in the range of 7.57-7.79 except on occasions when the tide was low where the pH was less than pH 7. Generally, the pH readings for the Port Dickson beaches were suitable for both coliform and coliphages growth.

Dissolved oxygen will influence microbial activity and the chemical oxidation state of various metals. Sampling during low tide may reduce the value of dissolve oxygen as stated by Mill et al. (2006) who did a study in an estuarine creek in Australia which showed that the readings for temperature, pH, salinity, conductivity and dissolved oxygen were lower during the low (ebb) tide than those taken during the flood (high) tide. A study carried out by Kadaruddin (1997) reported that the dissolve oxygen values at Teluk Kemang (C) and Blue Lagoon (D) were 5.5 mg/L and 5.2 mg/L, respectively. When compared to the current study, the dissolve oxygen values at Teluk Kemang (C) and Blue Lagoon (D) have increased, and this showed the water quality at these sites has been improved.

The turbidity at Kemang Indah beach (B) was higher due to the shoreline erosion in this area, together with the dissolved organic matter from the sewage outfalls coming from the sewage pipe installed at this location. Turbidity also resulted in inability of sunlight to pass through the water, which can lead to suitable conditions for the coliform and coliphage to grow. Blue Lagoon beach (D) always showed lower value in turbidity because its position in a lagoon protected from strong current in mixing the sediment. In a study on New South Wales estuaries, turbidity proved informative as an indicator, and was linked to nutrient loads and to the percentage of the catchment under urbanisation (Scanes et al. 2007).

In term of bacteriological loads, beaches at Port Dickson were polluted based on numbers of total coliform (TC), fecal coliform (FC), fecal streptococci (FS) and also coliphages. There were not many studies on microbiological loads in coastal water at Malaysia. A study done by Kadaruddin (1997) found that the numbers of total coliform at Teluk Kemang and Blue Lagoon were in the range of 2 to 0 MPN/100 mL. The most probable number (MPN) value is not as accurate as the colony forming unit value determined by the membrane filtration method. The guidelines for recreational marine water recommended or implemented by various US governmental agencies was at geometric mean of 1000/100 mL of total coliform (U.S. EPA 1986). From this study showed the number of total coliform at all sampling points at Port Dickson beaches exceeded the U.S. EPA limit. Furthermore, there is a positive and strong correlation between total coliform and fecal coliform (R value, 0.75) at all sampling sites. Unfortunately, there is still no total coliform and fecal coliform standard for bathing water in Malaysia to be used as comparison in this study.

According to the Malaysian Interim Marine Water Quality Standard (IMWQS) (DOE 2006), the acceptable *E. coli* count is 100 MPN/100 mL or 200 cfu/100 mL by U.S. EPA guidelines (U.S. EPA 1986). Based on this standard, none of the seawater at these locations is safe for body contact. A study done by Kadaruddin in 1997 stated that the number of fecal coliform at Blue Lagoon was at 141 MPN/100 mL, and 1950 MPN/100 mL at Teluk Kemang; both values exceeded the IMWQS standards. Countries such as United States, Phillipines and Canada introduced standards for *E. coli* for recreational seawater at maximum geometric mean of 200 counts/100 mL water, while Australia and Hong Kong at 150 and 180 counts/100 mL, respectively (Wang 1999). The evaluation by the U.S. EPA of the bacteriological data indicated that using fecal coliform count at 200 counts/100 mL would cause an estimated 19 illnesses per 1,000 swimmers at marine beaches (U.S. EPA 1986).

In addition to fecal coliform and total coliform, fecal streptococci is also used as indicators of bathing water quality by United States and European Union legislative schemes especially in seawater, because enterococci are more resistant to physico-chemical and environmental stress than *E. coli* (U.S. EPA 1986; EU 2006). The current standard for marine waters for recreational purposes/ bathing waters, the standard set by US Environmental Protection Agency (U.S. EPA 1986), is 35 cfu of enterococcus /100 mL while according to WHO Standards (2003) for category B water the intestinal enterococci was ≤40 per 100 mL water (95th percentile). Based on this standard, all of the public beaches at Port Dickson were not suitable for body contact purposes. The high number of fecal streptococci is related to soil erosion, which can cause a high turbidity of the water. Sunlight will reduce the activity of fecal streptococci survival (Robert et al. 1994) and in this study, the number of fecal streptococci increased proportionally with turbidity. According to Fujioka et al. (1981), the presence of sunlight is a major factor controlling the survival of fecal coliforms and fecal streptococci in seawater. Fecal streptococci also have a high tolerance to the high salinity of seawater and it gave good reason to include fecal streptococci as marine water indicator for fecal pollution. Being a tropical county which received sunlight all year round, the effect of sunlight as killing factor for *E. coli* and fecal streptococci are more prominent.

Another biological indicator used in this study was coliphage levels. Coliphage has been suggested as an alternative indicator for fecal pollution in seawater (Havelaar et al. 1990). The advantage of using coliphage is that it is a good indicator of enteroviruses due to similar seasonal variations, propensities for removal and resistances to environmental stress (WHO 1999; 2003). The incremental increase in the numbers of coliphages was caused by the surrounding conditions, including improper sewage pipe management, beach conditions (deep or shallow), weather and water tides (which diluted the coliphages colonies). Coliphages have been shown to have a close correlation with the presence of pathogenic microorganisms, and with the fecal pollution levels of recreational seawaters. There is still no specific numbers of coliphage in the any guidelines except Dionisio et al. (2002) suggested for bathing waters the level of coliphage Ph_{80}= 300 pfu/100 mL and Ph_{95}= 2000 pfu/100 mL. In this study, the number of coliphage at all sites of Port Dickson beaches was below 100 pfu/100 mL. In this study, there is a weak correlation between fecal coliform and fecal streptococci (R value, 0.41) and a negative correlation between fecal coliform and coliphages. However, Brezina and Baldini (2008) found a high correlation between coliphages and *E. coli* in estuarine waters. On the other hand, Elmanama et al. (2005) found a significant correlation between fecal coliform and streptococci and between *Salmonella* and *Vibrio* at Gaza beach contaminated with sewage outfall. The variation of results may exist; depend of location of study, source of pollutants, climate changes, topography of the beach, etc.

This study showed that all the bacteriological parameters, total coliform, fecal coliform, and fecal streptococci showed counts above the recommended standards for seawater for recreational purposes. The presence of these fecal indicators was due to the existence of many sewage outfalls along the beaches, which carried effluent from domestic dwellings and hotels. At some sampling locations, the garbage disposal area was located on the beach. All of these factors contributed to the presence of high fecal bacteria in the water. The Strait of Malacca is among the busiest waterways and many ships and tankers do their cleaning at the sea; this oil contaminated wastewater is brought up to the beach during high tide. The high total coliform count is also influenced by temperature since the best growth temperature for coliform bacteria is around 30^{o}C, which is equivalent to the temperature of tropical seawater. According to Banat et al. (1998) the number of total coliform was highest when the temperature was at 30^{o}C, at around 650/100 mL, while in a study by Galindo et al. (1997), the level was 230 MPN/100 mL. On the other hand, coliforms were more rapidly inactivated in sunlight than in the dark. The solar radiation was found to be the most significant factor affecting the mortality of coliform bacteria (Yukselen et al. 2003).

Conclusion

For marine water quality, total coliform, fecal coliform, fecal streptococci except coliphages can be used as pollution indicators especially for public places like beaches, but total coliform and fecal coliform are better indicators than fecal streptococci and coliphages for marine waters. This study demonstrated that Port Dickson beaches were not safe for human activities with body contact such as swimming. The rank order in terms of level of pollution at Port Dickson are the Kemang Indah Beach (B) > the Teluk Kemang Public Beach (C) > the Blue Lagoon (D) > the Saujana Beach (A).

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