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ASSESSMENT OF HAZARDOUS SUBSTANCES IN THE SNAIL, Nerita lineata GMELIN, AND SEDIMENTS FROM SELECTED MANGROVE AREAS OF PENINSULAR MALAYSIA

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By

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Rapid growth of the land and marine based industries has generated various contaminations to the coastal areas of Malaysia. Thus, continuous assessments and monitoring of pollutants especially trace metals and polycyclic aromatic hydrocarbons (PAHs) are crucial. The aim of this study is to determine the baseline data, ecological risk and health risk assessments of trace metals and PAHs in mangrove snails, *Nerita lineata* and surface sediments of at least 9 sampling sites in Peninsular Malaysia between 2010 − 2012. Metal analysis was done by using the Atomic Absorption Spectrophotometer (AAS) and Inductively Coupled Plasma-mass Spectrometer (ICPMS). Analysis of PAHs was carried out by using gas chromatography-mass spectrophotometry (GC-MS) while Inter-Simple Sequence Repeat (ISSR) markers were applied for genetic polymorphism study in the snails. All 18 metals in the snails ranged from BDL (below detection limit) – 3235 µg/g dw, with higher accumulation of essential metals in the soft tissues and non-essential metals in the shells and opercula. For sediments, all 18 metals ranged from 0.003 – 48916 µg/g dw. The ∑16 PAHs concentrations (ng/g dw) ranged from 871 – 3066 and 273 – 18030 for soft tissues and sediments, respectively. To determine the ecological risks of habitat on surface sediments, the sediment quality guidelines (SQGs), geochemical indices, potential ecological risk index (PERI) and mean ERM quotient (m-ERM-q) were used. Based on the SQGs, all the metals investigated were unlikely to cause any adverse ecological effects. The ecological risk indices revealed that the pollution and ecological risks of all metals and PAHs were minimal, except for PAHs in JPKetam being 'moderately to highly polluted'. Potential health risks were assessed from calculations of estimated daily intakes (EDI), target hazard quotients (THQ) and total THQ (TTHQ) of the snails' soft tissues for trace metals, while excess cancer risks (ECR) were applied for PAHs assessments. Non-carcinogenic risks estimation (THQ and TTHQ) for trace metals showed that average snails consumers were exposed to low potential health risk whereas the high consumers were at high risks (TTHQ>1). The carcinogenic risks approach (ECR) showed that the snail consumption from most sites were in a range of moderate to high carcinogenic risks exposure (1×10^{-6}< ECR< 1×10^{-4}) to PAHs, implying moderation (<17.86 g/day) in snail consumption. The ISSR markers were found to have positive relationships from comparison of patterns of genetic clustering and trace metal levels of the snail muscle tissues but not PAHs. This implied that the

polymorphic loci could be used as potential biomarkers for metals pollution. Generally this study suggested that all metals and PAHs from the sampling sites were not likely to cause hazardous effects to the environment. Consumption of the snails should be of moderation especially to high consumers of the snails as the data showed possible health risks for both trace metals and PAHs. This study has produced important data of the hazardous pollutants in the snails and sediments which can be used for environmental management in future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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PENILAIAN BAHAN PENCEMAR DALAM SIPUT, *Nerita lineata* GMELIN, DAN SEDIMEN DARI KAWASAN BAKAU DI SEMENANJUNG MALAYSIA

Oleh

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Pertumbuhan pesat dalam industri (daratan dan marin) telah menjana pelbagai pencemaran ke atas kawasan pantai di Malaysia. Oleh itu, penilaian dan pemantauan pencemaran yang berterusan terutamanya logam surih dan hidrokarbon polisiklik aromatik (PAHs) adalah penting. Tujuan kajian ini dijalankan adalah untuk menentukan data asas, risiko ekologi dan risiko kesihatan bagi logam surih dan PAHs dalam siput bakau, *Nerita lineata* dan permukaan sedimen dalam sekurang-kurangnya 9 tapak persampelan di Semenanjung Malaysia dari tahun 2010 - 2012. Analisis logam dilakukan dengan menggunakan 'Atomic Absorption Spectrophotometer' (AAS) dan 'Inductively Coupled Plasma-mass Spectrometer' (ICPMS). Analisis PAH dilakukan dengan menggunakan gas chromatography-mass spectrophotometry (GC-MS) manakala 'Inter-Spesific Sequence Repeat' (ISSR) telah digunakan untuk menkaji polimorfisme genetik dalam siput. Julat kandungan semua 18 logam dalam siput adalah BDL – 3235 μg/g berat kering, dengan akumulasi logam esensial yang lebih tinggi dalam tisu lembut dan logam tidak esensial dalam cengkerang dan operkula. Dalam sedimen, julat semua 18 logam adalah 0.003 – 48916 μg/g berat kering. Kepekatan Σ16 PAH (ng/g berat kering) adalah antara 871 – 3066 and 273 – 18030 untuk tisu-tisu lembut siput dan sedimen, masing-masing. Untuk menentukan risiko ekologi habitat pada permukaan sedimen, garis panduan kualiti sedimen (SQGs), indeks geokimia, indeks risiko potensi ekologi (PERI) dan hasil bahagi min ERM (m-ERM-q) telah digunakan. Berdasarkan kepada SQGs, semua logam dalam kajian ini tidak berkemungkinan untuk menyebabkan kesan ekologi yang buruk. Indeks risiko ekologi menunjukkan bahawa pencemaran dan risiko ekologi untuk semua logam dan PAHs adalah minimum, kecuali PAHs di JPKetam yang dikategorikan sebagai 'sederhana hingga sangat tercemar'. Risiko potensi ke atas kesihatan dinilai melalui anggaran pemakanan harian (EDI), 'total hazard quotient' (THQ) dan jumlah THQ (TTHQ) di dalam tisu lembut siput bagi logam surih, manakala risiko kanser (ECR) telah digunakan untuk penilaian risiko PAHs. Anggaran risiko bukan karsinogen (THQ dan TTHQ) telah menunjukkan bahawa risiko potensi kesihatan yang disebabkan oleh pendedahan kepada logam surih bagi konsumer siput sederhana adalah rendah manakala konsumer siput tinggi adalah berisiko tinggi (TTHQ>1). Anggaran risiko karsinogenik (ECR) pula menunjukkan bahawa risiko yang disebabkan oleh pendedahan kepada PAHs bagi konsumer siput adalah tinggi (1×10^{-6} <ECR <1×10^{-4}) dan pemakanan siput secara sederhana (<17.86 g/day) adalah digalakkan. Analisis

kluster (melalui petanda ISSR) telah mendapati hubungan positif antara corak pengelompokan genetik dan kandungan logam surih di dalam tisu otot siput tetapi tidak untuk PAHs. Ini telah membuktikan bahawa lokus polimorfik berpotensi sebagai penanda biologi untuk pencemaran logam. Secara keseluruhannya, kajian ini telah mencadangkan bahawa semua logam dan PAH dari semua sampel tidak akan membawa kemudaratan terhadap persekitaran. Pengambilan siput secara kesederhanaan dari segi pemakanan terutamanua kepada konsumer tinggi siput. Kajian ini telah menghasilkan data bahan pencemar merbahaya yang penting dalam siput dan sedimen untuk pengurusan alam sekitar pada masa akan datang.

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

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LIST OF ABBREVIATIONS

AC	Average consumers
Ace	Acenapthene
Acy	Acenaphthylene
AHR	Aryl hydrocarbon receptor
Ant	Anthracene
BaA	Benzo(a)anthracene
BaP	Benzo(a)pyrene
BbF	Benzo(b)fluoranthene
BghiP	Benzo(g,h,i)perylene
BkF	Benzo(k)fluoranthene
BSAF	Biota-sediment accumulation factors
Cf	Contamination factor
CF	Conversion factor
Chry	Chrysene
CYP1A	Cytochrome P4501A
DahA	Dibenzo(a,h)anthracene
DCM	Dichloromethane
DDW	Double distilled water
dw	dry weight
EADI	Estimated average daily intake
ECD	Electron capture detector
ECR	Excess cancer risks
ECRA	Excess cancer risks for average consumers
ECRH	Excess cancer risks for high consumers

EDI	Estimated daily intake
EDI	Estimated daily intakes
EF	Enrichment factor
EHDI	Estimated high daily intake
Er^i	Potential risk of individual metal
ERL	Effect range low
ERM	Effect range median
FID	Flame ionization detector
Flu	Fluorene
Fth	Fluoranthene
HC	High consumers
HMW	High molecular weight polycyclic hydrocarbons
IcdP	Indeno[1,2,3-cd]pyrene
IIS	Internal injection Standard
ISQV-high	Interim sediment quality value-high
ISQV-low	Interim sediment quality value-low
LMW	High molecular weight polycyclic hydrocarbons
lw	lipid weight
MS	Mass spectrophotometry
m-ERM-q	Mean effect range median quotient
Nap	Naphthalene
PEL	Probable effect level
Phe	Phenanthrene
Pyr	Pyrene
RI	sum of potential risk of individual metal
SD	Standard deviation

SIS	Surrogate internal standard mixture
SQGs	Sediment quality guidelines
ST	Soft tissues
TCD	Thermal conductivity detector
TEF	Toxic equivalent factor
TEL	Threshold effect level
TEQ/B(a)P_{eq}	Benzo(a)pyrene toxic equivalent quotient
TFM	Tetraflouroethylene Modified
THQ	Target hazard quotients
tPAH	Total polycyclic aromatic hydrocarbons
TTHQ/HI	Total target hazard quotient/hazard index
ww	wet weight

CHAPTER 1

INTRODUCTION

In the field of ecotoxicology studies, marine coastal pollution has always been a matter of concern. With the rapid growth of the industries in Malaysia, various organic and inorganic contaminations were generated to the coastal areas of Malaysia. Inorganic contaminants such as heavy metals occur naturally in the environment. Human activities which cause elevated levels of metals input to the environment will cause detrimental effects on the marine coastal ecosystem. Organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) on the other hand, is an important class of persistent pollutants known to have carcinogenic and mutagenic properties and they are introduced to the environment by two sources of anthropogenic activities namely, pyrogenic and petrogenic (Wang et al., 2014). Such pollution will not only threaten the ecology of marine system but also pose danger to humans, as most marine organisms are consumed by humans. Therefore, a continuous study on monitoring of heavy metal pollution is crucial.

In this study the analyses of pollution levels of trace metals and PAHs were conducted through the assessment biomonitors, sediments and snails, from mangrove areas of Klang (Selangor), Negeri Sembilan and Johore between the period of 2010 – 2011. These sites are chosen due to their location on important shipping lane across the coastal line of Peninsular Malaysia and susceptible to various marine base pollution sources. Various land base industries were observed in these sites. On top of that, mangrove areas occupying majority of the coastal area in Peninsular Malaysia, is an important ecosystem and exchange point of numerous organic and inorganic substances from various sources, i.e. marine, terrestrial or atmospheric (Alongi et al., 2004).

Biomonitoring is a widely procedure that has been practically utilized as a monitoring and assessment tool for heavy metal pollutions in order to remedy the aggravation of the environment. While sediment has a reputation as an ultimate biomonitor for environmental pollution, application of local or endemic biological species are confronted with challenges of the wide range of environmental conditions that may limit the availability of the species (Kang et al., 2000). Apart from their massive species number and ubiquitous distribution in marine coastal ecosystems, the biological data from these species are directly related to the ecological condition and or 'ecological health' of the marine aquatic ecosystem (Campbell, 2002).

The species collected for the current study were the mangrove snails, *Nerita lineata*. The *N. lineata* is a common snail found mainly in the intertidal area of Malaysia, Indonesia and Singapore. This species is studied for it fulfils some basic criteria of a good biomonitor such as easily collected and weighed, abundant, sedentary and of relatively high longevity. It is also observed that many people in this region are taking

N. lineata as source of food. Therefore it is essential to provide the heavy metal data of the snails as a guideline for safety consumption.

Assessment of risks of pollutants, such as metal and PAHs, in sediments were done through three approaches namely, i) established Sediment Quality Guidelines (SQGs), ii) ecological risks indices, and iii) geochemical pollution indexes. Screening-level of risk assessment in the *N. lineata* was conducted through consideration of internationally accepted dietary guidelines and the calculation of estimated daily intakes (EDI), target hazard quotients (THQ) and excess cancer risks (ECR). These guidelines were developed by researchers or organizations (USEPA) and they are widely used as a reliable risk assessment tool.

However, ecotoxicological studies should not be restricted to the context of metal levels as the impact of the pollution is also revealed in the biological response of the organisms. Marine organisms exhibit genetic polymorphisms due to adaptation and response towards environmental contaminants (Basu et al., 2014). Thus, inter-simple sequence repeat (ISSR) markers were used for the analysis of genetic polymorphisms in the *N. lineata*.

The objectives of this study were:

1. To determine the baseline levels of trace metals and PAHs in different parts of the snails, *N. lineata* and sediments from selected mangrove areas of Peninsular Malaysia,

2. To determine the ecological risk assessment of trace metals and PAH on the surface sediments at the mangrove snail's habitats,

3. To determine the human health assessments based on the estimated daily intake and risk indices of trace metals and PAHs in the *N. lineata* collected from selected mangrove areas of Peninsular Malaysia,

4. To investigate the relationships between genetic variation and hazardous substances (trace metals and PAHs) in *N. lineata* collected from selected mangrove areas of Peninsular Malaysia.

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