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BIOAVAILABILITY OF PETROLEUM HYDROCARBONS TO MANGROVE OYSTER (CRASSOSTREA BELCHERI G.B. SOWERBY) FROM SEDIMENT IN MANGROVE ECOSYSTEMS OF WEST COAST OF PENINSULAR MALAYSIA

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FPAS 2015 2

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By

VAHAB VAEZZADEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia In Fulfilment of Requirements for Degree of Doctor of Philosophy

July 2015

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DEDICATION

To my Mother

To my Father and my Brother

for their nonstop encouragement

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

BIOAVAILABILITY OF PETROLEUM HYDROCARBONS TO MANGROVE OYSTER (*CRASSOSTREA BELCHERI* G.B. SOWERBY) FROM SEDIMENT IN MANGROVE ECOSYSTEMS OF WEST COAST OF PENINSULAR MALAYSIA

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July 2015

Chairperson: Professor Mohamad Pauzi Zakaria, PhD
Faculty: Environmental Studies

West coast of Peninsular Malaysia which faces to the Strait of Malacca has gone through rapid industrialization and urbanization and is susceptible to both sea-based and land-based petroleum pollution. Bioavailable petroleum hydrocarbons (PHC) can be toxic to aquatic organisms and pass along the food chain to higher levels, including humans. Consequently, a clear understanding of distribution and sources of PHC is of high importance in the region. Surface sediment samples and mangrove oyster *(Crassostrea belcheri)* were collected from five locations including the Merbok River, Prai River, Klang River, Muar River and Pulau Merambong in west coast of Peninsular Malaysia and investigated for the levels of PHC. Normal alkanes (n-alkanes), hopanes and polycyclic aromatic hydrocarbons (PAHs) fractions were extracted through soxhlet extraction, first step and second step column chromatography and injected to gas chromatography-mass spectrometry (GC-MS) for analysis. The total concentrations of n-alkanes ranged between 33697 and 290471 $ng.g^{-1}$ dry weight (dw) in the sediments. The concentrations of n-alkanes in the sediments collected from different stations are in the order: Klang River > Prai River > Pulau Merambong > Merbok River > Muar River. Petroleum origin n-alkanes were predominant in the lower parts of the estuaries, while higher plant origin n-alkanes were predominant in the upper parts of the Rivers. Concentrations of n-alkanes in the oysters ranged between 56661 to 262515 $ng.g^{-1}$dw. The concentrations of n-alkanes in the oysters from different stations are in the order: Klang River > Prai River > Merbok River > Pulau Merambong > Muar River. Low molecular weight (LMW) n-alkanes were more predominant in the oysters. Hopanes diagnostic ratios revealed used crankcase oil as the main source of hopanes in the sediment as well as in the oysters in the majority of sampling locations. The concentrations of total PAHs ranged between151and 4973 $ng.g^{-1}$ dw in the sediments. The concentrations of PAHs in the sediments from various sampling stations are in the order: Klang River > Prai River > Merbok River > Muar River > Pulau Merambong. A predominance of pyrogenic source PAHs were detected in the sediments. The concentrations of PAHs in the oysters ranged from 309 to 2225 $ng.g^{-1}$ dw. The concentrations of PAHs in the oysters from various stations follow the order: Klang River > Prai River > Merbok River > Pulau Merambong > Muar River. PAHs in the oysters were detected to be from mixed petrogenic and pyrogenic sources. A predominance of 2-3 ring PAHs was detected over 4 ring PAHs and 5-6 ring PAHs in the oysters. Significant correlations ($p<0.05$) were found between n-alkanes, hopanes and HMW PAHs in the sediments and oysters indicating that PHC body burden of the oysters is responsive to changes of PHC in the sediments. Moreover, biota accumulation factors (BAFs) of approaching or exceeding one were calculated for PHC in the majority of sampling locations, especially for LMW PHC indicating the ability of mangrove oyster to accumulate high levels of PHC. Overall, this study indicates

that mangrove oyster *(Crassostrea belcheri)* can be a good biomonitor, especially for LMW PHC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

BIOAVAILABILITI HIDROKARBON PETROLEUM TERHADAP TIRAM BAKAU (*CRASSOSTREA BELCHERI* G.B. SOWERBY) DARIPADA SEDIMEN BAKAU EKOSISTEM DI PANTAI BARAT SEMENANJUNG MALAYSIA

Oleh

VAHAB VAEZZADEH

Julai 2015

Pengerusi: Professor Mohamad Pauzi Zakaria, PhD
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Pantai Barat Semenanjung Malaysia terletak di sepanjang Selat Melaka mengalami perindustrian dan perbandaran yang amat pesat. Oleh itu, ianya sangat mudah terdedah kepada pencemaran tanah dan laut yang berasaskan petroleum. Hidrokarbon petroleum boleh memasuki ke dalam rantaian makanan ke tahap yang lebih tinggi termasuk manusia di bahagian atas. Oleh itu, kajian yang jelas tentang pengedaran, dansumber-sumber PHC amat penting di rantau ini. Sampel diambil pada permukaan enapan dan tiram bakau *(Crassostrea belcheri)* yang berada di Sungai Merbok, Sungai Perai, Sungai Klang, Sungai Muar dan Pulau Merambong di pantai barat Semenanjung Malaysia dan disiasat untuk mendapatkan tahap-tahap PHC. Normal alkanes (n-alkanes), hopanes dan polycyclic aromatic hydrocarbons (PAH) telah diekstrak menggunakan soxhlet extraction. Cara pertama sampel disuntik ke dalam Coloumn Gas Chromotography dan cara kedua sampel disuntik ke dalam Gas Chromatography-Mass Spectrometry (GC-MS) untuk dianalisa. Jumlah kepekatan n-alkanes adalah di antara 33697 dan 290471 ng.g^{-1} dw di dalam sedimen. Kepekatan di stesen persampelan berada dalam keadaan berikut: Sungai Klang > Sungai Prai > Pulau Merambong > Sungai Merbok > Sungai Muar. Sumber Petroleum n-alkanes adalah signifikan di bahagian rendah di muara sungai, manakala tumbuhan n-alkanes adalah lebih dominan di bahagian tinggi sungai. Kepekatan n-alkanes di dalam tiram adalah antara 56661 dan 262515 ng.g^{-1} dw. Kepekatan adalah seperti berikut: Sungai Klang > Sungai Prai > Sungai Merbok > Pulau Merambong > Sungai Muar. Low molecular weight (LMW) n-alkanes adalah sumber unsur pencemaran hidrokarbon yang paling utama di dalam tiram. Nisbah Hopanes diagnostik menunjukkan minyak crankcase adalah sumber utama hopanes di dalam enapan dan juga tiram di kebanyakan lokasi persampelan. Kepekatan jumlah PAH adalah antara 151dan4973 ng.g^{-1}dw dalam sedimen. Kepekatan adalah seperti berikut: Sungai Klang > Sungai Prai > Sungai Merbok > Sungai Muar > Pulau Merambong. A penguasaan PAH sumber pirogenik dikesan dalam sedimen. Kepekatan PAH dalam tiram antara 309 to 2225 ng.g^{-1} dw. Kepekatan diikuti perintah iaitu: Sungai Klang > Sungai Prai > Sungai Merbok > Pulau Merambong > Sungai Muar. Pencemaran dari petrogenik dan pirogenik dikesan sebagai sumber utama PAH di dalam tiram. Jumlah 2-3 ring PAHs adalah lebih banyak daripada 4 ring PAHs dan 5-6 ring PAHs di dalam tiram. Sejumlah korelasi ($p<0.05$) yang telah dikesan diantara n-alkanes, hopanes and HMW PAHs didalam endapan-endapan dan tiram-tiram menunjukkan bahawa beban badan PHC tiram-tiram adalah responsif kepada perubahan-perubahan PHC didalam endapan-endapan. Tambahan pula, faktor-faktor pengumpulan biota (BAFs) yang menghampiri atau melebihi telah dihitungkan untuk PHC dalam kebanyakan tempat-tempat pensampelan, terutamanya untuk LMW PHC yang menunjukkan kebolehan tiram bakau dalam mengumpulkan tahap-tahap PHC yang tinggi. Secara keseluruhannya, penyelidikan ini

menunjukkan bahawa tiram bakau *(Crassostrea belcheri)* merupakan satu biomonitor yang baik terutama bagi LMW hidrokarbon.

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I certify that a Thesis Examination Committee has met on 3 July 2015 to conduct the final examination of Vahab Vaezzadeh on his thesis entitled "Bioavailability of Petroleum Hydrocarbons to Mangrove Oyster (*Crassostrea belcheri* G.B. Sowerby) from Sediment in Mangrove Ecosystems of West Coast of Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

PHC	Petroleum hydrocarbons
n-Alkanes	Normal alkanes
PAHs	Polycyclic aromatic hydrocarbons
APM	Atmospheric particulate material
SPM	Suspended particulate matters
PEL	Probable effects level
ERM	Effect range median
ERL	Effects range low
SLC	Screening level contamination
TEL	Threshold effect level
SQGs	Sediment quality guidelines
DCM	Dichloromethane
Tg	Tetra gram (million tons)
TOC	Total organic carbon
SIM	Selected Ion Monitoring
m/z	Mass to charge ratio
eV	Electron Volt
ppm	Parts per million
dw	Dry weight
HMW	High Molecular Weight
USEPA	United States Environmental Protection Agency
LMW	Low Molecular Weight
BHT	Bacteriohopanetetrol
Phytane	Ph
Pristane	Pr
Pr/Ph	Ratio of pristane over phytane

UCM	Unresolved complex mixture
C_{17}/pristane	Ratio of C_{17} n-alkane to pristane
CPI_s	Carbon preference indices
C_{18}/phytane	Ratio of C_{18} n-alkane to pristane
ACL	Average chain length
TARs	Terrigenous/aquatic ratios
MH	Major hydrocarbon
GC-MS	Gas Chromatography-Mass Spectrometry
SIS	Surrogate Internal Standard
IIS	Internal Injection Standard
UNCLOS	United Nation Convention of the Law of the Sea
MARPOL	Marine Pollution Convention
OPRC	Oil Pollution Response Cooperation
CLC	Civil Liability Convention
SOMCP	Straits of Malacca Contingency Plan
DOE	Department of Environment
SCSCP	South China Sea Contingency Plan
NOSCP	National Oil Spill Contingency Plan
EEZ	Exclusive Economic Zone
PIMMAG	Petroleum Industry of Malaysia Mutual Aid Group
ASEAN	Association of Southeast Asian Nations
OSRAP	Oil Spill Response Action Plan
MP/P	Ratio of methylphenanthrenes over phenanthrene
(MFlu+MPyr)/Flu	Ratio of methylfluoranthenes plus methylpyrenes over fluoranthene
Flu/(Flu +Pyr)	Ratio of fluoranthene to fluoranthene plus pyrene
Ant/(Ant+Phe)	Ratio of anthracene to phenanthrene plus anthracene
B(a)An/(B(a)An+Chry)	Ratio of benz(a)anthracene to benz(a)anthracene plus chrysene

IcdP/(IcdP+BghiP)	Ratio of indeno(1,2,3-cd)pyrene to indeno(1,2,3-cd)pyrene plus benzo(g,h,i)perylene
Phe/Ant	Ratio of phenanthrene to anthracene
Flu/Pyr	Ratio of fluoranthene to pyrene
LMW/HMW	Ratio of Low Molecular Weight over High Molecular Weight
SPMD	Semi-permeable membrane devices
EROD	Ethoxyresorufin-O-deethylase
µs/cm	Microsiemens per centimeter
ppt	Parts per thousand
NTU	Nephelometric Turbidity Unit
Na_2SO_4	Sodium sulfate
LABs	Linearalkylbenzenes
BC	Black carbon
K_{ow}	octanol:water partition coefficient
ST	Station
nd	Not detected
∑cPAHs	Sum of carcinogenic PAHs
BAF	Biota bioaccumulation factor
T_s	18α(H)-22,29,30-trisnorneohopane
T_m	17α(H)-22,29,30-trisnorhopane
MECO	Middle East Crude Oil
SEACO	South East Asia Crude Oil
T_m/T_s	Ratio of 17α-22,29,30-trisnorhopane over 18α-22,29,30-trisnorhopane
C_{29}/C_{30}	Ratio of 17α, 21β (H)-30-norhopane to17α, 21β (H)-hopane
C_{29}/C_{30}	Ratio of 17α, 21β (H)-30-norhopane to17α, 21β (H)-hopane
ΣC_{31} -C_{35}/C_{30}	Ratio of sum of 17α, 21β (H)-C_{31} homohopane to 17α, 21β (H)-C_{35} homohopane relative to 17α, 21β (H)-hopane
FRI	Fisheries Research Institute

CHAPTER 1

INTRODUCTION

1.1 General introduction

It is beyond dispute that environmental pollution is one of the most challenging issues in the world today. Human's ambition towards economic growth and improvement of life quality led to development of cities in 20th century. It was followed by serious environmental problems, in particular pollution produced by petroleum. Petroleum and petroleum products consist of various chemicals including hydrocarbon compounds. Normal alkanes (n-alkanes), hopanes and polycyclic aromatic hydrocarbons (PAHs) are among these hydrocarbon compounds (Youngblood and Blumer, 1975).

Peninsular Malaysia is located in the Southeast Asia, land bordering with Thailand in north, and maritime bordering with Indonesia, Singapore, and Vietnam. Climatic condition in Peninsular Malaysia is characterized by heavy rainfall as is common for tropical rainforest climate. Peninsular Malaysia is surrounded by the South China Sea in the east and the Strait of Malacca in the west. Population of Malaysia was recorded at 30 millions in the year 2015 ("Department of Statistics Malaysia", 2015).

The Strait of Malacca is as wide as 300 miles in the north part, while it is as narrow as 3 miles in the south part near Singapore. The average depth of the Strait of Malacca is below 23 meter (Kasmin, 2010). Generally, the Strait of Malacca is known as a narrow water way. The Strait of Malacca is similar to a funnel in shape and is the shortest shipping route for transportation of oil tankers from Middle East to Asian countries such as Japan and China. Around 75000 ships go through the Strait of Malacca each year containing different dangerous materials including oil and oil products (Kasmin, 2010). Peninsular Malaysia, especially west coast has gone through rapid industrialization and urbanization during past few decades giving rise to release of petroleum pollutants from various anthropogenic sources.

1.2 Problem statement

Previous studies indicated that with increasing industrialization and urbanization, pollution problems related to petroleum and its products have become more significant in Malaysia (Bakhtiari et al., 2009; Mirsadeghi et al., 2013; Raza et al., 2013; Retnam et al., 2013; Sakari et al., 2011; Shahbazi et al., 2010a, 2010b; Zakaria et al., 2002). West coast of Peninsular Malaysia lies directly to the Strait of Malacca where a great deal of marine-based oil pollution occurs as a result of heavy oil tanker traffic. Indeed, west coast of Peninsular Malaysia is highly prone to accidental marine oil spills. The Strait of Malacca is narrower in south of Peninsular Malaysia, increasing the possibility of accidental oil spills (Figure 1.1). The accidents between Nagasaki Spirit and Ocean Blessing in 1992, and also Diego Silang in 1976 and Showa Maru in 1975 can be named as the three most significant oil spills in the history of Malaysia. Since 2007, over 80 oil spill incidents were reported by Malaysian Marine Department in the Strait of Malacca (Malaysian Marine Department, 2012).

West coast of Malaysia is highly populated and urbanized with numerous rivers flowing through the region that receive land-based pollutants such as municipal

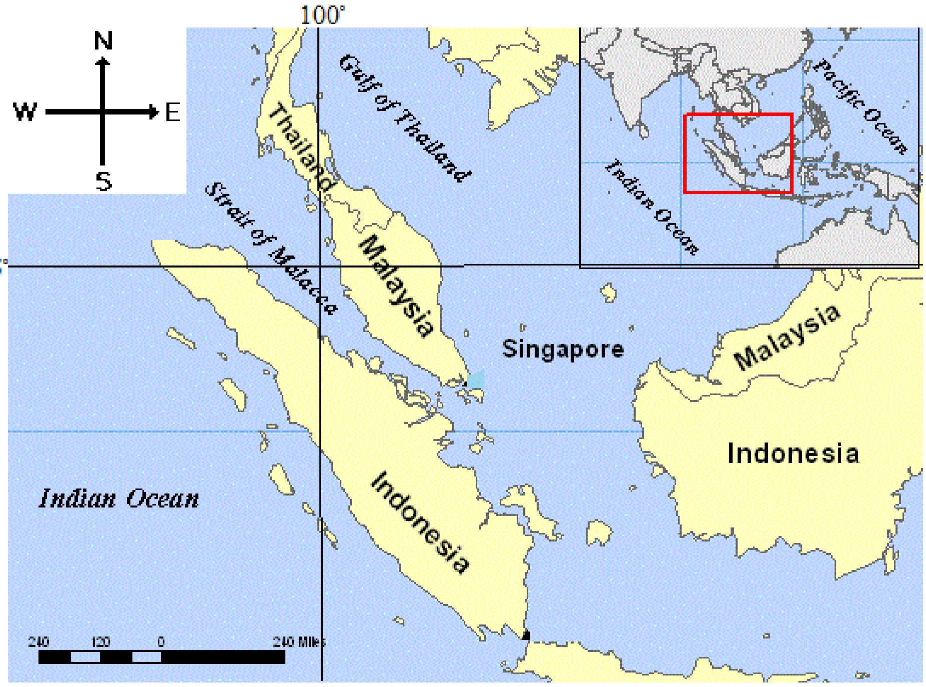

Figure 1.1 Map of the Strait of Malacca ("See-Seek", 2013)

effluents, agricultural effluents, and industrial discharges. The rise of automobiles itself can contribute enormously to anthropogenic pollutants especially in big cities such as capital city of Kuala Lumpur. Therefore, rivers in this region carry petroleum pollutants from various anthropogenic inputs (Shahbazi et al., 2010). There are more than 20 rivers in west coast of Peninsular Malaysia all ending in the Strait of Malacca. In addition, boating activities such as fishing, sailing, and recreational activities increase the possibility of petroleum pollutants entering into aquatic environment in west coast of Peninsular Malaysia (Mirsadeghi et al., 2011).

Nonetheless, pollution in east coast of Peninsular Malaysia commonly originated from urban locations since east coast is less industrialized. However, industrial areas are growing in east coast recently and petroleum pollution in east coast need to be addressed as well (Sakari et al., 2008a).

Petroleum pollution can also be transported from further areas by atmospheric transportation, global ocean circulation and oil transportation through international marine routes (Figure 1.2). In Malaysia, the highest petroleum pollution is recognized in coastal areas where majority of the human activities are performed (Mirsadeghi et al., 2011).

Mangrove oyster *(Crassostrea belcheri)* habitat is mangrove ecosystems located in coastal areas, therefore, petroleum pollutants entering in coastal areas can be available to the oysters. These organic pollutants can be absorbed by the oysters through filtration of water and pass through food chain to humans at the top of the food chain. Some of these hydrocarbons have mutagenic and carcinogenic characteristics (Neff, 1979).

1.3 Significance of study

As it was mentioned earlier, increasing industrialization and urbanization has given rise to release of various petroleum hydrocarbon pollutants from different anthropogenic sources to the aquatic environment in west coast of Peninsular Malaysia. Some of these pollutants are known as carcinogens and mutagens causing serious health issues to organisms including humans. Controlling measures are inevitable in order to reduce the implications caused by the presence of these pollutants in the aquatic environment. Therefore, it is of great significance to determine the distribution and sources of petroleum hydrocarbons (PHC) as the prerequisite for any major steps to control the release of these pollutants to the environment.

Once PHC enter aquatic environment, aquatic organisms are exposed to these pollutants, therefore, they can be absorbed by the aquatic organisms. However, PHC are not available to aquatic organisms at the same degree. In other words, different bioavailability of PHC to aquatic organisms determine organisms body burden of these pollutants.

Therefore, this study aims at determining the sources and distribution of PHC including normal alkanes (n-alkanes), hopanes and polycyclic aromatic hydrocarbons (PAHs) in the sediment and mangrove oyster *(Crassostrea belcheri)* in the west coast of Peninsular Malaysia. In addition, the bioavailability of PHC to the oysters from the sediment is another purpose of this study to realize absorption pattern of PHC by the oysters.

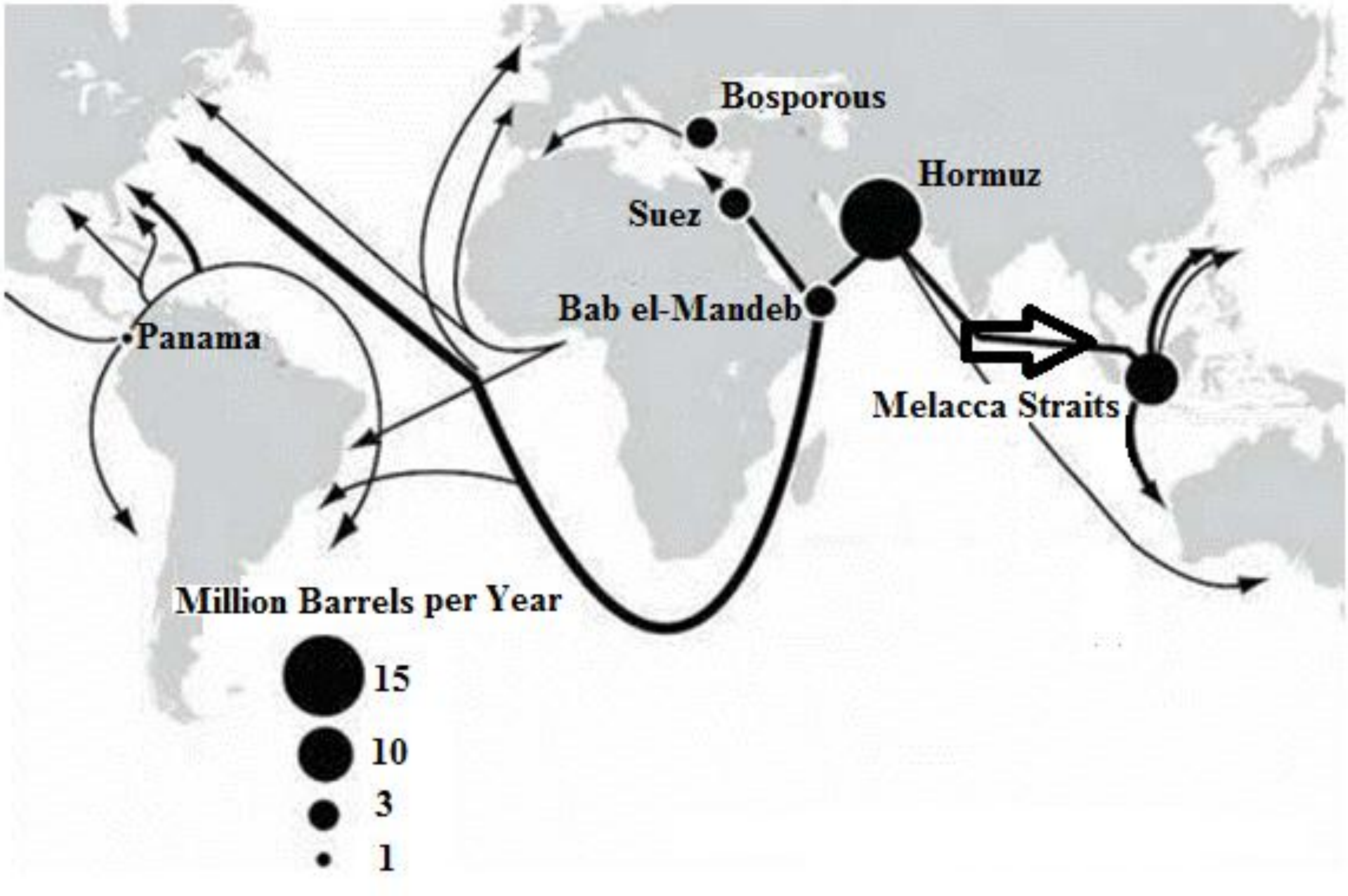

Figure 1.2 International marine routes for oil transportation (EIA, 2011)

1.4 Research objectives

1. To assess the concentrations of n-alkanes, hopanes, and PAHs in sediment and mangrove oyster *(Crassostrea belcheri)*;
2. To identify source of n-alkanes, hopanes and PAHs, in sediment and mangrove oyster *(Crassostrea belcheri)* using diagnostic ratios; and
3. To determine the utility of mangrove oyster *(Crassostrea belcheri)* as a biomonitor species for petroleum hydrocarbons.

1.5 Research scope

In order to investigate distribution, source and bioavailability of PHC including n-alkanes, hopanes and PAHs from sediment to mangrove oyster *(Crassostrea belcheri)*, surface sediment and oyster samples were collected once from west coast of Peninsular Malaysia between January to May 2013. All sampling locations were covered with mangrove trees. Moreover, based on field observation mangrove ecosystems were less impacted by human activities in the Merbok River, Muar River and Pulau Merambong, while they were more impacted in the Klang River and Prai River with lower tree densities. Various diagnostic ratios and indices were applied to distinguish petrogenic, pyrogenic and biogenic sources of PHC in the sediment and oyster. The focus of this study was on 16 parent PAHs and among alkylated PAHs, only methylphenanthrene was analysed.

1.6 Research hypotheses

The hypotheses for this research are as below:

1. West coast of Peninsular Malaysia receives petroleum hydrocarbons from various anthropogenic sources.
2. More industrialized and urbanized areas with more intense human activities receive higher amounts of petroleum hydrocarbons.
3. Bivalve molluscs including oysters accumulate high amounts of petroleum hydrocarbons in their body.

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