UNIVERSITI PUTRA MALAYSIA

THE KINETICS OF UPTAKE AND RELEASE OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE GREEN MUSSELS *Perna viridis* FOR BIOMONITORING OF MARINE POLLUTION

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By

ALI MASHINCHIAN MORADI

Thesis Submitted in Fulfilment of the Requirement for the Degree of Doctor of Philosophy in the Graduate School Universiti Putra Malaysia

December 2001

DEDICATION

To my dear family, my parents, wife and sisters who have been my source of inspiration, wisdom and strength through the most difficult times of my life.

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

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Chairman: **Associate Professor Jambari Hj. Ali, Ph.D.**

Faculty: **Science and Environmental Studies**

The kinetics of accumulation and depuration of polycyclic aromatic hydrocarbons (PAHs) in green mussel *Perna viridis* has never been investigated under field conditions. In this study, green mussels were used to assess the spatial distribution of PAH pollution along the west coast of Peninsular Malaysia, and to determine PAHs uptake and release kinetics in this species under field conditions. In addition, the effect of sex and exposure duration on the uptake and release kinetics of these contaminants were investigated. Overall more than 1500 individual mussels were collected, soft tissues were homogenized, extracted, cleaned up, fractionated and concentration of 17 individual PAH compounds were analysed using Gas Chromatography-Mass Spectrometry (GC-MS). Among the eleven stations sampled in 1997 and 1998 along the Straits of Malacca, mussels from Pasir Puteh (01° 26.05' N and 103° 55.94' E) had the highest (2855-3450 ng/g lipid weight) and from Pasir Panjang (02° 24.96' N and 101° 56.54' E) the lowest (278-366 ng/g lipid weight) levels of PAHs. As a result, Pasir Puteh was selected as the "hot-spot" and Pasir

Panjang as the "clean site" to further conduct the kinetics experiments. The PAH levels in mussels from Malaysian coastlines in comparison with the polluted areas worldwide were found to be relatively low. At the end of both uptake and release experiments (28 and 36 days, respectively), PAH levels in transplanted and back-transplanted mussels were not significantly different ($p>0.05$) from those found in natives (controls in Pasir Puteh and Pasir Panjang, respectively). It was found that sex did not make a significant difference ($p>0.05$) in the uptake and release rates. Similarly, duration of exposure did not significantly affect the release rates. However, significant differences ($p<0.05$) were found both in uptake and release rates when target PAHs were divided into three, four and five to seven-ring compounds. In general, rapid rates of uptake and release of relatively less lipophilic PAHs were observed, indicating that the primary mechanism of bioaccumulation of these lipophilic pollutants in *Perna viridis* complies with the concept of lipid-water partitioning equilibrium. The biological half-life of individual PAHs ranged from 7 to 29 days. This study illustrated that green mussels are a useful tool in monitoring of PAHs. However, due to relatively rapid rates of uptake and release of these compounds, to get an average level of PAHs over a long period, "time-bulking" should be considered for future biomonitoring programmes using this species.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KINETIK PENGAMBILAN DAN PELEPASAN POLISIKLIK AROMATIK HIDROKARBON (PAHs) DI DALAM KUPANG *Perna viridis* BAGI PEMANTAUAN BIOLOGI PENCEMARAN MARIN

Oleh

ALI MASHINCHIAN MORADI

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Kinetik pengumpulan dan depurasi polisiklik aromatik hidrokarbon (PAHs) di dalam kupang *Perna viridis* tidak pernah dikaji di bawah keadaan lapangan. Dalam kajian ini, kupang telah digunakan untuk mengenalpasti ruang taburan pencemaran PAHs di sepanjang persisiran pantai barat Semenanjung Malaysia, dan menentukan kinetik pengambilan dan pelepasan bagi PAHs di dalam spesies ini di bawah keadaan lapangan. Disamping itu, kesan jantina dan jangkamasa pendedahan kepada kinetik pengambilan dan pelepasan terhadap pencemar ini telah dilakukan. Keseluruhannya, lebih daripada 1500 individu kupang telah disampel, tisu kupang telah dihomogenat, diekstrak, dicuci, dipecah dan kepekatan bagi 17 individu sebatian PAHs telah dianalisis dengan menggunakan gas kromatografi spektrometri jisim (GC-MS). Keputusan bagi fasa satu menunjukkan antara 11 stesen yang disampel di Selat Melaka, kupang dari Kg. Pasir Puteh (01° 26.05' N dan 103° 55.94' E) mempunyai nilai tertinggi (2855-3450 ng/g berat lipid) dan Pasir Panjang (02° 24.96' N dan

101° 56.54' E) mempunyai nilai terendah (278-366 ng/g berat lipid) bagi tahap PAHs yang dikaji. Oleh itu, Kg. Pasir Puteh telah dipilih sebagai "stesen tercemar" dan Pasir Panjang sebagai "stesen bersih" untuk eksperimen kinetik. Keputusan kajian menunjukkan tahap PAHs dalam tisu kupang di persisiran pantai Malaysia adalah rendah jika dibandingkan dengan tahap di kawasan tercemar lain di dunia. Pada akhir eksperimen biopenimbunan dan depurasi (28 hari, dan 36 hari) tahap PAHs bagi kupang yang dipindah dan yang dikembalikan ke tempat asal tidak mencapai tahap yang signifikan ($p>0.05$) berbanding dengan kupang asal (kawalan - Kg. Pasir Puteh dan Pasir Panjang). Keputusan kajian telah menunjukkan jantina tidak menunjukkan perbezaan yang signifikan ($p>0.05$) dalam kadar pengambilan dan pelepasan. Pada masa yang sama, masa pendedahan tidak memberi kesan ke atas kadar pelepasan. Bagaimanapun, perbezaan yang signifikan ($p<0.05$) telah didapati bagi kadar pengambilan apabila PAHs sasaran dibahagikan kepada sebatian gegelang tiga, empat, lima hingga tujuh. Secara amnya, kadar pengambilan dan pelepasan yang cepat bagi PAHs telah berlaku bagi PAHs yang lebih lipofilik. Ini telah menunjukkan bahawa mekanisme primer untuk pencemar lipofilik didalam *Perna viridis* menepati konsep keseimbangan pembahagian "lipid-air" Separuh hayat biologi individu PAHs berjulat 7 hingga 29 hari. Kajian in menunjukkan kupang adalah bahan yang sangat berguna untuk pemantauan pemonitoran. Bagaimanapun, oleh kerana kadar pengumpulan dan pelepasan yang cepat bagi sebatian tersebut demi mendapatkan tahap hitung panjang PAHs "time-bulking" haruslah diambilkira dalam kajian pemonitoran PAHs dimasa hadapan.

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I certify that an Examination Committee on 26th January 2002 to conduct the final examination of Graduate Student on his Doctor of Philosophy thesis entitled "The Kinetics of Uptake and Release of Polycyclic Aromatic Hydrocarbons in the Green Mussels, *Perna viridis*, for Biomonitoring of Marine Pollution" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra 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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Date: 14 MAR 2002

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citation 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.

Ali Mashinchian Moradi

Date: 07/02/2002

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LIST OF ABBREVIATIONS

PAHs:	Polycyclic aromatic hydrocarbons
BCF:	Biological concentration factor
BHL:	Biological half-life
$t_{90\%}$:	Time required to reach 90% uptake equilibrium
Kow:	Octanol-water partition coefficient
k_1:	Uptake rate constant
k_2:	Release rate constant
GC-MS:	Gas chromatography mass spectrometry
IIS:	Internal injection standard
SIS:	Surrogate internal standard
Lipid wt.:	Lipid weight basis
Wet wt.:	Wet weight basis
DBT:	Dibenzothiophene
PHN:	Phenanthrene
ANT:	Anthracene
2MANT:	2-methyl anthracene
2MPHN:	2-methyl phenanthrene
FLU:	Fluoranthene
PYR:	Pyrene
RET:	Retene
1MPYR:	1-methyl pyrene
BaNT:	Benzo(a)anthracene
CHR:	Chrysene
BkFLU:	Benzo(k)fluoranthene
BeP:	Benzo(e)pyrene
BaP:	Benzo(a)pyrene
BeACPH:	Benzo(e)acephenanthrylene
DBahANT:	Dibenz(a,h)anthracene
COR:	Coronene

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The coastal zones of West Peninsular Malaysia are heavily populated and lined by urban, industrial, agricultural areas and shipping ports (DOE, 1995). As a result, the coastal waters of this region receive a broad range of anthropogenic organic micropollutants from various land-based and marine-based sources (Law, 1994). Organic micropollutants are also transported from other regions via atmospheric and global ocean transport systems (Zubir, 1992; Iwata *et al.*, 1993). The Straits of Malacca is one of the busiest waterways in the world and subject to various types of pollution (Zubir, 1992; Law, 1994; DOE, 1995). Oil spills in the Straits of Malacca have been occurring very frequently in the past and have been affecting coastal ecosystems (Law, 1994; Zakaria *et al.*, 1999). Monitoring reports have shown that the coastal waters of West Peninsular Malaysia are polluted by organic contaminants (Sittig, 1985; WHO, 1989; Law and Ravinthar, 1989; Law *et al.*, 1990; Denker *et al.*, 1994; WHO, 1994). These substances may include polychlorinated biphenyls (PCBs), DDT insecticide and its derivatives, and polycyclic aromatic hydrocarbons (PAHs) (Iwata *et al.*, 1993; Iwata *et al.*, 1994; Dominic, 1994). The spatial distribution, levels and sources of PAHs in the Straits of Malacca are not well known.