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Baseline

Determination of linear alkylbenzenes (LABs) in mangrove ecosystems using the oyster *Crassostrea belcheri* as a biosensor

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ABSTRACT

The current study aimed to develop a suitable molecular marker [Linear alkylbenzenes (LABs)] approach for pollution determination in mangrove oysters of peninsular Malaysia. *C. belcheri* species were collected from rivers of Merbok, Perai, Klang, Muar and PulauMerambong (An Island). The LABs were extracted from *C. belcheri* and determined using GC–MS. The LABs indices which included I/E, L/S and C13/C12 were applied to describe the sources and biodegradation of LABs. The results revealed that the maximum concentrations were detected in oysters from Klang (27.91 ng g^{-1}dw), while the lowest concentrations were detected in oysters from Merbok (8.12 ng g^{-1}dw). Moreover, I/E ratios varied between 2.83 and 6.40, indicating the secondary treatment effluents being discharged to coastal zones. The results of this study suggested that the oysters absorbed LABs mainly in dissolved phase. Therefore, mangrove oysters are a good biosensor for LABs contamination in the aquatic environment.

1. Introduction

Linear alkylbenzenes (LABs) are one of the main groups of organic contaminants that are used as molecular markers of sewage pollution. LABs are classified as lipophilic, with high adsorption affinity to organic matter, suspended particulate matter and sediment in aquatic ecosystems. The availability of LABs has been reported in river water, detergent, wastewater and soil (Ni et al., 2008; Ni et al., 2009; Zhang et al., 2012; Wei et al., 2014). However, the determination of LABs only in water or sediment is insufficient to evaluate the effects of sewage on the organisms and ecosystems (Allan et al., 2006; Cajaraville et al., 2000).The scientists in the literature suggested investigating the chemical and biological impacts during the monitoring process(Aguirre-Rubí et al., 2018). The word 'biosensor' is used here to mean an organism that has the ability to accumulate specific chemical substances. Therefore, the presence of these chemicals in the biosensor is an indication of the presence of chemicals in the surrounding environment and confirms the transmission of the contamination into organism tissue. The application of bivalves as a biosensor for the biological effect of organic pollutants in the coastal area has been reported in the literature due to their ability to bio-accumulate high risk organic chemicals such as petroleum hydrocarbons (Vaezzadeh et al., 2017a; Keshavarzifard et al., 2016b). The extent of bioaccumulation by these organisms compared to the concentrations in the water is often 1:10 ngg^{-1}.The abundance of bivalves, easy sampling, tolerance towards different environmental pollutants, very low-level activity of organic contaminant metabolising enzymes, reasonably long life span to differentiate accumulation of pollutants in different ages and reasonable size make them the most efficient sentinel among the several organisms living in the coastal area (Hamza Chaffai, 2014).Among the several available bivalve species, Aguirre-Rubí et al. (2018) used the mangrove cupped oyster, *C. rhizophorae*, as a sentinel for monitoring pollution in the Caribbean mangroves and coastal zones. Furthermore, Mangrove oyster *C. belcheri* species are easy to distinguish from other species based on their morphological alterations and white adductor mussel scars (Visootiviseth et al., 1998).

Malaysia is surrounded by the Malacca Strait in the West and the South China Sea in the East. The Malacca strait is 480 km wide in the north and 5 km wide in the southern part which is close to Singapore, with water depth < 23 m (Kasmin, 2010). The west coast of peninsular Malaysia has experienced rapid urbanization and industrialization over the last few decades leading to an increase in the volume of wastewater

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inputs into the rivers from different anthropogenic sources. Most rivers in the West coast discharge to the Malacca Strait and the sediment accumulation in Malaysian estuaries results from heavy precipitation and then consequently movement of suspended particulate matters via surface runoff and flow into these rivers. Mangrove oysters are ubiquitous in coasts and rivers of Malaysia and they are consumed by coastal communities (Tan et al., 2014). Sewage pollution also occurs as a result of anthropogenic activities such as boating, fishing, sailing and recreation in the West coast of Malaysia (Shahbazi et al., 2010; Keshavarzifard et al., 2016a).

LAB indices such as I/E ratio (internal to external isomer), L/S (Long Chain/Short Chain) and C_{13}/C_{12} were calculated as (6-C_{12} LAB + 5-C_{12} LAB) / (4-C_{12} LAB + 3-C_{12} LAB + 2C_{12} LAB), C_{13}/C_{12} [ΣC_{13}-LAB / ΣC_{12}-LAB], and L/S = (5C_{13} + 5-C_{12}) / (5-C_{11} + 5-C_{10}), and used to describe the sources and/or degree of biodegradation of LABs in the environment (Dauner et al., 2015). The high level of I/E ratios indicates high LABs degradation in the aquatic environment which means high degradation of LABs external isomer (Takada and Ishiwatari, 1990). Scientists found I/E ratios in synthetic detergents and raw sewage around 0.7, 0.5–0.9 in primary sewage treatment and 2.0–7.0 in secondary treatment (Takada and Eganhouse, 1998). The degradation level for LABs homologs in raw sewage and synthetic detergents is very low due to the intensity of LABs inputs and presence of LABs isomers in stable condition. In the primary stages, the I/E ratio systemically changes under aerobic conditions and due to the physical elimination of sewage inputs; thus, the limitation of aerobic microbial degradation through this process might occur. On other hand, the secondary treatment shows high levels of I/E ratios because this process involves the biological removal of organic contaminants and thereafter discharges into the aquatic environment through natural microbial degradation (Takada and Eganhouse, 1998).

The degradation percentage for LABs (D %) was suggested by Takada and Ishiwatari (1990), and it is found to increase with the increase of I/E ratio. Likewise, the ratios of C_{13}/C_{12} and L/S in raw sewage and detergent were lesser than those in river water (Ni et al., 2008) and sediment (Luo et al., 2008) due to the stability of long-chain and short-chain alkylbenzenes present in raw sewage.

C. belcheri has not been used as a biosensor for the biological impacts of LABs in the coastal area. Therefore, the current study aimed to measure and interpret the concentration, spatial distribution, sewage sources and degradation of LABs in ecosystems using *C. belcheri* as a biosensor.

The investigated locations included Merbok, Perai, Klang, Muar and Pulau Merambong mangrove ecosystems (Fig. 1). The sampling sites, along with the selected areas of Peninsular Malaysia, are in Table 1. The sampling sites were chosen based on the presence of mangrove oysters and included areas that might be contaminated and those thought to be less contaminated across the north and the south of west Peninsular Malaysia.

Twenty *C. belcheri* samples were collected form each study site. The samples were transported to the laboratoryin an ice box (−20 °C). Subsequently, the *C. belcheri* samples were thawed overnight under room temperature. The soft tissues of *C. belcheri* were detached and homogenised at 8000–10,000 rpm for 12 min with an NISSEI AM-7, (0−200) rpm × 100 homogeniser (Japan).

The blended *C. belcheri* tissues were freeze-dried and subjected to the digestion process in order to extract the target hydrocarbons according to methods described by Perugini et al. (2007).In brief; A fixed volume (10 mL) of $C_2H_7KO_2$(1 M) was added to a sample and spiked with 50 μL of 10 mg/L of surrogate internal standards (SIS:n-octylbenzene, n-nonylbenzene, n-decylbenzene, n-undecylbenzene, n-dodecylbenzene, n-tridecylbenzene, and n-tetradecylbenzene, Restek company, Bellefonte, PA 16823, USA)for LABs. The samples were then put in a reflux system for 3 h at 80 °C. Subsequently, the liquid portion of each sample was isolated and extracted with 10 mL of cyclohexane (C_6H_{12}) in a separation funnel with 30 min of rigorous shaking. The aqueous part was then put in a separation funnel and extracted with 10 mL of C_6H_{12} two more times. Thereafter, the combined extract was transferred through an anhydrous sodium sulphate column and then through a florisil column, while the final volume was reduced by a rotary evaporation. The oyster extract was transferred onto the top of 5% H_2O deactivated silica gel column chromatography (1 cm i.d. × 9 cm, 60–200 mesh size) for purification and then the hydrocarbon fraction was eluted with 20 mL hexane/DCM (3:1 *v*/v). For fractionation, the resulted eluate was concentrated by a rotary evaporator and transferred onto the a fully activated silica gel column chromatography (0.47 cm i.d, 18 cm height). LABs fraction was collected using the second 4 mL of high purity n-hexane. The LABs fraction was then reduced to 1 mL using rotary evaporator and moved to amber vials. The fraction was reduced to near dryness by a gentle stream of nitrogen and 200 μL of biphenyl-d10 in isooctane as LABs internal injection standard (IIS) was added to the amber vial before GC–MS procedure.

A GC–MS using 7890A series, Agilent Technologies interfaced with a MSD 5972 split/splitless injector. A 30-m fused silica capillary column of 0.25-mm internal diameter (i.d.) and DB-5MS capillary column of 0.25-μm film thickness were used. The carrier gas was helium at a pressure of 60 kg cm^{-2}. GC–MS was set with selected ion monitoring (SIM mode) and was kept at 70 eV for ionization with a source at 200 °C and electron multiplier voltage at ~1250 eV. The injection port was maintained at 300 °C when the sample was injected via split mode followed by a 1-min purge after the injection. The column temperature was kept at 70 °C for 2 min, then programmed at a rate of 30 °C/min^{-1} to 150 °C and finally at a rate of 4 °C/min^{-1} to 310 °C and maintained for 15 min. Target LABs compounds were identified using standard mixtures and the scan method before SIM programming. LABs compounds were quantified by comparing selected ion and IIS peak areas and matching their compound ions and retention times with the LABs standard mixture. The LABs concentrations were calculated based on the peak area to determine the 26 LABs congeners.

Surrogate standards (SIS) of LABs were recovered with a reasonably efficient proportion showing only minimal loss of target compounds through the analytical procedures. The range of recoveries for LABs surrogates was within 87%–98% for samples analysed in this study. Procedural blanks for LABs went through similar analysis procedures with every samples batch to avoid any possible cross-contamination from different sources during the analytical procedures. Internal injection standards (IIS) was spiked to the LABs fraction prior to instrumental analysis and is identified at m/z = 164 to check probable errors during injection. GC–MS was set in SIM mode at m/z = 91, 92 and 105 to analyse target LABs congeners. Five different concentrations of LABs standard mixtures within the range of 0.25–5 mg/L were injected together with every batch of sample isolates of LABs and a five-point calibration curve was used to quantify LABs target compounds. Detection limits were estimated for LAB compounds as three times the baseline of measured chromatograph. Reproducibility of the analyses was tested by performing duplicate analysis. The variation coefficient was below 10% for LABs.

In this study, gravimetrical method was used to obtain the lipid content. The oyster soft tissues were weighed and dried using anhydrous sodium sulphate and dried samples were then soxhlet extracted for 10 h. The extract was then separated and diethyl ether and hexane with 3 to 1 volume ratio was added and stirred. Then the extract was dried by a gentle stream of nitrogen and weighed (Tanabe et al., 1987). The subsequent equation was used to obtain lipid content:

$$Lw = Wr \times Vf/(Ws \times Va) \times 0.1$$

Lw = lipid weight (content) %; Wr = residual weight of extract (mg); Ws = weight of sample (g); Vf = final volume of extract; Va = volume of separated extract.

The collected data were subjected to one-way analysis of variances with three replicates. The differences between the LABs concentrations

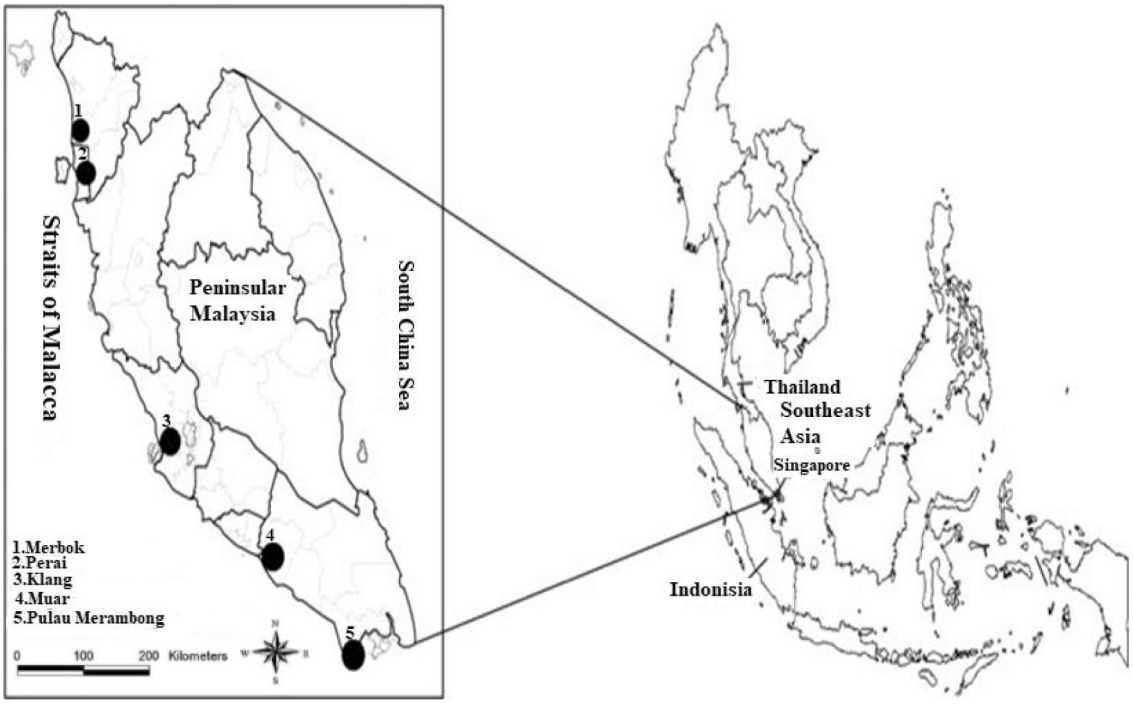

Fig. 1. Locations of oyster sampling areas in west and south coast of Peninsular Malaysia.

among the collected samples were compared using LSD test (ANOVA). Analysis of variance (ANOVA) was performed in order to determine the significance of the differences between the sampling sites. The differences were considered significant at $p < 0.05$ (95% of the confidence level). The data were analysed using a Statistic program (SPSS for windows, version 20). The Pearson correlation at $p < 0.05$ was used in this study.

The concentrations of LABs in the soft tissues of *C. belcheri* from different locations on the western coast of Malaysia are illustrated in Table 2. It was noted that the concentrations of total LABs were significantly different in the investigated locations as analysed using ANOVA ($p < 0.05$). The LABs ranged from 8.10 to 27.90 ng g^{-1}dw (Table 3a, b). The highest concentrations of LABs (27.90 ng·g^{-1}dw) was detected in the *C. belcheri* samples obtained from Klang, while the lowest concentrations were recorded for the samples obtained from Merbok (8.00 ng g^{-1}dw) (Fig. 2). The total LABs in *C. belcheri* tissue from mangrove ecosystems of Malaysia has not been investigated before. A previous study reported that the highest concentrations of total LABs in tissues ofgreen mussels (*Pernaviridis*) and oysters (*Crassostrea brasiliana*) from South and South East Asian coasts and estuarine aquaculture areas in Southern Brazil were 1640 and 152 ng·g^{-1}dw, respectively, which are higher than those in presented study (Tsutsumi et al., 2002; Zacchi et al., 2018). Similarly, some other earlier studies revealed that the concentration of LABs in sediment from Klang River was 8590 ng g^{-1} dw, while it was 32 ng g^{-1}dw in sediment from Muar River (Isobe et al., 2004). Magam et al. (2015) revealed that the concentrations of LABs in sediment samples from Merbok River was 1017 ng g^{-1} dw (Table 4). The concentrations of LABs in *C. belcheri*

Table 2
ΣLABs concentration (ng·g^{-1}·dw) and relative ratios in the mangrove oyster tissue.

Compounds	Merbok	Perai	Klang	Muar	Pulau Merambong	Average
Σ C10-LABs	1.20	1.62	1.80	2.72	1.51	1.77
Σ C_{11}-LABs	2.62	6.01	8.21	7.71	5.11	5.93
Σ C_{12}-LABs	1.41	1.62	7.83	3.21	4.93	3.80
Σ C_{13}-LABs	1.81	2.21	7.01	3.62	4.82	3.89
Σ C_{14}-LABs	1.23	1.62	3.32	2.91	2.41	2.29
Σ-LABs[a]	8.12	13.10	27.91	20.21	18.72	17.61
LC-LABs[b]	3.00	3.80	10.32	6.50	7.21	6.16
SC-LABs[c]	3.81	7.63	10.01	10.41	6.60	7.69
I/E[d]	2.83	3.12	6.40	4.20	4.72	4.25
L/S[e]	1.14	1.01	2.92	1.33	2.41	1.76
C_{13}/C_{12}[f]	3.42	4.70	13.02	7.40	8.30	7.37
LAB degradation (%)[g]	51	55	80	65	69	64
Lipid content (%)	1.20	1.91	1.52	0.90	0.90	1.29

[a] Σ-LABs, Sum of the 26 LAB congeners ranging from 5-$C_{10}$to 2-C_{14}.
[b] LC-LABs, sum of LABs ranging from 6-C_{13} to 2-C_{14}.
[c] SC-LABs, sum of LABs ranging from 5-C_{10} to 2-C_{11}.
[d] I/E (C_{12}-LABs), ratio of (6-C_{12}LAB + 5-C_{12}LAB) relative to (4-C_{12}LAB + 3-C_{12}LAB + 2-C_{12}LAB).
[e] L/S, ratio of (5-C_{13}LAB + 5-C_{12}LAB) relative to (5-C_{11}LAB + 5-C_{10}LAB).
[f] C_{13}/C_{12}, ratio of (6-,5-,4-,3- and 2-C_{13} / (6-, 5-, 4-, 3-, and 2-C_{12}LAB).
[g] LAB Degradation (D%), LAB deg = 81 × log (I/E ratio) + 15 (r^2 = 0.96).

Table 1
Description of sampling site along the study areas of Peninsular Malaysia.

No	Sampling location	Latitude (°N)	Longitude (°E)	Description of location
1	Merbok	5° 39′	100° 24′	Less urban area
2	Perai	5° 24′	100°24′	Urban and industrial area
3	Klang	4° 00′	100° 45′	Urban and industrial area
4	Muar	02° 04′	102° 33′	Urban and industrial area
5	Pulau Merambong	01° 22′	103° 38′	Uninhabited area

Table 3
Total LABs concentration in oyster tissues among diffirent locations in Malaysia with the a) ANOVA, b) Post hoc tests.

a) ANOVA

	Sum of squares	df	Mean square	F	Sig.*
Between Groups	673.447	4	168.362	222.878	0.000
Within Groups	7.554	10	0.755		
Total	681.001	14			

b) Post hoc tests

Dependent variable: LABs concentration

LSD

(I) Location	(J) Location	Mean difference (I–J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
1.00	2.00	−4.98000*	0.70965	0.000	−8.2351	−1.7249
	3.00	−19.79000*	0.70965	0.000	−23.0451	−16.5349
	4.00	−12.09000*	0.70965	0.000	−15.3451	−8.8349
	5.00	−10.60000*	0.70965	0.000	−13.8551	−7.3449
2.00	1.00	4.98000*	0.70965	0.000	1.7249	8.2351
	3.00	−14.81000*	0.70965	0.000	−18.0651	−11.5549
	4.00	−7.11000*	0.70965	0.000	−10.3651	−3.8549
	5.00	−5.62000*	0.70965	0.000	−8.8751	−2.3649
3.00	1.00	19.79000*	0.70965	0.000	16.5349	23.0451
	2.00	14.81000*	0.70965	0.000	11.5549	18.0651
	4.00	7.70000*	0.70965	0.000	4.4449	10.9551
	5.00	9.19000*	0.70965	0.000	5.9349	12.4451
4.00	1.00	12.09000*	0.70965	0.000	8.8349	15.3451
	2.00	7.11000*	0.70965	0.000	3.8549	10.3651
	3.00	−7.70000*	0.70965	0.000	−10.9551	−4.4449
	5.00	1.49000	0.70965	0.062	−1.7651	4.7451
5.00	1.00	10.60000*	0.70965	0.000	7.3449	13.8551
	2.00	5.62000*	0.70965	0.000	2.3649	8.8751
	3.00	−9.19000*	0.70965	0.000	−12.4451	−5.9349
	4.00	−1.49000	0.70965	0.062	−4.7451	1.7651

* The mean difference is significant at the 0.05 level.

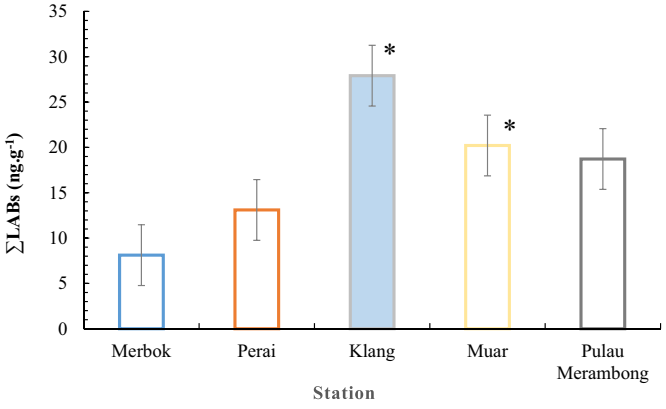

Fig. 2. Concentration of LABs in oyster samples from west and south coast peninsular Malaysia (concentrations are expressed as ΣLAB C_{10}-C_{14}). (*) indicate for the high significance differences ($P < 0.05$).

from Klang and Muar River in this study were compared with those reported by previous studies (Isobe et al., 2004; Magam et al., 2015). The results revealed that there is a significant Pearson correlation ($p < 0.05$, $r = 0.59$) between the concentrations of LABs in the sediment and *C. belcheri*, indicating the potential of *C. belcheri* to be a biosensor for monitoring LABs (Table 5).

Possibly, the transfer mechanisms of congener specificity, partitioning phase and degradation potentials for LAB homologs from source to receptor are causes for this differentiation. Lateral transport and hydrodynamic flow might be the major inputs for hydrophobic pollutants (Wania et al., 1998; Jones and de Voogt, 1999).

Table 6a, b, displays a significant difference ($p < 0.05$) for C_{11}-LABs and C_{12}-LABs values in oyster soft tissues among the investigated locations in the west coast of peninsular Malaysia.

The results revealed that Σ C_{11}-LABs homologs recorded the highest concentrations (average 5.00 ng g^{-1}·dw), while Σ C_{10}-LABs were observed with the lowest concentrations (average 1.80 ng g^{-1} dw). C_{11}-LABshomologswere recorded in all investigated samples from five locations, with the highest proportion in the Perai mangrove ecosystem at 46%, followed by the Muar mangrove ecosystem at 38% of total LABs (Fig. 3). The LAB homologs in the oyster's tissues were found to be opposite to those in sediments (Luo et al., 2008). A major reason can be the higher biodegradation of short homologs such as C11 in the sediment with higher K_{ow} of long chain LABs such as C_{13}, and therefore, C_{13} homologs tend to be attached to particulate matter and settle down in sediment.

The comparison between the long chain (LC) and short chain (SC) of LABs in the soft tissues of *C. belcheri* are presented in Fig. 4. The short chain homologs were predominant over the long chain ones where the proportion of short chain homologs was between 48% and 67% of the total LABs with the highest level in the Perai mangrove oysters at 67%. The long chain homologs were between 33% and 52% of total LABs with the highest abundance in the Pulau Merambong mangrove ecosystem at 52%. However, SC was predominantly found in Muar, Perai and Merbok. At Klang and Pulau Merambong, LC was predominantly found (Table 6a, b). This is an indication that SC-LABs homologs, due to their high biodegradability, are more easily uptaken than LC homologs by oysters.

The results of I/E ratios are depicted in Fig. 5, Tables 2 and 4. It was

Table 4
Total concentrations of ΣLABs from different areas around Malaysia and the world.

Location	Sample type	Maximum ΣLABs (ng/g^{-1})[a]	I/E ratio[b]	Degradation[c] (%)	Reference
South and South East Asian	Mussels	1640	8.2	89	Tsutsumi et al. (2002)
Southern Brazil	Oysters	152	1.2	21	Zacchi et al. (2018)
Port Klang, Malaysia	Sediment	8590	0.7	3	Isobe et al. (2004)
Muar River, Malaysia	Sediment	32	2.8	51	Isobe et al. (2004)
Prai River, Malaysia	Sediment	25	3.4	58	Isobe et al. (2004)
Selangor River	Sediment	90	1.0	67	Masood et al. (2015)
Merbok River, Malaysia	Sediment	1017	1.4	25	Magam et al. (2015)
Prai River, Malaysia	Sediment	2910	0.8	5	Magam et al. (2015)
Perak River, Malaysia	Sediment	329	0.8	5	Magam et al. (2015)
Brunei Bay	Sediment	41	1.9	38	Alkhadher et al. (2015)
Johor Coast, Malaysia	Sediment	189	2.7	49	Alkhadher et al. (2016)
Kim Kim River, Malaysia	Sediment	119	1.9	38	Alkhadher et al. (2016)
Merbok, Malaysia	Oyster	8	2.8	51	Present study
Perai, Malaysia	Oyster	13	3.1	55	Present study
Klang, Malaysia	Oyster	30	6.4	80	Present study
Muar, Malaysia	Oyster	20	4.2	65	Present study
Pulau Merambong, Malaysia	Oyster	19	4.7	69	Present study
South China Sea	Sediment	23	0.9	11	Luo et al. (2008)
The Pearl River Delta	Water	634	1.7	34	Ni et al. (2008)
Dongjiang River	Sediment	410	1.4	27	Zhang et al. (2012)
Outfalls of paper mills	Wastewater	3270	1.3	24	Zhang et al. (2012)
Jakarta Bay	Sediment	86,745	0.9	12	Rinawati et al. (2012)
Coastal shelf of China	Soil	77	1.2	21	Wei et al. (2014)
Admiralty Bay, Antartica	Sediment	47	0.9	11	Martins et al. (2012)
Anzali Wetland	Sediment	109,305	1.3	24	Bakhtiari et al. (2018)
Detergent	powder		1.2	21	Ni et al. (2008)

[a] ΣLAB = sum of concentrations of all secondary LAB congeners having C_{10}–C_{14} alkyl chain.
[b] I/E = (6_C_{12} + 5_C_{12}) / 4_C_{12} + 3_C_{12} + 2_C_{12}).
[c] LAB deg = 81 ∗ log(I/E ratio) + 15 (r^2 = 0.96).

Table 5
Pearson correlation between total LABs in oyster tissues from different sampling locations.

	Location	Total LABs concentration
Pearson Correlation	1	0.594*
Sig. (2-tailed)		0.020
N	15	15

* Correlation is significant at the 0.05 level (2-tailed).

observed that I/E ratio varied between 2.80 and 6.40, showing that secondary treatment effluents were released into the coastal zones. I/E ratio results for the current study are similar to those reported in sediment from Japan, Malaysia and Thailand (Chalaux et al., 1995; Isobe et al., 2004; Magam et al., 2015; Masood et al., 2015; Alkhadher et al., 2015) and higher than those of commercial detergents in Jakarta Bay, and Admiralty Bay in Antartica (Ni et al., 2008; Rinawati et al., 2012; Martins et al., 2012). I/E ratio results beside that predominated SC-LABs of this study are explained by that oyster uptakes high degradable LABs congeners such as external isomers of LABs homologs. This is consistent with a previous study conducted by Vaezzadeh et al. (2017b) which proved that oysters (*C. belcheri*) were found to uptake low molecular weight (LMWPAHs) more than high molecular weight (HMW) ones.

The LABs ratios of L/S were valued from 1.00 to 2.90 (average 1.70) in oysters of sampling locations which were relatively higher than those observed in Perai and Merbok (Magam et al., 2015) and were lower than those in the sediment of Selangor River (Masood et al., 2015). Similarly, C_{13}/C_{12} were detected from 3.40 to 13.00 (average 7.40), which is higher than those observed in sediment reported by Magam et al. (2015). Therefore, L/S and C13/C12 values suggested that short-chain LABs were significantly uptaken in oyster samples of mangrove ecosystems compared with long-chain LABs. Moreover, the occurrence of LAB congener inter-conversions involving phenyl group migration or the enrichment of external isomers (Luo et al., 2008) may have been the reason behind the anticipated increase in I/E values.

I/E ratio in oysters was ranged from 2.80 to 6.40 in the Merbok and Klang mangrove ecosystems, respectively, presenting secondary treated sewage effluent sources of LABs with high LABs degradation in aquatic environment. In treated sewage effluents or secondary effluents, the internal isomers of LABs are predominant over external ones due to higher selective degradation of the external isomers than internal ones (Takada and Ishiwatari, 1990). Therefore, the I/E ratios results suggested that oysters are in the areas that receive predominantly treated effluents.

The results of the current study suggest that the oysters mostly uptake LABs from water, especially from dissolved phase and the LABs associated with particulate matters can be absorbed by oysters as well. Murray et al. (1991) reported that the LABs in *Blue mussel* species are not available for ingestion by mussels in the gut. Therefore, the gills may have been the sites of LAB absorption. This is consistent with the observation of Stegeman and Teal (1973) where, in relation to the site uptake of cyclic compounds by oysters, they found that the deficiency of sediment LABs absorption may occur as a result of high binding of LABs congeners. Another reason for the deficiency of ingestion of these particles is that LABs are broken down by the flora gut before uptake. Biodegradation of LABs could be assessed by the changing of each isomer amount within a particular chain length homolog. This occurs because the bacterial enzyme breaks down the external isomer more than internal isomer (high substituent).

Erhardt (1972) reported that hydrocarbon homologs accumulated by oysters maybe modified by digestive tract bacteria. Previous measures depended on the evidence that oysters cannot metabolise hydrocarbons, e.g. LABs. Currently, it is evidenced that bivalve has enzymes that can metabolise hydrocarbons (Livingstone and Farrar, 1984), that the enzyme action is remarkably low and that it may not show significantly in LAB homologs modification. In an incubation experiment with domestic waste, Takada and Ishiwatari (1990) matched the selective biodegradation of C11 isomers to the total level of LABs biodegradation. Their paper values showed a good linear correlation (r = 0.97) among the ratio of externals to internals isomers and the

Table 6
Distribution of LABs homologs total LABs in oyster tissues from different sampling locations. a) ANOVA, b) Post hoc tests.

a) ANOVA

	Sum of squares	df	Mean square	F	Sig.
Between Groups	52.950	4	13.238	3.755	0.020
Within Groups	70.503	20	3.525		
Total	123.453	24			

b) Post hoc tests

Location (I)	(J) VAR00001	Mean difference (I–J)	Std. error	Sig.	95% confidence interval	
					Lower bound	Upper bound
1.00	2.00	−4.16200*	1.18746	0.002	−6.6390	−1.6850
	3.00	−2.03000	1.18746	0.103	−4.5070	0.4470
	4.00	−2.12400	1.18746	0.089	−4.6010	0.3530
	5.00	−0.52800	1.18746	0.661	−3.0050	1.9490
2.00	1.00	4.16200*	1.18746	0.002	1.6850	6.6390
	3.00	2.13200	1.18746	0.088	−0.3450	4.6090
	4.00	2.03800	1.18746	0.102	−0.4390	4.5150
	5.00	3.63400*	1.18746	0.006	1.1570	6.1110
3.00	1.00	2.03000	1.18746	0.103	−0.4470	4.5070
	2.00	−2.13200	1.18746	0.088	−4.6090	0.3450
	4.00	−0.09400	1.18746	0.938	−2.5710	2.3830
	5.00	1.50200	1.18746	0.220	−0.9750	3.9790
4.00	1.00	2.12400	1.18746	0.089	−0.3530	4.6010
	2.00	−2.03800*	1.18746	0.102	−4.5150	0.4390
	3.00	0.09400	1.18746	0.938	−2.3830	2.5710
	5.00	1.59600	1.18746	0.194	−0.8810	4.0730
5.00	1.00	0.52800	1.18746	0.661	−1.9490	3.0050
	2.00	−3.63400*	1.18746	0.006	−6.1110	−1.1570
	3.00	−1.50200	1.18746	0.220	−3.9790	0.9750
	4.00	−1.59600	1.18746	0.194	−4.0730	0.8810

* The mean difference is significant at the 0.05 level.

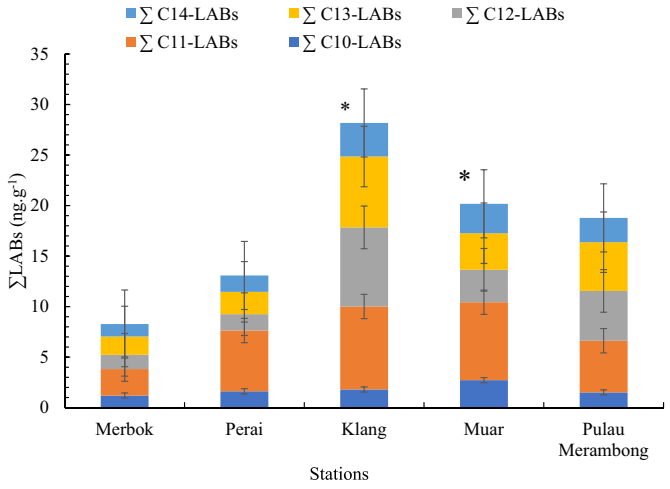

Fig. 3. Compositional profiles of linear alkylbenzenes in oyster samples from West and South coast Peninsular Malaysia. (*) indicate for the high significance differences ($P < 0.05$).

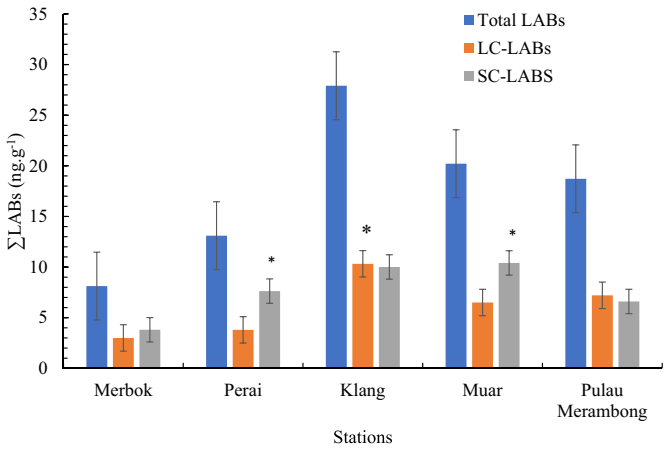

Fig. 4. Concentration of short chain (SC-LABs), long chain (LC-LABs) and total linear alkylbenzenes (LABs) in oyster samples from West and South coast Peninsular Malaysia. (*) indicate for the high significance differences ($P < 0.05$).

percentage residual of overall LAB concentrations.

The study results showed that C11 homologs were the most abundant in all sampling locations and that they are a sign of LAB biodegradation in each location. Therefore, they were chosen in this study as the index of bioavailability of LABs group by oysters. LAB in oyster soft tissue was significantly high biodegraded than those in sediment (Alkhadher et al., 2016; Magam et al., 2015). This occurs due to the bacteria attached to the oysters, and such processes could probably be more active in the gut.

Due to the low value of the standard deviation of lipid contents (0.40), the lipid content in different locations of mangrove oysters was not the key factor for LABs distribution (Vaezzadeh et al., 2017b). Bioconcentration is one of the main feeding factors in the uptake of contaminants such as LABs by oysters.

The data of this study showed that short chain-LABs homologs are bio-accumulated more than long chain-LABs by oysters. This is perhaps due to their low length distribution that ensures that they are absorbed readily by oysters (Mirsadeghi et al., 2013; Patel and Eapen, 1989; Menon and Menon, 1999; Boscolo et al., 2007). Higher bioavailability of short chain-LABs and their absorption than long chain-LABs in oysters was well reported previously (Murray et al., 1991). However, L/S

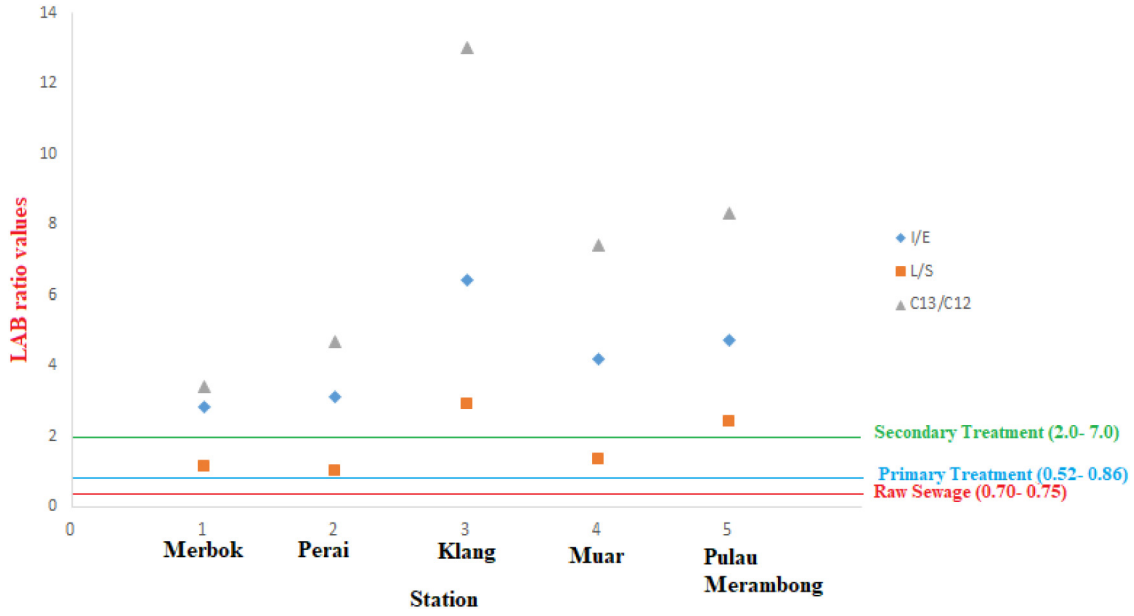

Fig. 5. LAB cross plots for the ratios of I/E, L/S and C_{13}/C_{12} in the Oyster samples from the west and south coast of peninsular Malaysia. The horizontal lines are drawn as stated in Takada and Eganhouse (1998).

and C_{13}/C_{12} were found to be more sensitive ratios and less reliable than I/E ratios for evaluation of LABs degradation (Ni et al., 2008).

Because the sediment contaminants such as LABs are absorbed by deposit feeders, the oysters are filter-feeders and they uptake pollutants commonly from water column through filtration (Boehm et al., 1978; Baumard et al., 1998; Farrington et al., 1983; Vaezzadeh et al., 2017b). On the other hand, the re suspension of sediment may be happened in shallow estuaries as result of Malaysian heavy rainfall, high turbidity and tidal current beside the bottom dwellers reworking in the sediment. All above activities may contribute to directly touching of sediment to oysters gill or assimilation of sediment and subsequently absorption through the digestive tract (Sakai et al., 2017; Thomes et al., 2019).

The percentage values of LAB biodegradation of the present study (D %) is very high and ranged from 51 to 80%. The study results suggest the presence of sewage input with different treatment efficiencies. LAB ratios in oyster's tissue from western Peninsular Malaysia were compared to other worldwide sampling locations (Table 4). It is observed that I/E ratios were relatively higher with those found in Zacchi et al. (2018) and higher than those found in other matrices such as water, sediment and soil. Therefore, the oysters *C. belcheri* appear to mostly uptake LABs from areas with secondary treated effluents.

2. Conclusion

This is the first study to assess the use of mangrove oyster (*C. belcheri*) as a biosensor for organic contaminants (LABs) in the ecosystem environment of Malaysia. LABs found in the oyster samples indicate significant distribution in the studied areas ($p < 0.05$). The trend of LABs concentration in oyster samples from all locations was: Klang > Muar > Pulau Merambong > Perai > Merbok. LAB ratios indicated the prevalence, at source, of secondary treatment effluents into the oysters. High level of short chain homologs(SC-LABs) were detected in oyster samples among other LABs homologs indicating that oysters more easily bio-accumulate short, rather than long, chain LABs compounds from the aquatic environment. Furthermore, I/E ratios are more than 2in all oyster sampling areas, indicating the secondary treated effluent sources of LABs. The Klang mangrove ecosystem showed approximately similar values for SC and LC-LABs, probably due to intense inputs of secondary treated effluents –LABs into this ecosystem. The results of this study evidenced mangrove oysters as a good biosensor organism for LABs in aquatic environments.

CRediT authorship contribution statement

Sadeq Abdullah Abdo Alkhadher: Investigation, Writing - original draft.**Aeslina Abdul Kadir:**Supervision, Writing - review & editing, Resources.**Mohamad Pauzi Zakaria:**Writing - review & editing, Formal analysis.**Adel Al-Gheethi:**Supervision, Data curation, Resources.**Basim H. Asghar:**Writing - review & editing, Resources.

Declaration of competing interest

The authors of the manuscript “Monitoring of Linear Alkybenzenes (LABs) Disturbance in Mangrove Ecosystems using the Oyster Crostrassea belcheri as a Biosensor” confirm that there is no conflict of interest.

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