

## Distribution of Linear Alkylbenzenes (LABs) in Sediments of Sarawak and Sembulan Rivers, Malaysia

Sami Muhsen Magam<sup>a</sup>, Mohamad Pauzi Zakaria<sup>a\*</sup>, Normala Halimoon<sup>b</sup>,  
Najat Masood<sup>a</sup>, Murad Ali Alsalahi<sup>c</sup>

<sup>a</sup> Center of Excellence for Environmental Forensics, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

<sup>b</sup> Department of Environmental Science, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Malaysia

<sup>c</sup> School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

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### Abstract

The current study is one of the first studies evaluating the levels of linear alkylbenzenes (LABs) in surface sediments of Sarawak and Sembulan rivers which are located in the east coast of Malaysia. The LABs, which are molecular tracers of sewage contamination, were measured in 15 surface sediment samples collected from these rivers. The samples were extracted, fractioned and analyzed by gas chromatography mass spectrometry (GC-MS). The findings revealed that the concentrations of  $\Sigma$ LABs ranged from 156.47 to 7386.19 ng/g dry weight (dw) in the sediments of Sarawak River and from 643.18 to 5567.12 ng/g dw in the sediments of Sembulan River. The highest LABs levels were detected in the sediments collected from the sampling location SS9 in Sembulan River whereas the lowest levels were observed in the SS1 sampling location in Sarawak River. The *I/E* ratios (ratio of internal to external isomers of LABs) for Sarawak River sediments ranged from 0.52 to 0.98 while for Sembulan River they fell within the range 0.87-1.79. The *I/E* ratio at the sampling station SS4 was much lower than the *I/E* ratios at the other stations, thus indicating that the wastewater discharged into Sarawak River from the areas surrounding station SS4 was poorly treated.

**Keywords:** LABs; molecular tracers; surface sediment; Sarawak River; Sembulan River; *I/E* ratio

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### 1. Introduction

Over the past few decades, Malaysia has been rapidly heading towards being a manufacturing country. Lately, there has been a growing concern over the possible harmful effects of industrialization-related pollutants, which are steadily released into the aquatic environment, on the living aquatic organisms. Human activities concomitant to urban and industrial development produce varying types and amounts of contaminants, especially untreated municipal wastewater, into the marine environment. This, besides urban runoff, resulted in growing levels of marine pollution.

Sarawak River in Kuching and Sembulan River in Kota Kinabalu are located in the eastern part of Malaysia and are considered as two of the most important rivers in the country. Rapid population growth, land development, urbanization, and industrialization within the river basins have subjected them to increasing stress which gave rise to environmental pollution and water quality deterioration. Densely-populated cities stretch

along the coastlines of the two rivers and are expected to be, directly and indirectly, releasing bulky volumes of sewage and domestic wastewater into these rivers. Besides these, tourism, which is one of the prominent human activities in these cities, is thought of as a potential source of water pollution in the study areas. Consequently, sewage pollution is a severe health risk to people living near these rivers and the associated waterways. Direct discharge of domestic waste, poorly maintained septic tanks, and improper management of farm wastes are suspected to be the major sources of water-borne diseases.

The LABs with C<sub>10</sub>-C<sub>14</sub> normal alkyl chains have been used in production of linear alkylbenzene sulfonates (LASs) which are one of the most widely used anionic surfactant groups in the detergents industry since the 1960s. As a result, it is presumed that small amounts of LABs are likely contained in LAS-type detergents. Hence, use of these detergents and subsequent disposal of them into the environment brings LABs into the river systems (Eganhouse and Ruth,

1983; Takada and Ishiwatari, 1987). The LABs have been widely associated with monitoring sewage inputs to aquatic environments due to their high resistance to microbial degradation (Takada and Ishiwatari, 1987; 1991). They proved to be more resistant to microbial degradation than the LASs (Takada and Ishiwatari, 1987; 1991). In addition, the LABs are characterized by being hydrophobic. In consequence, they are normally associated with particulate matter in sewage and in the water column and are therefore readily integrated into the bottom sediments. The LABs are made up of isomers with different phenyl-substitution positions on the alkyl chains and are broadly classified as external and internal isomers. The external isomers are characterized by that the phenyl groups are located near the terminal end of the alkyl chain and hence they are more susceptible to aerobic microbial degradation than the internal isomers which have the phenyl groups positioned close to the center of the alkyl chain. In light of this, it has been pointed that the isomeric distribution of LABs provides information indicative of their susceptibility to biodeg-

radation (Takada and Ishiwatari, 1990). Furthermore, as indicated by Tsutsumi and Yamaguchi (2002), the isomeric distribution of LABs can provide information on the various kinds of sewage discharged into the aquatic environment (e.g., raw sewage or secondary effluent). So, it is essential to characterize, through LAB analysis, the types of sewage/wastewater inputs to the river(s) of interest for the design and efficient operation of sewage treatment systems.

The LABs have been studied by several researchers in many countries. However, investigations of the levels and distribution patterns of LABs in the east Malaysian rivers are still limited. Therefore, this paper presents findings of a study conducted at selected coastal areas in Kuching and Kota Kinabalu in east Malaysia. The aim of the study was to assess the concentrations of LABs in the sediments of these rivers and to explore their distribution, with particular attention to an examination of the potential utilization of LABs as molecular biomarkers of sewage pollution in the aquatic environment.

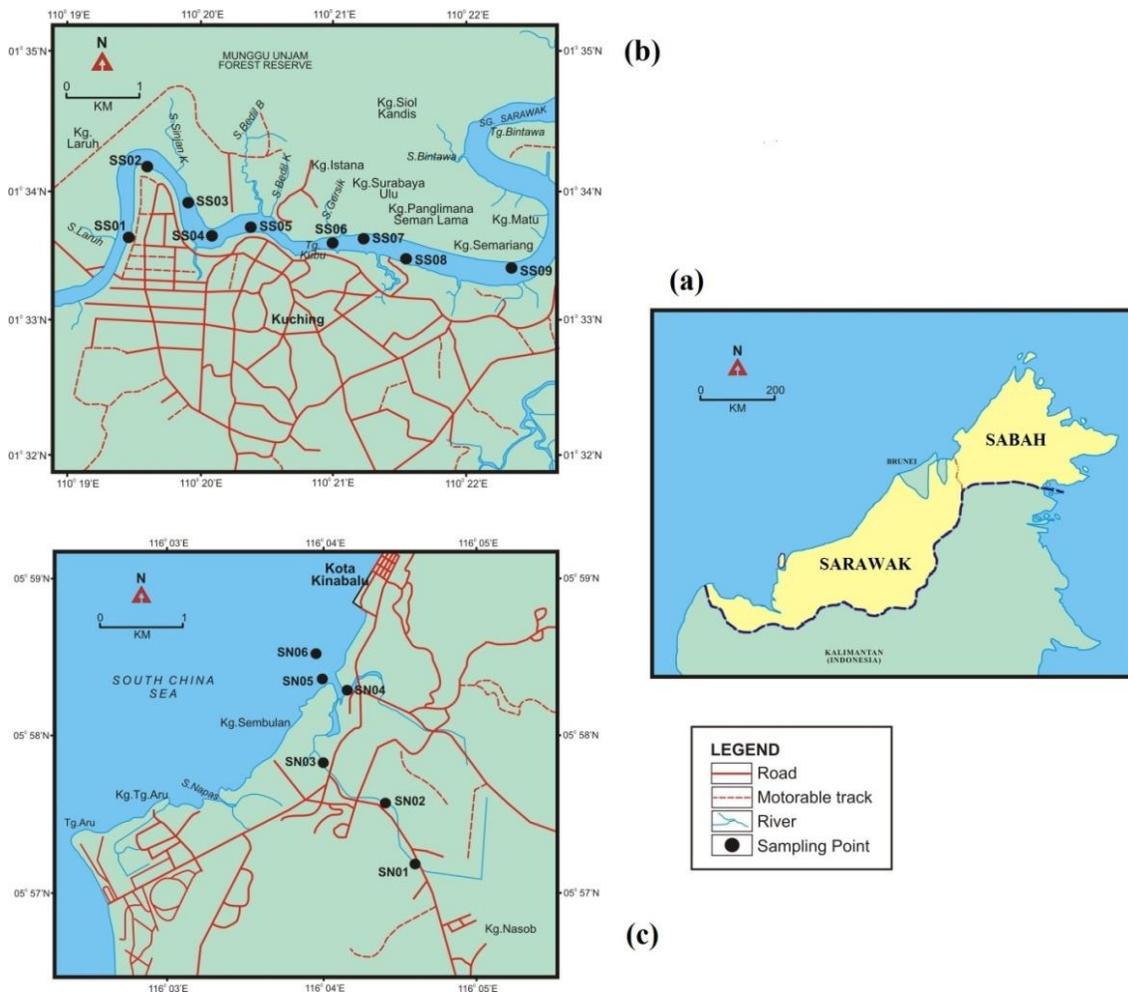


Figure 1. (a) General location of the study area, (b) Sarawak River, and (c) Sembulan River.

## 2. Materials and Methods

### 2.1. Sampling area and sample collection

The study areas are shown in Fig. 1. Fifteen surface sediment samples were collected during the period from December 2008 to May 2009, corresponding to nine surface sediment samples from the top 2-5 cm, representing recent inputs, from Sarawak River and six surface sediment samples from Sembulan River. The samples were kept in zip-lock bags, transported to the laboratory in an ice box and stored at -20°C for further analysis. Details on the sampling stations including sampling site code, GPS coordinates, sampling dates, dominant human activities, and weather and tidal conditions are provided in Table 1.

### 2.2. Reference Standards, Reagents and Materials

The standard mixtures of LABs used in this study contained isomers of C<sub>10</sub>-LABs, C<sub>11</sub>-LABs, C<sub>12</sub>-LABs, C<sub>13</sub>-LABs, and C<sub>14</sub>-LABs. The organic solvents methanol, acetone, n-hexane, isooctane, and dichloromethane (DCM) besides authentic standard solutions for LABs were purchased from Wako Pure Chemical (Japan), Chiron (Norway), and Sigma (US). The DCM, n-hexane, and isooctane were distilled before use. All glassware and metallic tools were washed with diluted detergent; thoroughly rinsed with tap water; cleaned with distilled water; rinsed with methanol, acetone,

and n-hexane (HPLC Grade) in the order listed; and then dried at 60°C for several hours. Activated silica gel was prepared by heating the reagent at 400°C for four hours, cooling, and deactivating with 5% (w/w) distilled H<sub>2</sub>O.

### 2.3. Analytical procedure

The surface sediment samples were homogenized and then about 20 g of each sample were dried with baked anhydrous sodium sulphate to remove any moisture, placed in cellulose thimbles, and soxhlet-extracted for 8-10 hours with distilled DCM. All the samples were spiked with 1-CnLAB mixture ( $n = 8-14$ ) in isooctane (50 µl) as an alkylbenzene surrogate internal standard. Volumes of the solvents were reduced to near dryness using a rotary evaporator. Few activated copper chips were added to the samples and left overnight. The extracts were then subjected to purification, fractionation, and instrumental analysis following the procedure established by Hartmann *et al.* (2000) and Zakaria *et al.* (2002). Briefly, the extracts were purified and fractionated using two-step silica gel column chromatography and the alkylbenzene fraction was determined by GC-MS in the selected ion monitoring mode at  $m/z = 91, 92$  and  $105$ . The limits of detection (LOD) varied from 0.3 to 1.0 µg/L for the different LABs based on a signal-to-noise ratio (S/N) of 3, and the limits of quantification (LOQ) varied from 2.5 to 8.3 µg/L at a S/N ratio of 10.

Table 1. Chief characteristics of the sampling sites in Sarawak and Sembulan Rivers

Station Code*	Longitude (E)	Latitude (N)	Location	Dominant Human Activities	Weather	Tide
SS 01	01 33. 837'	110 19. 641'	Kuching	Mangrove and port for fish used	Raing	Low
SS 02	01 34. 157'	110 19. 795'	Kuching	Tourism activity	Cloudy	Low
SS 03	01 33. 864'	110 20. 073'	Kuching	Hospital/Queen Elizabeth	Cloudy	Low
SS 04	01 33. 661'	110 20. 292'	Kuching	Across River apartment Kuching	Cloudy	Low
SS 05	01 33. 640'	110 20. 541'	Kuching	Tourism activity	Cloudy	Low
SS 06	01 33. 601'	110 20. 880'	Kuching	Hotels at Kuching city center	Cloudy	Low
SS 07	01 33. 566'	110 21. 067'	Kuching	Tourism activity	Cloudy	Low
SS 08	01 33. 490'	110 21. 552'	Kuching	Hotels at Kuching city center	Cloudy	Low
SS 09	01 33. 275'	110 22. 160'	Kuching	Across River apartment Kuching	Cloudy	Low
SN 01	05 57. 197'	116 04. 380'	Kota Kinabalu	small village and port for fish used	Partly cloudy	High
SN 02	05 57. 308'	116 04. 249'	Kota Kinabalu	Tourism activity	Partly cloudy	High
SN 03	05 57. 474'	116 04. 083'	Kota Kinabalu	Hotels at Sembulan city center	Partly cloudy	High
SN 04	05 58. 108'	116 04. 134'	Kota Kinabalu	Hospital and Tourism activity	Partly cloudy	High
SN 05	05 58. 210'	116 03. 562'	Kota Kinabalu	Estuary & Tourism activity	Partly cloudy	High
SN 06	05 58. 317'	116 03. 561'	Kota Kinabalu	In south china sea	Partly cloudy	High

\* The first letter stands for station, the second letters indicate Sarawak (S) and Sembulan (N) rivers, and the numbers refer to the sampling stations' sequential numbers.

### 3. Results and Discussion

As displayed in Fig. 2, the LABs consist of 26 congeners. The structures of individual congeners are expressed as n-CmAB where n indicates the phenyl substitution position on the alkyl chain and m indicates the number of alkyl carbons. The sum of the 26 congeners is represented as  $\Sigma$ LABs. In some samples, tetrapropylene-based alkylbenzenes (TABs) were superimposed on the C<sub>10</sub> and C<sub>11</sub> congeners in the chromatograms, therefore preventing reliable determination of  $\Sigma$ LABs. In these cases, the sum of C<sub>12</sub>, C<sub>13</sub>, and C<sub>14</sub> homologues ( $\Sigma$ C<sub>12</sub>-C<sub>14</sub> LABs) was used for comparing the concentrations of LABs between the samples.

According to the findings of the present study, the concentrations of  $\Sigma$ LABs in ng/g dw in the surface sediment samples of Sarawak River and Sembulan River ranged from 156.47 to 7386.19 and from 643.18 to 5567.12 ng/g dw, respectively (Table 2). The highest levels of LABs were found in the sediments collected from the sampling station SS9 in Sarawak River

whereas the lowest LABs levels were detected in the sampling station SS1 in Sarawak River. Based on the I/E ratios obtained, the highest levels of LABs were associated with the sampling sites SS8 and SS9 in Sarawak River where the sediments were accumulating LABs from raw sewage inputs to the river. These sampling points were located in the centres of residential areas characterized with high population densities. The site SS7, which belongs to Sarawak River as well, showed a relatively high concentration of  $\Sigma$ LABs; 5051.15 ng/g dw. High concentrations of LABs in this site are explained by that this sampling location is close to the sampling stations of highest LABs levels. On the other hand, Fig. 3 reveals that the concentrations of LABs in the sediments of Sarawak River increased from upstream to downstream. However, the LAB concentrations in SS5 and SS6 decreased because of presence of sewage treatment plants in their neighborhood. It can hence be inferred that the sewage treatment plants around SS5 and SS6 were effective in treating the domestic wastewater they used to receive and thus

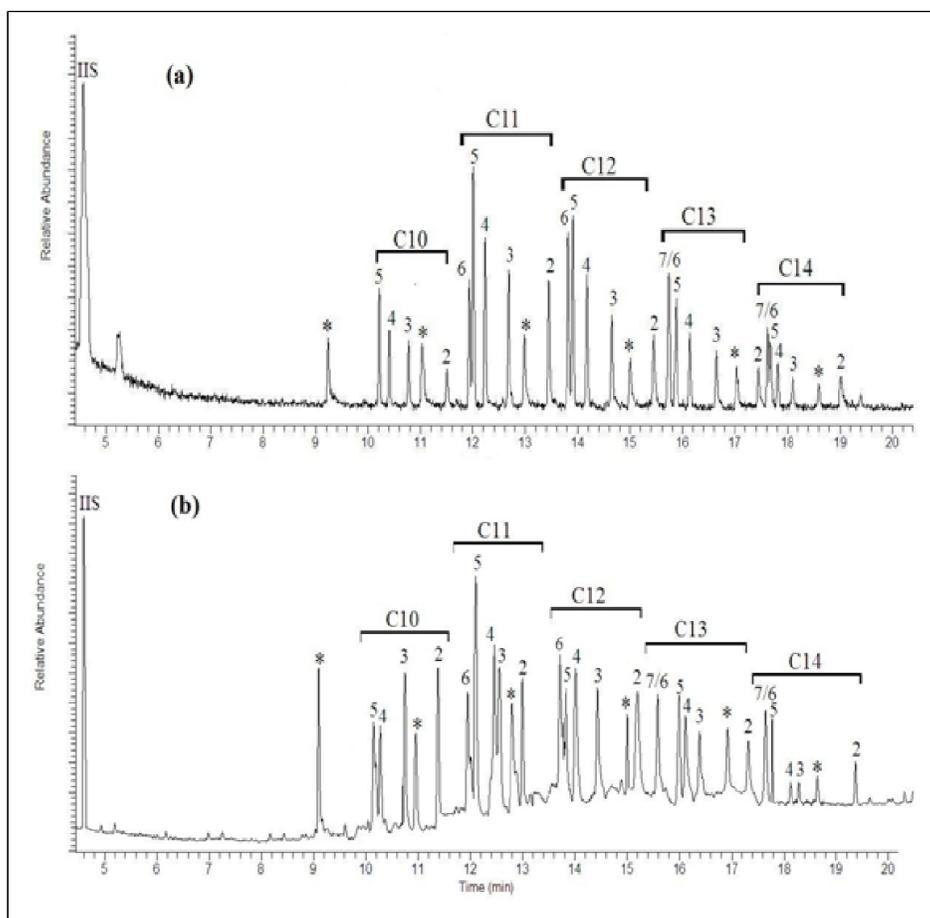


Figure 2. Gas chromatogram of alkylbenzenes in sediments collected from (a) Sarawak River, and (b) Sembulan River. IIS (internal injection standard, *biphenyl-d*<sub>10</sub>) was monitored at *m/z* = 164. Surrogates (1-C<sub>m</sub>, *m*: 8-14; from left to right) indicated by asterisks were partially erased during figure processing. Subscripts denote the alkyl chain length. Numbers on the peaks indicate the positions of phenyl substitution on the alkyl chain.

Table 2. The  $\Sigma$ LABs concentrations in the sediments of Sarawak and Sembulan rivers.

River	Station No	Wet Weight (g)	Final Dry Weight (g)	Dry Weight (%)	Moisture Content (%)	$\Sigma$ LABs Concentration ng/g-dry <sup>a</sup>	<sup>b</sup> I/E Ratio
Sarawak River	SS 01	20.02	12.17	60.79	39.21	156.47	cND
	SS 02	20.03	8.43	42.13	57.87	1333.38	0.86
	SS 03	20.00	8.94	44.69	55.31	3228.95	0.79
	SS 04	20.01	10.08	50.39	49.61	4010.75	0.52
	SS 05	20.01	8.12	40.58	59.42	3356.07	0.97
	SS 06	20.01	11.63	58.15	41.85	3852.09	0.90
	SS 07	20.00	10.94	54.68	45.32	5051.15	0.98
	SS 08	20.09	9.70	48.51	51.49	6929.73	0.85
	SS 09	20.02	14.55	72.68	27.32	7386.19	0.86
Sembulan River	SN 01	20.11	8.89	44.21	55.79	643.18	0.96
	SN 02	20.12	8.817	43.82	56.18	1851.96	1.79
	SN 03	20.10	10.01	49.77	50.23	1945.84	1.32
	SN 04	20.37	10.48	51.42	48.58	3621.44	1.37
	SN 05	20.24	9.54	47.11	52.89	4498.33	0.92
	SN 06	20.04	8.74	43.61	56.39	5567.12	0.87

<sup>a</sup> Sum of the concentrations of the 26 LAB congeners.

<sup>b</sup> Ratio of (6-C<sub>12</sub>AB+5-C<sub>12</sub>AB) to (4-C<sub>12</sub>AB+3-C<sub>12</sub>AB+2-C<sub>12</sub>AB).

<sup>c</sup> Not detected

water in these areas is relatively clean. This conclusion was further evidenced by the high I/E ratios for these stations.

Similarly, Fig. 3 shows that the levels of LABs in the sediment samples found in this river increase upon moving from the up-, to the down-stream, especially in SS3 and SS4 due to the high human populations and activities around the sampling points. It is also noticed that limited human activities prevail the areas surrounding the sampling stations SS1 and SS2 which had low

LABs concentrations, presumably as a direct result of the limited human activities in these areas.

The concentrations of LABs in the sediments of Sembulan River increased consistently from the first station to the last one. However, as shown in Fig. 2, the concentrations of LABs were higher in the middle of the study area (stations SN4-SN6) than in the sampling stations SN1 through SN3 since the stations SN4, SN5, and SN6 were close to the center of the town which undergoes many human activities in hospitals, hotels,

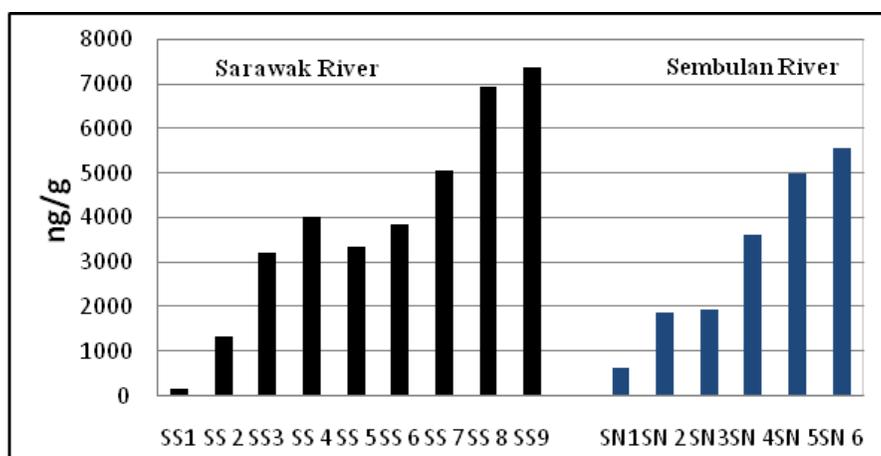


Figure 3. Concentration of LABs in Sarawak and Sembulan rivers (Concentrations are expressed as  $\Sigma$ C<sub>10</sub>-C<sub>14</sub> which is the sum of the concentrations of the 26 LABs congeners).

governmental institutions, shop lots, and restaurants. Hence, the LABs might have originated from those anthropogenic activities as well as from the activities in the residential areas. The low concentrations of LABs in SN1 and SN3 can be attributed to the lack of human activities in the surroundings of these two stations. In other respects, the *I/E* ratios characteristic of the sampled stations indicate that most of the stations were receiving primarily-, and secondary-treated wastewater inflows.

In general, the concentrations of  $\Sigma$ LABs in the sediments were higher in Sarawak River than in Sembulan River (Fig. 3). The differences in industrialization and urbanization rates in the watersheds of these two rivers are responsible for these spatial distributions. The results also point out that the sewage treatment plants (STPs) directly influence the concentrations of LABs as generally low concentrations of LABs are observed wherever there are many STPs, and vice versa.

In comparison with the findings of previous studies, the total concentrations of LABs observed in the current study were relatively low compared to those in Klang River and the Straits of Malacca; 4-8,590 ng/g (Isobe and Zakaria, 2004) and in the northern Tokyo Bay; 1,000-3,000 ng/g (Takada et al., 1992).

The relationships between  $\Sigma$ LABs concentrations in Sarawak and Sembulan rivers and distances of the sampling sites from the headwaters were tested statistically and found to be linear (Fig. 4). The values of the coefficients of determination ( $R^2$ ) for Sarawak and Sembulan rivers were 0.916 and 0.965, respectively. The implication of these strong, linear relationships is that the LABs in the two rivers are associated with sewage-derived particulates and that they have almost the same, or similar, sources.

On the other hand, the findings revealed that phenyltetradecane was the most abundant LAB

homologue in both rivers. The LAB homologue phenyltridecane ranked next to phenyltetradecane. However, in Sarawak River phenyl-decane and tridecane were the homologues which ranked second in abundance. This demonstrates that the LABs had different homologue compositional patterns and abundance in the two rivers (Fig. 5). Based on these results, the observed variations in homologues abundance between the sampling points possibly indicate that there are differences in the LAB source characteristics and/or degradation rates in the two riverine environments.

In relating the findings of the present study to those of previous studies, the laboratory experiments and field observations have demonstrated that the isomer composition of the LABs systematically changed (Eganhouse et al., 1983; Bayona et al., 1986; Takada and Ishiwatari, 1990; Gledhill et al., 1991; Takada et al., 1992b; Gustafsson et al., 2001). The external isomers are preferentially biodegraded over the internal counterparts. To quantitatively express the isomer composition, the ratio of internal to external isomers (i.e., the *I/E* ratio was proposed as an index of the degree of LAB degradation (Takada and Ishiwatari, 1990). Takada and Eganhouse (1998) observed that raw sewage has a low *I/E* ratio (0.7-0.8) while the *I/E* ratio for primary effluent ranges from 0.5-0.9. This is ascribed to that the primary treatment of wastewater is usually physical that essentially removes the suspended solids and organic matter from the wastewater. According to this study, sedimentation was found to allow a limited opportunity for aerobic degradation. On the other hand, secondary effluents show a much higher *I/E* ratio; ranging from 2 to 7, than the primary ones due to the biological removal of biodegradable organic matter and suspended solids during the secondary treatment of wastewater. A high value of this ratio means a greater depletion, through degradation, of external isomers than

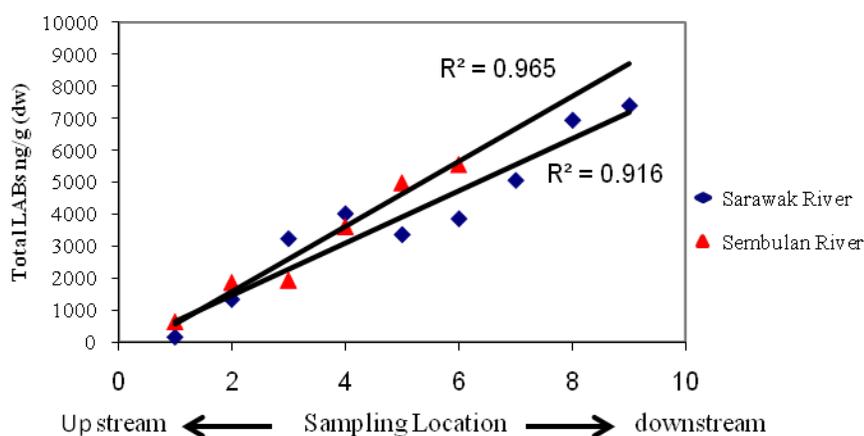


Figure 4. Scatter plot of the relationship between total LABs concentrations (ng/g dw) and sampling locations in Sarawak and Sembulan rivers.

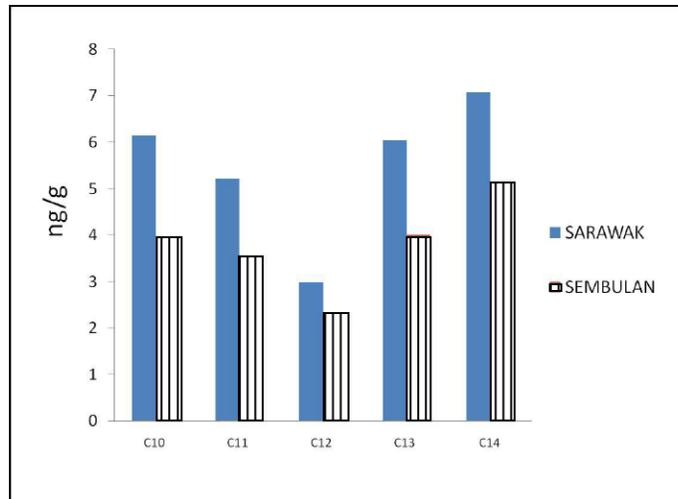


Figure 5. Relative abundance of LABs in the sediment samples of Sarawak and Sembulan rivers.

of internal ones. In the present study, the *I/E* ratios for the sediment samples were calculated (Table 2). The *I/E* ratios in the sediment of Sarawak and Sembulan rivers ranged from 0.52-0.98 and from 0.87-1.79, respectively. Furthermore, the *I/E* ratio at SS4 was much lower than the *I/E* ratios reported at the other stations, thereby indicating that the sewage discharged to Sarawak River was poorly treated. Hence, based on the *I/E* ratio, it appears that untreated sewage was directly discharged into the aquatic environment in the study area. It is worthwhile to highlight that the sediments collected from Sarawak and Sembulan rivers further away from the wastewater input sources manifested considerably lower *I/E* ratios than those collected from stations close

to the wastewater sources such as SS3 and SS8 in Sarawak River and SN5 in Sembulan River (Fig. 6).

#### 4. Conclusion

The sediments used in this study were collected as part of a comprehensive study conducted on sewage pollution in Sarawak River in Kuching and Sembulan River in Kota Kinabalu in the east of Malaysia. The high  $\Sigma$ LABs concentrations and low *I/E* ratios observed in the sediments of sampling stations surrounded by populous cities in Sarawak and Sembulan rivers indicate a high contamination with untreated domestic waste. The LABs proved to be effective indicators of

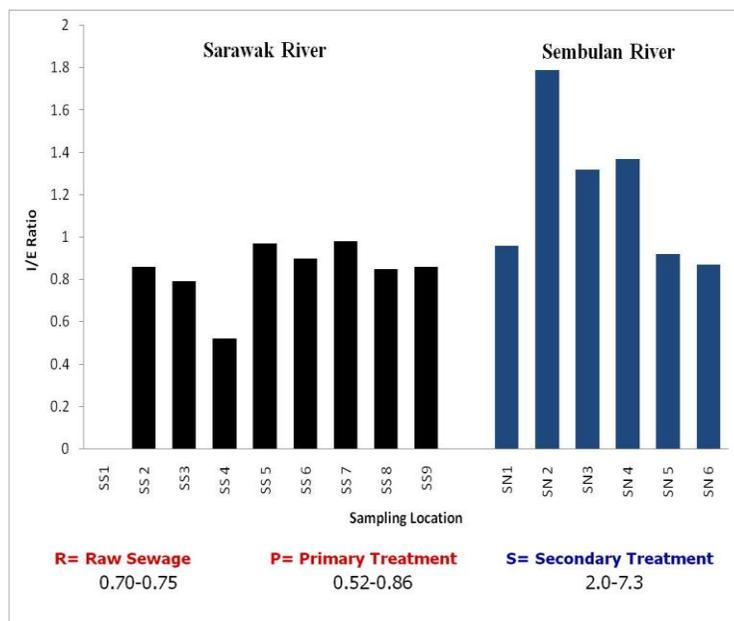


Figure 6. The *I/E* ratios in the sediments of Sarawak and Sembulan rivers.

land-derived pollutants that are transported laterally to the surface water. To sum up, partially-treated sewage will still contain LABs. The growth in tourism activities in marine parks and reclamation of sea areas for land use activities also can escalate the discharge of LABs into the water body. The expansion of coastal development probably results in a large number of anthropogenic pollutants, including LABs, being discharged into the surface water bodies. Human activities continue to grow and to increase the variety of organic compounds input to the environment, which in turn tend to alter the amounts and ratios of naturally-occurring compounds as well as anthropogenic ones.

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#### Correspondence to

Professor Dr. Mohamad Pauzi Zakaria  
Center of Excellence for Environmental Forensics,  
Faculty of Environmental Studies,  
Universiti Putra Malaysia,  
43400, UPM Serdang,  
Selangor,  
Malaysia  
Email: mpauzi@env.upm.edu.my