
**UNIVERSITI PUTRA MALAYSIA**

# *POLYCYCLIC AROMATIC HYDROCARBONS IN THE COASTAL ENVIRONMENT OF PENINSULAR MALAYSIA AND THEIR BIOAVAILABILITY TO Paphia undulata BORN*

**MEHRZAD KESHAVARZIFARD**

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**By**

**MEHRZAD KESHAVARZIFARD**

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

**March 2015**

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**DEDICATION**

This research is dedicated to my father, my mother, my sisters and my brother. I would like to express a warm appreciation to my family for their encouragement, motivating, unwavering support, patience and love during this research.

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

**POLYCYCLIC AROMATIC HYDROCARBONS IN THE COASTAL ENVIRONMENT OF PENINSULAR MALAYSIA AND THEIR BIOAVAILABILITY TO *PAPHIA UNDULATA* BORN**

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**March 2015**

**Chairman: Professor Mohamad Pauzi Zakaria, PhD**
**Faculty: Environmental Studies**

Polycyclic aromatic hydrocarbons (PAHs) with two or more benzene rings are one of the most important classes of pollutants. PAHs contamination is a great global concern due to their negative effects on human health and environmental consequences, such as toxic, mutagenic, carcinogenic, teratogenic and hepatotoxic effects. Therefore, their sources, pathways, distributions and fate in the environment and ecosystems are important to be monitored. Samples were collected from selected rivers and estuaries in Peninsular Malaysia from January to May 2013. Samples were homogenized, freeze-dried, extracted, cleanup, fractionated and analyzed using gas chromatography mass spectrometry.

In this study, the distributions and sources of PAHs in the surface sediments from the Kedah, Merbok, Prai, Perak, Klang and Malacca Rivers and Estuaries and *Paphia undulata* (short-neck clam) from the Kedah, Merbok, Prai, Perak, Klang and Malacca Estuaries were monitored, and also the bioavailability of PAHs to *Paphia undulata* from sediments were evaluated. The concentrations of total PAHs in sediments varied from 195.2 to 7938 ng $g^{-1}$ dry weight (dw) and in *Paphia undulata* varied from 179.97 to 1657.5 ng $g^{-1}$ dw. PAHs in sediments can be classified as moderate in the Kedah and Perak, moderate to high in the Merbok and Malacca and high to very high in the Prai and Klang Rivers and Estuaries. PAHs in the soft tissues of *Paphia undulata* can be classified as moderate.

The comparison of PAHs with Sediment Quality Guidelines (SQGs) indicates that occasionally adverse biological effects may occur at the Stations 1, 2 and 3 of the Klang River, Station 1 of the Prai River and at Station 2 of the Merbok River, and severe adverse biological effects may occur from low molecular weight (LMW) PAHs at the Stations 1 of the Prai River. The diagnostic ratios of individual PAHs in sediments and *Paphia undulata* indicated both petrogenic and pyrogenic origin PAHs with significantly dominance of pyrogenic source.
The calculated biota-sediment accumulation factor (BSAF) values of total PAH ranged from 0.27 to 0.84. The results clearly showed that *Paphia undulata* from the Merbok, Prai, Perak, Klang and Malacca Estuaries had larger BSAF values for LMW PAHs, while those from the Kedah Estuary showed higher BSAF values for high molecular

weight (HMW) from the sediment. The results of this study indicated that acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe) and naphthalene (Nap) had the highest bioavailability, while benzo[k]fluoranthene (BkF), anthracene (Ant), dibenzo [a, h] anthracene (DBA) and benzo [b] fluoranthene (BbF) had the lowest bioavailability. Based on the findings of this study *Paphia undulata* can be introduced as a good biomonitor of contamination and bioavailability of hydrocarbon pollution in estuary ecosystems of Peninsular Malaysia. The results also indicated that under environmental conditions, the sedimentary load of hydrocarbons appears to be one of the factors controlling their bioavailability.

The concentrations of total *n*-alkanes ($nC_{10}$-$nC_{36}$) ranged from 1365 to 15850 and from 1875 to 34270 µg $g^{-1}$ dw, in sediment and short-neck clam samples, respectively. The results of this study indicated that the *n*-alkanes in the sediment and *Paphia undulata* samples originated from diverse sources. Biogenic sources such as algal remains, aquatic organisms, and terrestrial plants are all considered to be a source of *n*-alkanes. The $C_{29}/C_{30}$ hopanes ratios were similar with those of Middle East Crude Oil (MECO), suggesting MECO as a major source of petroleum hydrocarbons for Malaysian sedimentary.

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

**HIDROKARBON AROMATIK POLISIKLIK DALAM PERSEKITARAN KAWASAN PERAIRAN SEMENANJUNG MALAYSIA DAN BIOKETERSEDIAANNYA BERBANDING *PAPHIA UNDULATA* YANG DILAHIRKAN**

Oleh

**MEHRZAD KESHAVARZIFARD**

**Mac 2015**

**Pengerusi: Profesor Mohamad Pauzi Zakaria, PhD**
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Hidrokarbon aromatik polisiklik (PAHs) yang mempunyai dua atau lebih gelang benzena merupakan salah satu kelas bahan pencemar paling penting. Pencemaran PAHs menjadi satu isu global kerana ia menyebabkan kesan negatif kepada kesihatan manusia dan alam sekitar melalui peninggalan kesan toksik, mutagenik, karsinogenik, teratogenik dan hepatotoksik. Oleh itu sumber, laluan, taburan, dan takdir PAHs dalam persekitaran dan ekosistem mesti dipantau. Sampel telah dikumpul dari sungai dan muara sungai terpilih di Semenanjung Malaysia bermula dari Januari sehingga Mei 2013. Kemudian, sampel ini dihomogenkan, dikeringsejukbekukan, diekstrak, dibersihkan, dipecahkan, dan dianalisa menggunakan kromatografi gas spektrometri jisim.

Dalam kajian ini, taburan dan sumber PAHs di permukaan sedimen sungai dan muara sungai di Kedah, Merbok, Prai, Perak, Klang dan Melaka dan dalam *Paphia undulata* (kepah berleher pendek) di muara sungai Kedah, Pantai Merbok, Prai, Klang dan Melaka telah dipantau. Bioketersediaan PAHs berbanding *Paphia undulata* dalam sedimen turut diuji. Kepekatan keseluruhan PAHs dalam sedimen berubah-ubah dari 195.2 hingga 7938 ng $g^{-1}$ berat kering dan variasi kepekatan keseluruhan PAHs dalam *Paphia undulata* pula berada dalam lingkungan 179.97 hingga 1657.5 ng $g^{-1}$ berat kering. Tahap PAHs dalam sedimen boleh dikelaskan sebagai sederhana di Kedah dan Perak, sederhana ke tinggi di Merbok dan Melaka, dan tinggi hingga sangat tinggi di sungai dan muara sungai Prai dan Klang. PAHs dalam tisu lembut *Paphia undulata* pula boleh dikelaskan sebagai sederhana.

Perbandingan antara PAHs dengan Garis Panduan Kualiti Sedimen (SQG) menunjukkan bahawa kesan-kesan buruk biologi mungkin akan berlaku sekali-sekala di Stesen 1, 2 dan 3 di Sungai Klang, Stesen 1 di Sungai Prai dan Stesen 2 di Sungai Merbok. Kesan-kesan buruk biologi yang teruk mungkin akan berlaku disebabkan oleh PAHs yang mempunyai berat molekul rendah (LMW) di Stesen 1 yang terletak di Sungai Prai. Nisbah diagnostik untuk PAHs dalam sedimen secara individu dan dalam *Paphia undulata* menunjukkan PAHs tersebut berpunca daripada sumber petrogenik dan pirogenik dan didapati PAHs dari sumber pirogenik lebih dominan.

Nilai faktor pengumpulan sedimen biota (BSAF) yang dikira daripada keseluruhan PAHs mempunyai julat daripada 0.27 hingga 0.84. Keputusan ini jelas menunjukkan bahawa *Paphia undulata* dari muara sungai Merbok, Prai, Perak, Klang dan Melaka mempunyai nilai BSAF untuk PAHs LMW yang tinggi. Sebaliknya, *Paphia undulata* di muara sungai Kedah menunjukkan nilai BSAF yang lebih tinggi bagi PAHS dalam sedimen dengan berat molekul tinggi (HMW). Hasil kajian menunjukkan acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe) dan naphthalene (Nap) mempunyai bioketersediaan paling tinggi, manakala benzo[k]fluoranthene (BkF), anthracene (Ant), dibenzo[a, h]anthracene (DBA) dan benzo[b]fluoranthene (BbF) mempunyai bioketersediaan paling rendah. Kesemua hasil penelitian ini menunjukkan bahawa *Paphia undulata* boleh digunakan sebagai bioawas yang baik untuk pencemaran dan untuk bioketersediaan pencemaran hidrokarbon di ekosistem muara sungai Semenanjung Malaysia. Hasil yang didapati turut menunjukkan bahawa dalam keadaan persekitaran, beban sedimen hidrokarbon menjadi salah satu faktor yang mengawal bioketersediaan hidrokarbon tersebut.

Jumlah kepekatan *n*-alkana ($nC_{10}$-$nC_{36}$) mempunyai julat dari 1365 hingga 15850 µg $g^{-1}$ berat kering dalam sedimen dan 1875 hingga 34270 µg $g^{-1}$ berat kering dalam sampel kepah berleher pendek, Hasil kajian ini menunjukkan *n*-alkana dalam sedimen dan sampel *Paphia undulata* berpunca dari sumber yang berlainan. Sumber biogenik seperti sisa alga, organisma akuatik, dan tumbuh-tumbuhan darat semuanya dianggap sebagai sumber *n*-alkana. Nisbah hopana $C_{29}/C_{30}$ menyerupai hopana sedimen Minyak Mentah di Timur Tengah (MECO). Justeru, MECO merupakan sumber hidrokarbon petroleum yang utama dalam sedimen di Malaysia.

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I certify that a Thesis Examination Committee has met on 17 March 2015 to conduct the final examination of Mehrzad Keshavarzifard on her thesis entitled "Polycyclic Aromatic Hydrocarbons in the Coastal Environment of Peninsular Malaysia and their Bioavailability to *Paphia undulata* Born" 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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| | |
|---|---|
| Ace | Acenaphthene |
| Acy | Acenaphthylene |
| Ant | Anthracene |
| BaA | Benzo[a]anthracene |
| BAF | Bioaccumulation Factor |
| BaP | Benzo (a) pyrene |
| BbF | Benzo[b]fluoranthene |
| BkF | Benzo (k) fluoranthene |
| BgP | Benzo[g, h, i]perylene |
| BSAF | Biota Sediment Accumulation Factor |
| Chr | Chrysene |
| DBA | Dibenzo (a, h) antharacene |
| DCM | Dichloromethane |
| di- | Di-aromatic PAHs (Naphthalene) |
| DO | Disolved Oxygen |
| dw | Dry Weight |
| Fl | Fluorene |
| Fluo | Fluoranthene |
| GC-MS | Gas Chromatography- Mass Spectrometry |
| HMW | High Molecular Weight |
| IIS | Internal Injection Standard |
| InP | Indeno[1,2,3-cd]pyrene |
| LMW | Low Molecular Weight |
| µg | Microgram |
| MP/P | Ratio of Methylphenanthrenes to phenanthrene |
| 1-MPhe | 1-Methylphenantherene |
| 2-MPhe | 2-Methylphenantherene |
| 3-MPhe | 3-Methylphenantherene |
| 9-MPhe | 9-Methylphenantherene |
| Nap | Naphthalene |
| Phe | Phenanthrene |
| PAHs | Polycyclic Aromatic Hydrocarbons |

| | |
|---|---|
| penta- | Penta Aromatics PAHs (Sum of BbF, BkF and BaP) |
| Py | Pyrene |
| tetra- | Tetra-aromatic PAHs (Sum of Fluo, Py, BaA and Chr) |
| TOC | Total Organic Carbon |
| tri- | Tri-aromatic PAHs (Sum of Acy, Ace, Fl, Phe and Ant) |
| USEPA | United States Environmental Protection Agency |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Malaysia is geographically situated in south of China, east of India, west of New Guinea and north of Australia. During the last few decades industrialization and urbanization have expanded rapidly in Malaysia and could be associated with the increase in PAHs level, which is a concern in this region. Beside this, the strategic location of the Strait of Malacca made it as one of the busiest and largest shipping route and it causes the huge volume of petroleum trading from the Middle-East countries to Japan, China and the other countries around Malaysia.

The coasts of Peninsular Malaysia are important for fisheries, recreational and marine activities, tourism, and maintaining the biodiversity in the tropical area. However, the Malaysian coasts are threatened constantly by of various pollutions. The identification of land and sea-based sources of pollution in Malaysia which contributes to the pollution load in the Straits has been overviewed. One of the major threats is petroleum pollution. There are a variety of potential sources of petroleum in the coastal zones. With increasing industrialization and urbanization since the 1980s, pollution problems have featured more prominently in Malaysia and public awareness has increased. Hazards to the coastal environment in Malaysia include domestic discharge, sewage, industrial and agricultural effluents, as well as hydrocarbon pollution (Zakaria et al., 2002) caused by major engineering and development projects. PAHs are the widespread environmental contaminants that occurred in atmosphere, water, soil, sediment and organism.

During the last few decades, Southeast Asian countries such as Malaysia have experienced a drastic increase in urbanization and industrialization, resulting in immense increase in petroleum hydrocarbon consumption. These changes had caused significant input of Polycyclic Aromatic Hydrocarbon (PAHs) to freshwater and marine ecosystems in theses areas (Zakaria et al., 2002, Mirsadeghi et al., 2013). Recent studies indicated that rivers and coastal areas of Malaysia are still receiving significant input of petroleum hydrocarbon pollution (Bakhtiari et al., 2009, Bakhtiari et al., 2010, Sakari et al., 2008, Shahbazi et al., 2010, Mirsadeghi et al., 2013, Masood et al., 2014, Vaezzadeh et al., 2014, Vaezzadeh et al., 2015). Zakaria et al. (2002) have also documented an intensive input of petroleum hydrocarbons from petrol and petroleum products such as crankcase oil to the coastal area of Malaysia.

## 1.2 Problem statement

In the recent years, population of Southeast of Asia such as Malaysia has been increasing with the increase of industrialization and urbanization. Petroleum pollution becomes one of the major threats in the Malacca Strait. In this study, PAHs were

selected because they are persistent, toxic, mutagenic, and carcinogenic and also they have teratogenic and hepatotoxic effects (Cao et al., 2009, Karami et al., 2012a, Masiol et al., 2012), therefore these contaminants are important to the public health. Also, they can be concentrated in the human body following the breathing air that contains PAHs (usually stuck to particles or dust) and consumption of contaminated bivalves and may pose major human health concerns. Thus, monitoring these contaminants is very important in terms of ecotoxicology. Stranded or remobilized PAHs attached to suspended sediments may become available to filter feeders, particularly clams that inhabit in the inter-tidal and shallow subtidal seabeds. One of these filter feeder organisms is *P. undulata*, an important protein source in West Malaysia, with the ability to sequester many lipophilic organic compounds such as PAHs can be a good biomonitor. Different species of PAHs may have different bioavailability to *P. undulata* and merely bioavailable part of contaminants can pass through the food chain. Therefore, study of bioavailability of PAHs to *P. undulata* is very important. There is no investigation about bioavailability of PAHs to *P. undulata* from sediments. At present, no information is available regarding the concentrations of PAHs in short-neck clam or carpet clam (*Paphia undulata*) from the estuaries of Peninsular Malaysia. Therefore, this study evaluates the distribution and sources of anthropogenic contaminations in this region and also determines that which species of PAHs are more bioavailable to *Paphia andulata*.

## 1.3 Significance of study

Malaysia is geographically situated in the heart of Southeast Asia. In the past decades, Malaysia has witnessed an accelerated growth in industrial development that saw a tremendous increase of usage of oil and oil derivatives. Moreover, the strategic location of the Malacca Strait has made it one of the busiest and largest shipping routes. The huge amount of petrol and petroleum products are transported from the Middle-East countries to Japan, China, Korea and other neighboring countries via the narrow Straits of Malacca. The oil tankers transportation in this strait, the population increase in Malaysia and therefore, using more petroleum, growing of industrialization and industrial activities along rivers and Malaysian coastal zone have increased PAHs contamination in the rivers and Malaysian coastal areas. PAHs is one of the most important classes of pollutants that can occur in all ecosystems such as air, water, sediments and soil. PAHs are hydrophobic, lipophilic and persistent in the environment; therefore they are not disposed and removed easily from the environment. The hydrophobic and lipophilic PAHs accumulate in fatty tissues of aquatic organisms and consequently transfer through the food chain (Shahbazi, 2009). PAHs, especially high molecular weight (HMW) PAHs, are mutagenic, carcinogenic and they are featured by teratogenic and hepatotoxic effects. PAHs toxicity to marine organisms has been proven by a number of reports (Mirsadeghi, 2010, Mirsadeghi et al., 2011, Karami et al., 2012a). Since protecting the environment and reducing of pollutants releasing to ecosystems are the main concern of the $20^{th}$ and $21^{st}$ centuries, determination of sources, pathways, distribution and fate of micropollutants such as PAHs are important in order to decrease the input of these contaminants into the environment and therefore, reduce their harmful biological adverse effects.

There have been some studies on marine organisms such as clams, oysters and fish to evaluate the concentrations and bioaccumulation of PAHs. Bivalve can be good biomonitor for contaminants such as PAHs, because of living in sessile style, high abundance, easy to be cultured in the lab, easy for sampling, tolerating high amount of pollutants without death and occupying an important position in the food chain. Many researches on PAHs have been done in order to understand their concentrations, distributions and origins, but there is no investigation about bioavailability in *P. undulata*. Therefore, *P. undulata*, as a species of bivalve, was selected. In this study the concentrations of PAHs in sediments and *P. undulata* and also bioavailability of PAHs from sediments to *P. undulata* were determined.

Malaysia has had environmentally-related legislation since the early 1920s. But the legislation is limited in scope and inadequate for handling complex emerging environmental problems. So through environment quality act 1974 (EQA, 1974), a more comprehensive form of legislation and an agency to control pollution was established. EQA is an enabling piece of legislation for preventing, abating and controlling pollution, and enhancing the environment, or for other related purposes. Pollution, as declared in EQA, includes the direct or indirect alteration of any quality of the environment or any part of it by means of a positive act or act of omission. The regulation is generally aimed at waste solution fractions of the environmental pollutants (EQA, 1974). Hence, with EQA 1974 there is a provision in dealing with persistent organic pollutants (POPs) as specified under the Schedule Waste (waste of oil or oily sludge) especially the oil spill and used crankcase oil. The Schedule Waste aims at reducing spilling of the oily waste with punitive damages. Over the last decade, the level of compliance and awareness among the industries, household and general public of the EQA 1974 has significantly increased. Hence, the results of the present study will add new information to the global database, and also provide data in order to improve the regulations and consequently the environmental quality of the selected rivers and estuaries. This study attempts to evaluate comparisons between the different stations and the relative biota–sediment bioaccumulation factor (RBSAF) for PAHs. At present, the concentrations and bioavailability of PAHs to short-neck clams from sediments in the west coasts of Peninsular Malaysia are not well documented. The suitability of *P.undulata* as sentinel organism is also evaluated.

### 1.4 Research questions

Benthic habitats are preferential environmental sink for many contaminants, since many pollutants originally introduced into the water column have affinities for sediment particles. Because sediments are also an important biological habitat, uptake of toxicants into the food web are influenced by toxicant concentrations such as PAHs in sediment. Pollutants which settle out with bottom sediments exist in an equilibrium state with the water phase which may be altered by natural and anthropogenic environmental disturbances. Besides, benthic organisms such as P. undulata which contain lipid can accumulate the contaminatnts in the environment. Therefore, in this study it is searched for understanding what the distribution and sources of PAHs in sediments and *P. undulata* samples are? Can *P. undulata* be used as indicator species for pollution of PAHs? How is the correlation between sediment and

*P. undulata* in the study areas? Finally, to what extent PAHs are bioavailable to *Paphia undulata* and which species of PAHs are more bioavailable to be accumulated by *P. undulata*?

## 1.5 Study objectives

I. To assess the distribution and sources of PAHs in sediments from the study areas
II. To assess the distribution and sources of PAHs in *P. undulata* tissues in the study areas
III. To evaluate the potential usage of *P. undulata* as indicator species for bioavailability of PAHs
IV. To evaluate the relationship between PAHs in sediments and *P. undulata* samples from the study areas

## 1.6 Thesis structure

First chapter is introduction which is about background of study, problem statement, significant of study, research questions and study objectives.

Second chapter is literature review. This chapter provides a comprehensive litreture review regarding distribution, source, pathway and fate of organic contaminants in the environment and ecosystems.

Third chapter is Methodology which is about sampling, sample preparing and sample analyzing.

Forth chapter is results and discussion. The first, second and third sections are about distribution and sources of PAHs, alkanes and hopanes in sediments, respectively. These three sections cover first objective. The forth, fifth and sixth sections of result and discussion are about distribution and sources of PAHs, alkanes and hopanes in *P. undulate*, which cover second objective. The seventh section of result and discussion is about bioavailability of PAHs to *P. undulata* from sediments and introducing *P. undulata* as a good biomonitor for PAHs, which covers objective three and four.

Fifth chapter is conclusion and recommendation.

## 1.7 Research framework

As mentioned above, this thesis consists of five (5) chapters which include Introduction, Literature Review, Methodology, Results and Discussion, and Conclusion and Recommendations. Therefore, (Figure 1.1) shows the research process and also the relationship among research elements within the chapters of this thesis.

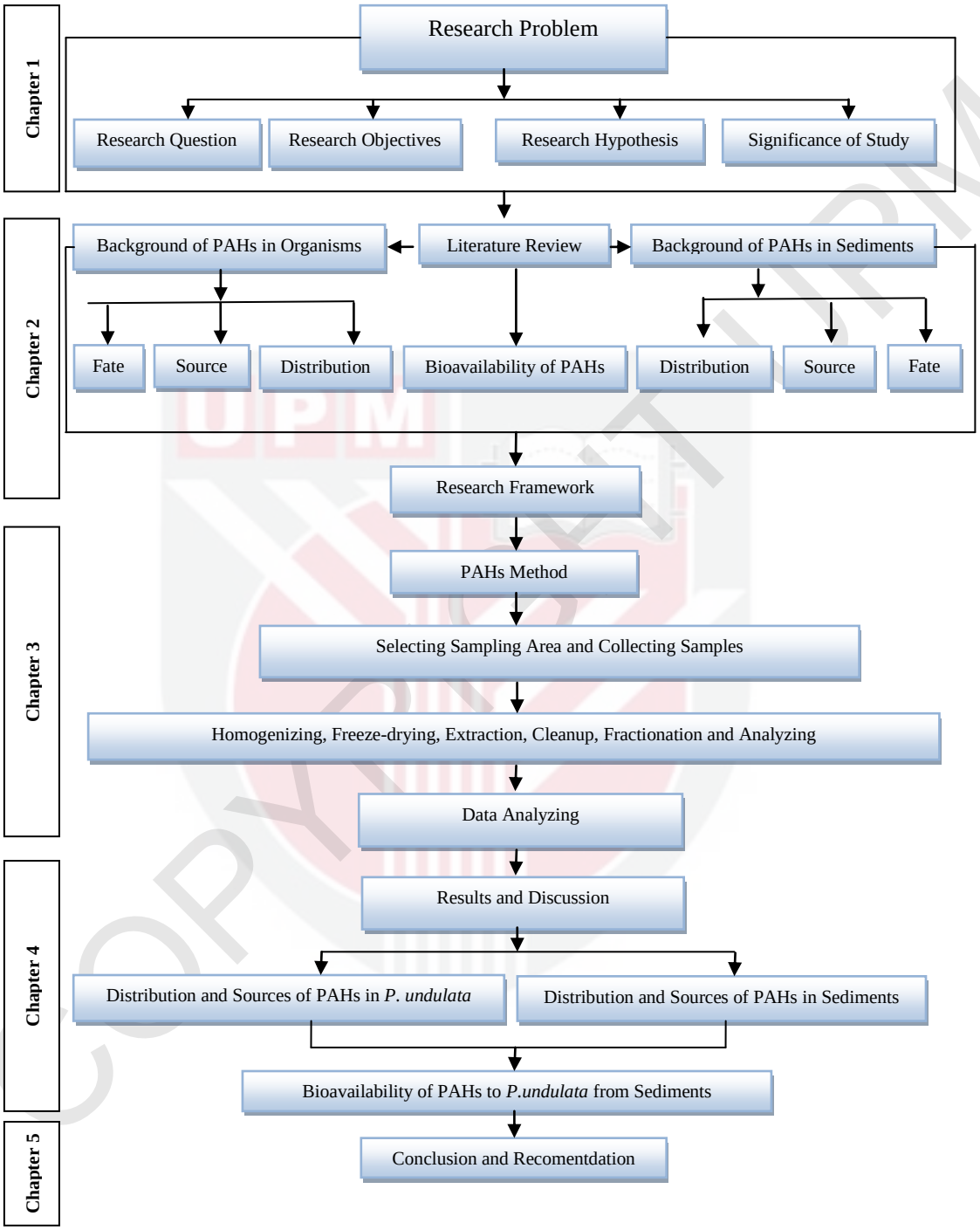


**Figure 1.1 Research framework**

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