



**UNIVERSITI PUTRA MALAYSIA**

**GREEN-LIPPED MUSSELS (*PERNA VIRIDIS*) AS BIOMARKERS OF  
PETROLEUM HYDROCARBON CONTAMINATION IN SELECTED  
COASTAL WATERS OF PENINSULAR MALAYSIA**

**AZADEH SHAHBAZI**

**FPAS 2009 4**



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**By**

**AZADEH SHAHBAZI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy of  
Science**

**April 2009**



## **DEDICATION**

This work is dedicated to my dear mother, and my sister, without whose support and love I would be lost, and to my supervisor, close friends, who always believed in me and encouraged me to do my best and to reach my goals.



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

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**April 2009**

**Chairman: Associate Professor Mohamad Pauzi Zakaria, PhD**

**Faculty: Environmental Studies**

Over the past few decades, Malaysia is rapidly developing towards being an industrial country. There has been a growing concern in recent years regarding possible harmful effects of industrialization to living aquatic organisms of pollutants released into the marine environment. Polycyclic aromatic hydrocarbons (PAHs) are one of the most important classes of micro-organic pollutants which give rise to this concern. PAHs consist of two or more fused aromatic benzene rings and because of their hydrophobic and persistent nature, they accumulate in tissue of aquatic biota such as mussels. Monitoring of toxic substances in the aquatic environment using green mussels (*P. viridis*) as a biomonitoring agent is commonly used because of its wide geographical distribution, immobile, easy sampling, tolerance of a wide range of salinity and comparatively long life-span. The present finding focuses on spatial distribution and major sources of petroleum hydrocarbons in green mussels (*P. viridis*) in Malaysian coastal environment.



The results of PAHs monitoring in total soft tissues of *P. viridis* showed that among the seven stations, mussels collected near Penang Bridge had the highest PAH concentrations (110500 ng/g lipid wt) and Sebatu had the lowest (766 ng/g lipid wt) concentrations of PAHs. The ratios of methyl phenanthrenes to phenanthrene (MP/P ratio) for Penang, Pasir Puteh and Tebing Runtuh (Johore Straits) were greater than 2, indicating extensive input of petrogenic PAHs. The results suggested that male individuals had significant higher concentrations of PAHs in their soft tissues when compared to female individuals. Negative significant correlations ( $r = -0.890$ ,  $p < 0.01$ ) and ( $r = -0.0655$ ,  $p < 0.05$ ), were found between weight and total of PAHs in female and male individuals respectively. This indicated that body weight of each individual was not affected by the PAHs concentrations.

This study investigates the distribution of trace organic contaminants in tissues (mantle, gills, foot, gonad, muscle, byssus remaining soft tissues), and shell of the *P. viridis* collected from 8 different geographical locations along the coastal waters of Peninsular Malaysia. The results showed along the eastern part of Johore Straits two stations namely Kg. Pasir Puteh and Senibong recorded the highest bioavailability and contamination by PAHs. Among the different organs studied, gonad was found to be the best organ for biomonitoring of PAHs. The results also indicated significant concentrations of lower molecular weight (LMW) PAHs in different tissues in comparison to higher molecular weight (HMW) of PAHs not only due to greater bioavailability of LMW PAHs but also it could be related to a partial biotransformation of the HMW PAHs rather than to a larger bioavailability of the more water soluble compounds. Molecular indices based on isomeric PAH ratios used to differentiate the pollution sources, and clearly indicate the differences in molecular distribution of PAHs in different soft tissues of *P. viridis*. Furthermore, the

composition and distribution of n-alkanes (C<sub>10</sub>-C<sub>36</sub>) and isoprenoid (pristane and phytane) hydrocarbons and hopanes were investigated in different STs and hard tissues of (*P. viridis*). Total n-alkanes and hopanes concentrations ranged from 154 to 1396597µg/g and 8 to 1681 µg/g dry weights, respectively. The highest levels of alkanes and hopanes were found in gills, and gonads of *P. viridis*, whereas adductor muscle and shell did not absorb significant amounts of hydrocarbons. The calculated hydrocarbon indexes suggest that petrogenic contamination was the main source of n-alkanes, while both natural and petrogenic sources contributed hopanes to the water column. All the present findings indicated that *P. viridis* is a good biomonitor of contamination and bioavailability of hydrocarbon pollution in the coastal waters of Peninsular Malaysia.

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

**KUPANG (*PERNA VIRIDIS*) SEBAGAI PENANDA PENCEMARAN  
HIDROKARBON PETROLEUM DALAM PERAIRAN SISIR PANTAI  
TERPILIH DI SEMENANJUNG MALAYSIA**

Oleh

AZADEH SHAHBAZI

**April 2009**

**Pengerusi : Profesor Madya Mohamad Pauzi Zakaria, PhD**

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Beberapa dekad yang lalu, Malaysia dengan pesatnya membangun sebagai negara perindustrian. Kesedaran tentang bahaya pencemar perindustrian yang dilepaskan ke dalam persekitaran laut terhadap organisma yang tinggal di persekitaran akuatik kian meningkat sejak beberapa tahun kebelakangan ini. Hidrokarbon polisiklik beraroma (PAHs) adalah salah satu daripada kelas pencemar mikro-organik yang penting di mana ia memberi kesedaran yang tinggi. PAHs mempunyai dua atau lebih gelang benzene beraroma yang bercantum dan oleh kerana sifat hidrofobik dan kebolehannya kekal secara natural, maka ia berkumpul dalam tisu biota akuatik seperti tiram. Pemerhatian bagi bahan toksik dalam persekitaran akuatik menggunakan tiram hijau (*P. viridis*) sebagai agen pemerhati sering digunakan kerana taburan geografinya adalah luas, tidak bergerak, mudah dikutip, boleh bertoleransi terhadap saliniti yang pelbagai dan juga mempunyai jangka hayat yang panjang. Penemuan terbaru memfokuskan kepada taburan dan sumber utama



hidrokarbon petroleum dalam tiram hijau (*P. viridis*) dalam sekitaran pantai di Malaysia.

Keputusan pemerhatian PAHs bagi 7 stesen untuk tisu lembut *P. viridis* menunjukkan tiram yang dikutip berhampiran jambatan Pulau Pinang mempunyai kepekatan PAHs yang paling tinggi (110500 ng/g berat lemak) manakala Sebatu mempunyai kepekatan PAHs yang paling rendah. Nisbah bagi methylphenanthrene terhadap phenanthrene (nisbah MP/P) mencadangkan individu jantan mempunyai kepekatan PAHs yang tinggi dalam tisu lembutnya berbanding individu betina. Hubungan negatif yang agak jelas iaitu  $r = -0.890$  dan  $p < 0.01$  bagi individu betina manakala  $r = -0.0655$  dan  $p < 0.05$  bagi individu jantan telah dikenalpasti antara berat dan jumlah PAHs. Ini menunjukkan berat badan bagi setiap individu tidak mempengaruhi kepekatan PAHs.

Kajian ini menyiasat taburan pencemar organic dalam tisu (lapisan luar, insang, kaki, organ pembiakan, otot, tisu lembut) dan cengkerang bagi *P. viridis* yang dikutip daripada 8 lokasi yang berbeza geografinya disepanjang perairan pantai Semenanjung Malaysia. Keputusan menunjukkan di sepanjang kawasan timur Selat Johor iaitu Kg. Pasir Puteh dan Senibong merakamkan kehadiran biologi dan pencemaran PAHs yang tinggi. Dalam organ-organ yang dikaji, organ pembiakan didapati sebagai organ terbaik untuk proses pemerhatian PAHs secara biologi. Keputusan juga menunjukkan kepekatan yang jelas bagi PAHs yang mempunyai berat molekul rendah (LMW) dalam tisu yang berbeza berbanding PAHs yang mempunyai berat molekul tinggi (HMW), di mana ia bukan sahaja dipengaruhi oleh PAHs LMW yang kehadiran biologinya tinggi, tetapi ia juga mungkin berkaitan dengan biotransformasi separa PAHs HMW berbanding kehadiran secara biologi



yang tinggi oleh sebatian yang lebih larut air. Indeks molekul berasaskan nisbah isomer PAHs digunakan bagi membezakan punca pencemaran dan dengan jelasnya menunjukkan perbezaan dalam taburan PAHs bagi tisu lembut *P. viridis*. Tambahan pula, komposisi dan taburan n-alkana (C<sub>10</sub>-C<sub>36</sub>) dan isoprenoid (pristine dan phytane) hidrokarbon serta hopane telah disiasat dari segi STs dan tisu keras. Jumlah kepekatan n-alkana dan hopane adalah berkala dari 154 kepada 1396597 µg/g dan 8 kepada 1681 µg/g berat kering mengikut turutan. Tahap tertinggi alkane dan hopane telah ditemui dalam insang dan organ pembiakan *P. viridis*, manakala otot adduktor dan cengkerang tidak menyerap jumlah hidrokarbon yang banyak. Indeks hidrokarbon yang dikira mencadangkan pencemaran petrogenik merupakan punca utama bagi n-alkana, manakal kedua-dua punca semulajadi dan petrogenik menyumbangkan hopane kepada saluran air. Kesemua penemuan terbaru ini menunjukkan *P. viridis* adalah biopemerhati yang baik bagi menentukan tahap pencemaran dan kehadiran secara biologi bagi pencemaran hidrokarbon di kawasan perairan pantai Semenanjung Malaysia.

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I certify that an Examination Committee has met on \_\_\_\_\_ to conduct the final examination of Azadeh Shahbazi on her Doctor of Philosophy thesis entitled “Distribution and Sources of Petroleum Hydrocarbons using Green-lipped mussels (*Perna viridis* in selected coastal waters of peninsular Malaysia” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 9 July 2009



## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotation and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**AZADEH SHAHBAZI**

Date: 5 June





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## LIST OF ABBREVIATIONS

Anth	Anthracene
B(a)A	Benzo (a) anthracene
BAF	Bioaccumulation Factor
B(a)P	Benzo (a) pyrene
BCF	Bioconcentration Factor
B(e)A	Benzo (e) acephenanthrylene
B(e)P	Benzo (e) pyrene
B(k)F	Benzo (k) fluoranthene
Chry	Chrysene
DB(a,h)A	Dibenzo (a,h) anthracene
DCM	Dichloromethane
Fluo	Fluoranthene
GC-MS	Gas Chromatography-Mass Spectrometry
LMW/HMW	Lower Molecular Weight/ Higher Molecular Weight
HMW	Higher Molecular Weight
IIS	Internal Injection Standard
LMW	Lower Molecular Weight
2-MA	2-methylanthracene
1-MP	1-methylphenanthrene
2-MP	2-methylphenanthrene
3-MP	3-methylphenanthrene
9-MP	9-methylphenanthrene



MP/P	Methylphenanthrene/Phenanthrene
1-MP	1-methylpyrene
Naph	Naphthalene
P-terph-d <sub>14</sub>	P-terphenyl-d <sub>14</sub>
PAHs	Polycyclic Aromatic Hydrocarbons
perylene	Perylene
Phe	Phenanthrene
Py	Pyrene
SIS	Surrogate Internal Standard
STs	Soft tissues



## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of study

During the past few decades, South and Southeast Asia has exhibited rapid population growth, and drastic industrialization and urbanization. Within this region the population exceeded 2 billion in 2000, with an annual growth rate of 3%. Large population, active industrialization and an increase in the use of vehicles have increased the potential threat of pollution to coastal environments in this region. In recent years, Malaysia's population, as well as its concomitant urban and industrial development has expanded rapidly (Zakaria et al., 2002; Boonyatumanond et al., 2006).

The coastal zone of west Peninsular Malaysia are heavily populated and lined by urban, industrial, agricultural areas and shipping ports, this area comprises about 100,000 hectares and this contributes significantly to the coastal productivity (Ong, 1985). In addition, the coasts of Peninsular Malaysia are important for fisheries, recreational and marine activities, tourism and maintaining the bio-diversity in the tropical area which monitoring reports have shown that the coastal waters are polluted by organic contamination (Zakaria et al., 2001). Trace organic contaminants present in the coastal ecosystem generally includes polychlorinated biphenyls

(PCBs), DDT insecticide and its derivatives, n-alkanes, and Polycyclic Aromatic Hydrocarbons (PAHs).

## **1.2 Problem statement**

Malaysian coasts are subjected to various threats of petroleum pollution including routine and accidental oil spill from tankers, spillage of crude oils from inland and offshore oil fields, and run-off from land-based human activities. Due to its strategic locations, the coasts of Peninsular Malaysia are experiencing a serious problem of pollution by hydrophobic organic micropollutants, including PAHs (Zakaria et al., 2001). In addition, geographical distribution and abundance of mussels along the east coast is not as high as that in the west coast of the Peninsular (Yap et al., 2004c).

In this study, use of marine mussels as “sentinel” organisms is one good approach to monitor or assess chemical organic pollution in the aquatic/marine environments. The major advantages of the use of mussels as biomonitoring tool are their direct measurement of the biological availability of pollutants and their time integration of the ambient pollution conditions at the site of collection. While most of the research work in Malaysia have been localized and limited to the analyses of heavy metals in sediments (Ismail et al., 1993; Mat et al., 1994; Yap et al., 2002)., Therefore, a more detailed and comprehensive study to monitor organic pollution, specific aspects of uptake, retention, and releases of organic contamination using different organs of mussels need more research. At present, only limited information is available concerning the levels of organic micropollutants such as PAHs in green mussels (*P. viridis*) from coastal waters of Peninsular Malaysia. Therefore, this study provides a

bench-mark for data on the distribution and sources of anthropogenic contaminations in this region, which is essential in evaluating temporal and spatial variation and effect of future regulatory measure.

### **1.3 Significance of study**

Environmental protection and the prevention of pollution became one of the main scientific, social and economic activities towards the end of the 20<sup>th</sup> century. However, the increasing concentrations of toxic substances in biota and bivalves and their accumulation along food chains reported in many coastal areas is a sign that degradation of the natural environment is still in progress (Porte and Albaigés 1993, Baumard et al. 1998b). Contamination of coastal areas can have an adverse effect on human health and on natural resource. Therefore, one of the primary aims of environmental quality studies is to understand the impacts of anthropogenic compounds such as organic micropollutants on the ecosystem, in order to minimize or prevent adverse effects.

Among the bivalves and intertidal molluscs, *P. viridis* is one of the species that can be found along the west coast of Peninsular Malaysia (Ismail, 2000; Yap et al., 2003a,b, 2004a,b). Although the use of green mussels (*P. viridis*) provided the integrated contamination by petroleum hydrocarbons in coastal waters, this information on hydrocarbon bioavailability is hardly provided by the analyses of hydrocarbon concentrations in seawater and sediment samples. Since the petroleum hydrocarbon bioavailability is of ecotoxicological significance, the use of green mussels as biomonitoring agent in coastal waters is suitable. Besides, knowledge of



the distribution of hydrocarbon contaminations in different organs/tissues of *P. viridis* is useful in order to identify specific organs that may be particularly selective and sensitive to accumulation of hydrocarbons. Therefore, petroleum hydrocarbons accumulated in the soft tissues (STs) of marine mussels will be a measure of the bioavailability of hydrocarbons originating from both natural and anthropogenic sources. However, the petroleum hydrocarbons levels in the different STs of marine mussels have not yet been as widely reported as those in the total STs in coastal waters of west Peninsular Malaysia.

Therefore, this study aimed to determine background concentrations of organic micropollutants such as Alkanes, hopanes and PAHs in the green-lipped mussels (*P. viridis*) as main seafood in Southeast Asia countries. In addition, the tissue distribution patterns of hydrocarbons in the different STs and shell of *P. viridis* collected from contaminated, less-contaminated, and clean sites were determined and compared which has not yet been documented in the literature in coastal waters of Peninsular Malaysia.

#### **1.4 Study Objectives**

Major objectives of this study are as follows:

1. To provide status of petroleum pollution at the compound-specific levels, especially PAHs, and to understand their sources and transport pathways.