# FORENSIC AND CHEMOMETRIC APPROACHES IN DISTRIBUTION, CHARACTERIZATION AND HEALTH RISK ASSESSMENT OF POLYCYCLIC HYDROCARBONS IN SELECTED AQUACULTURE FARMS IN PENINSULAR MALAYSIA

By

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

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

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This is a pioneering study and report on polycyclic aromatic hydrocarbons (PAHs) in aquaculture ecosystem in Malaysia. In this study the occurrence of PAHs in sediments, oysters, fish and fish feed were examined and the health risk assessment was conducted. Samples were collected from selected aquaculture farms in Peninsular Malaysia from January 2010 to November 2011 inclusive of remote and polluted areas. The results revealed the average concentration of total PAHs in sediment ranged from 20 to 1841, oysters 121 to 519, fish 22 to 228 and fish feed 150 to 366 ng/g dry weight. The levels of PAHs reflect human activities, with higher concentrations observed in industrial zones, port areas and urban settlements. Application of forensic and chemometric techniques on sample data revealed that the sediments collected from aquaculture farms and periphery were dominated by pyrogenic sources from vehicle emission (54%) and biomass burning (9%) while

petrogenic sources coming from petroleum oil (37%). The profile of PAHs obtained from fish and oyster farm sediments differ significantly ($p < 0.05$) implying additional pollution sources into fish farm. Enrichment of organic carbons, nitrogen and PAHs in fish farms revealed an average of 28.7%, 4.65% and 156% respectively. Enrichment of TOC is statistically significant to the enrichment of total PAHs, carcinogenic PAHs and 4-6 ring PAHs but not 2-3 ring PAHs. The compound benzo(a)pyrene was detected in all samples. This study found that acenaphthylene, fluoranthene and dibenzo(ah)anthracene concentrations were higher than the permissible levels in interim sediment quality guidelines. This might be harmful to aquatic animals and to human who consume them. The body burden of PAHs in oysters fall within moderate range and is comparable to other studies in bivalves. Temporal variation due to spawning was evident in oysters at Pulau Betong Batu Lintang and Pasir Panjang. PAHs in edible fish tissues range between low to moderate. Normalization of total PAHs with lipid content drew a meaningful result compared to dry weight normalization. Strong significant correlation was observed between lipid normalized total PAHs and total toxicity equivalence concentrations ($p < 0.05$ vs. $p > 0.05$). Fish feed samples were dominated by high molecular weight PAHs. The profile of PAHs in fish feed were similar regardless of locations, types and lipid content. PAHs concentrations were found to be higher or in par with other studies worldwide. Cluster analysis and discriminant analysis implied that fish feed was the main source of PAHs in edible fish tissue. Non-cancer risk assessment of PAHs in sediment, oyster and fish showed that the values were below the concern levels published by USEPA. Cancer risk due to consumption of red snapper from Jelutong and sea bass from Jelutong and Gelang Patah is higher than the safe levels recommended for all groups of population under study. Overall PAHs from vehicular emission dominated aquaculture sediment. The consumption of cultured seabass and red snapper from Jelutong and seabass from Gelang Patah are not safe for all group of populations.

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

**PENDEKATAN FORENSIK DAN KEMOMETRIK DALAM PENILAIAN PENGAGIHAN, PENCIRIAN DAN RISIKO KESIHATAN HIDROKARBON POLISIKLIK PADA LADANG AKUAKULTUR TERPILIH DI SEMENANJUNG MALAYSIA**

Oleh

**ANANTHY A/P RETNAM**

**Jun 2013**

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Ini merupakan satu kajian dan laporan perintis mengenai PAHs di dalam ekosistem akuakultur di Malaysia. Di dalam kajian ini kehadiran PAHs pada mendapan, tiram, ikan dan makanan ikan telah diuji dan risiko telah ditentukan. Sampel-sampel telah dikumpul daripada ladang akuakultur yang terpilih di Semenanjung Malaysia bermula dari Januari 2010 sehingga November 2011 merangkumi kawasan terpencil dan kawasan tercemar. Kesemua sampel-sampel tersebut telah di homogen, dibeku kering, di ekstrak, dibersihkan, dibahagikan kepada pecahan kecil dan dianalisa menggunakan kromatografi gas - spektrometri jisim. Daripada keputusan analisa ia menunjukkan purata jumlah kepekatan PAHs di dalam mendapan berjulat di antara 20 sehingga 1841, tiram 121 sehingga 519, ikan 22 sehingga 228 dan makanan ikan 150 sehingga 366 ng/g berat kering. Paras kandungan PAHs selaras dengan aktiviti manusia dengan kepekatan yang tinggi telah diperolehi di kawasan perindustrian,

perlabuhan dan kawasan bandar. Aplikasi forensik dan kemometrik telah menunjukkan mendapan yang dikumpul daripada kawasan akuakultur telah didominasi oleh sumber pirogenik seperti pelepasan asap kenderaan (54%) dan pembakaran biojisim (9%) manakala sumber petrogenik pula hadir daripada minyak petroleum (37%). Profil PAHs yang diperolehi daripada mendapan ladang ikan dan ladang tiram memberikan perbezaan yang penting ($p < 0.05$) mencadangkan ada tambahan sumber ke dalam ladang ikan. Peningkatan jumlah karbon organik, nitrogen dan PAHs telah ditentukan pada ladang ikan dengan purata masing-masingnya sebanyak 28.7%, 4.65% dan 156%. Peningkatan TOC menjadi penyumbang penting kepada peningkatan jumlah PAHs, karsinogenik PAHs dan 4-6 cincin PAHs tetapi tidak kepada 2-3 cincin PAHs. Benza(a)pyrene telah dikesan di dalam kesemua sampel. Kepekatan acenaphtylene, fluoranthene dan dibenza(ah)antracene telah dikesan pada paras tinggi jika dibandingkan dengan garis panduan kualiti mendapan interim. Ia mungkin menbahayakan haiwan akuatik dan juga kepada manusia yang memakannya. Isi tiram yang mengandungi PAHs berada pada julat sederhana jika dibandingkan dengan kajian lain yang melibatkan siput lokan. Perubahan PAHs mengikut masa telah dikesan pada tiram dari Pulau Betong, Batu Lintang dan Pasir Panjang. Kandungan PAHs didalam tisu ikan yang boleh dimakan berada pada julat rendah ke sederhana. Normalisasi nilai jumlah PAHs dengan kandungan lipid memberikan keputusan yang penting jika dibandingkan dengan normalisasi nilai berat kering. Hubungan yang kukuh dan penting diperolehi di antara normalisasi nilai lipid dengan jumlah PAHs dan jumlah keracunan setara kepekatan ($p < 0.05$ vs $p > 0.05$). Makanan ikan didominasikan oleh PAHs yang memiliki berat molekul tinggi. Profil PAHs di dalam makanan ikan adalah sama pada semua lokasi, jenis dan kandungan lipid. Paras kepekatan PAHs didapati tinggi ataupun setara dengan kajian lain yang dilakukan seluruh dunia. Analisa kelompok dan analisa diskriminasi menunjukkan makanan ikan sebagai sumber utama PAHs di dalam tisu ikan yang boleh di makan. Risiko penyakit kanser setelah memakan ikan merah dari Jelutong dan siakap dari Jelutong dan Gelang Patah adalah tinggi daripada tahap yang selamat bagi kesemua kumpulan. Kesimpulannya medapan akuakulture didominasi oleh PAHs dari pelepasan asap kenderaan. Pemakanan ikan siakap dan merah dari Jelutong dan siakap dari Gelang Patah tidak selamat untuk semua kumpulan penduduk.

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## TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

# LIST OF APPENDICES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| 12DMNAP | 1,2-dimethylnaphthalene |
| 1MNAP | 1-methylnaphthalene |
| 2MPHE | 2-methylphenanthrene |
| 2MANT | 2-methylanthracene |
| 36DMPHE | 3,6-dimethylphenanthrene |
| ACY | Acenaphthylene |
| ACE | Acenaphthene |
| AIC | Akaike Information Criteria |
| ANT | Anthracene |
| APCS | Absolute principle components scores |
| BaA | Benza(a)anthracene |
| BkF | Benzo(k)fluoranthene |
| BbF | Benzo(b)fluoranthene |
| BbkF | Benzo(b+k)fluoranthene |
| BeP | Benzo(e)pyrene |
| BaP | Benzo(a)pyrene |
| BghiP: | Benzo(ghi)perylene |
| BL | Batu Lintang |
| CA | Cluster analysis |
| CHR | Chrysene |
| *C. iredalei* | *Crassostrea iredalei* |
| *C. Belcheri* | *Crassostrea Belcheri* |
| CDI | Chronic daily intake |

| | |
|---|---|
| cPAHs | Carcinogenic PAHs (sum of BaA, CHR, BbF, BkF, BaP, DahA and IcdP) |
| COND | Conductivity |
| CR | Cancer risk |
| CTE | Central Tendency Exposure |
| DA | Discriminant analysis |
| DahA | Dibenza(ah)anthracene |
| DBT | Dibenzothiophene |
| DCM | Dichloromethane |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| DO | Dissolve oxygen |
| dw | Dry weight |
| FTH | Fluoranthene |
| FLU | Fluorene |
| GCMS | Gas chromatography mass spectrometry |
| GP | Gelang Patah |
| HB | Health Base concentration |
| HCH | Hexachlorocyclohexane |
| HI | Hazard Index |
| HQ | Hazard Quotient |
| HMW | High molecular weight PAHs (sum of FTH, PYR, RET, BaA, CHR, BkF, BbF, BeP, BaP, PERY, DahA, IcdP and BghiP) |
| IcdP | Indeno(1,2,3-cd)pyrene |
| IIS | Internal injection standard |
| IRIS | Integrated Risk Information System |

| | |
|---|---|
| ISQGs | Interim Sediment Quality Guidelines |
| JT | Jelutong |
| $K_{ow}$ | Octanol-water partition coefficient |
| KU | Pulau Kukup |
| KS | Kuala Setiu |
| LMW | Low molecular weight PAHs (sum of NAP, 1MNAP, FLU, ACY, 12DMNAP, ACE, DBT, PHE, ANT, 2MPHE, 2MANT, 36DMPHE) |
| LMW/HMW | Ratio of low molecular weight PAHs to high molecular weight PAHs |
| lw | lipid weight |
| Max | Maximum |
| MLR | Multiple linear regression |
| MP/P | Ratio of methylphenanthrene to phenanthrene |
| MT | Merchang |
| N | Nitrogen |
| NAP | Naphthalene |
| $NO_2$ | Nitrogen dioxide |
| $O_3$ | Ozone |
| OCPs | Organochlorine pesticides |
| PAHs | Polycyclic aromatic hydrocarbons |
| PCA | Principle component analysis |
| PHE | Phenanthrene |
| pg | Pictogram |
| PYR | Pyrene |
| PERY | Perylene |
| PEF | Potency equivalency factors |

| | |
|---|---|
| PB | Pulau Betong |
| PP | Pasir Panjang |
| PCBs | Polychlorinated biphenyls |
| PBDEs | Ploybrominated diphenyl ethers |
| RET | Retene |
| RfD | Reference dose |
| RME | Reasonable Maximum Exposure |
| SAL | Salinity |
| SBC | Shwarz Bayesian Criteria |
| SD | Standard deviation |
| SIS | Surrogate internal standard |
| $SO_2$ | Sulfur dioxide |
| SOD | Sediment oxygen demand |
| SF | Slope factor |
| SM | Sungai Muar |
| TOC | Total organic carbons |
| TC | Total carbons |
| TEF | Toxicity equivalency factors |
| TEMP | Temperature |
| TPAHs | Total PAHs (sum of all 24 PAHs analyzed in this study) |
| TTEC | Total toxicity equivalence concentrations |
| TJ | Teluk Jawa |
| USEPA | United States Environmental Protection |
| ww | Wet weight |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of study

Cancer has been recognized as the major health problem for urban human population worldwide. It is a growing affliction problem in Malaysia. Cancer stands as fourth leading cause of death in Malaysia with estimated 30 000 cases annually with an incidences ratio of 150 in 100 000 population having cancer (Lim, 2002). Cancer has become a national concern these past few decades due to rapid industrialization and urbanization of the country. Pollution is an associated problem with development of a country. The major concern is micro chemicals especially persistent organic pollutants (POPs) like halogenated pesticides, dioxin, flame retardants (PCBs, PBDEs) and polycyclic aromatic hydrocarbons (PAHs). These chemicals are categorized as known, probable and possible human carcinogens by United States Environmental Protection Agency (USEPA) in Integrated Risk Management System (IRIS) (USEPA, 2003b). PAHs having highly carcinogenic potencies linked to causing cancer to human. The PAHs exposure has been linked to elevated levels of DNA adducts formation, mutations and cancer in animals and human (European Commission, 2002).

PAHs enter into human through many routes. Excluding smokers and occupational exposure, dietary intake has been reported as an important route of PAHs to human (Moon et al., 2010; European Commission, 2002). PAH in the environment, contaminates vegetables, fruits and animals cultivated in polluted areas. Marine organisms in particular are known to accumulate these pollutants in concentration higher than the surroundings. Although seafood comprise of only about 10% of human diet but has proven to be one of the major route of contamination into human (Binelli and Provini, 2003). In recent years this is reflected by growing research interest in this field (Darilmaz and Kucuksezgin, 2012; Kelly et al., 2011; Mirsadeghi et al., 2011; Moon et al., 2010; Ling et al., 2009; Nghia et al., 2009; EFSA, 2008; Padula et al., 2008; Cheung et al., 2007; Moreau et al., 2007; Kong et al., 2005; Binelli and Provini, 2004).

The exponential increase in human population has placed serious demand for food supply. This compounded by higher living standard and need for quality protein has increased the demand for fish and seafood. The global consumption of fish and seafood has generally increased during recent decades by 31.5% from 2004 to 2009 (Martinez-Porchas and Martinez-Cordova, 2012; Verbeke et al., 2007). However the production of captured fisheries has been fully exploited or over-exploited in most part of the world (Russell et al., 2011). The unsustainable fishing habits with depleting wild fish and seafood stocks and increasing customer demands has forced aquaculture as a practical alternative. Aquaculture is the most rapidly growing food production sector in the world with 8.3% per year, compared to fisheries less than 2% and livestock 2.9% (Martinez-Porchas and Martinez-Cordova, 2012; Sather et al., 2006). The annual aquaculture production increased from 4 million metric tonnes in 1980 to 60 million metric tonnes (Martinez-Porchas and Martinez-Cordova, 2012; Verbeke et al., 2007).

In Malaysia, the tremendous increase in aquaculture production in the past 30 years (87%) (Food and Agricultural Organization, 2012a), is seen as a viable approach in securing fish and seafood and less stress on wild stocks. Despite the benefits, aquaculture is unfavourable because of environmental degradation that follows. Aquaculture introduces non-native species to the particular marine environment and the operation of aquaculture farms has impacted the cultured organisms and the marine habitat at or near the farms. Enrichment of nutrients and organic matters from the feed and feces cause the change of physical and chemical characteristics of seabed (Carroll et al., 2003). The changes include decrease of dissolved oxygen demand, production of methane and hydrogen sulfide gas and increase of bacterial production. Introduction of unintentional micro pollutants from aquaculture activities such as heavy metals and persistent organic pollutants (POPs) have been and/or are being investigated. The contamination caused by aquaculture said to reduce the natural population along side the detrimental downstream activity of trash fish and fish oil production also seen as unsustainable practice (Martinez-Porchas and Martinez-Cordova, 2012).

## 1.2 Problem statement

The Malaysian coastal areas are rich in natural resources and away from natural disasters, deemed ideal for aquaculture activities. Aquaculture industry showed a tremendous expansion over the 30 years in Malaysia. The cultivated organisms include bivalves such as mussels, cockles and oysters, finfish such as seabass, snappers and groupers to seaweeds. Malaysia is one of the largest producer of cultivated seabass in South East Asia (Alongi et al., 2003). Mariculture activities in Malaysia mostly focused in brackish and coastal zones. The coastal water of

Peninsular Malaysia especially west coast is experiencing micro chemical pollution including PAHs. The west coast of Peninsular Malaysia is densely populated with 58.62% of the nation's population residing here despite having only 20.46% of the total land area (Omar, 2012). Numerous industrial zones and ports are also located along this stretch. Adding to this pollution stress, The Straits of Malacca is known as one of the busiest trade route between Middle East and Far East accommodating over 100 000 oil tankers and cargo vessels carrying 3.23 million barrels of crude oil every day.

Recent studies demonstrated occurrence of extensive petroleum hydrocarbons pollution in Malaysia (Sakari et al., 2008a; Zakaria et al., 2002; Zakaria et al., 2001) and it is unique compared to other industrial countries which reported pyrogenic as the main source of PAHs (Liu et al., 2009; Larsen and Baker, 2003; Harrison et al., 1996). Petrogenic sources with lower molecular weight PAHs (2-3 rings) are more water soluble and readily bioavailable for aquatic animals. Higher level of petrogenic PAHs also were found to be accumulated in various organisms (Baumard et al., 1998a). Therefore the bioaccumulation of PAHs from sediments is greater for seafood animals. Extensive studies on PAHs in variety of environments have been reported (Mirsadeghi et al., 2011; Bakhtiari et al., 2009; Tahir et al., 2006b; Omar et al., 2002; Zakaria et al., 2002) but studies on PAHs in aquaculture remained scarce.

Generally aquaculture sites are located too near to potential sources of PAHs such as towns and oil tankers route especially along the Straits of Malacca. With cities, towns and industrial sites located in close vicinities to river mouth and coastal water, there is potentially high risk of PAHs pollution into the aquaculture sites. Furthermore the aquaculture sites are located at protected areas with less water circulations and less dilution enhancing bioaccumulation of PAHs in seafood animals. The high human activities at the aquaculture areas such as boating, seafood harvesting and cage cleaning resuspend and remobilize the sedimentary PAHs into water column with large amount of suspended particles. Therefore the PAHs pollution source for seafood animals can be from these suspended particles. The PAHs distribution in particles and sediments are partially reduced in the lower molecular weight PAHs enhancing exposure to high molecular weight compounds (Baumard et al., 1998a). Furthermore, in Malaysia the concentration of sedimentary PAHs is not regulated. Regulation is important in term of pollution control and to minimize the risk to human exposure. However, the effect of PAHs pollution on aquaculture has not been investigated to date.

Fish and seafood enjoy a good reputation as a cheap, nutritious and healthy food. It contains minerals, vitamin and protein which play essential role in human health. One of the most significant benefits is omega-3-fatty acid which has been associated with health benefits due to its cardio-protective effects (Verbeke et al., 2007). However, the levels of contaminants in fish and seafood together with poor management of fisheries are of particular interest because of the potential risk to humans who consume them. Many studies focused on PAHs contamination in wild captured fish and shellfish (Mirsadeghi et al., 2011; Shahbazi et al., 2010; Sim et al., 2010). Very few studies focused on contamination in cultured organisms which meant for human consumption, all on heavy metals (Najiah et al., 2008; Yap et al., 2004) and none on PAHs. Pollutants have been introduced directly into the marine environment as a result of aquaculture activities. Heavy metals such as cooper through antifoulants, pesticides, antibiotics and anesthetics though medication, compounded by unintended micro chemical contaminations such as PCBs, dioxins, pesticides and PAHs through fish feed and fish oils are detrimental pollutants (Tsapakis et al., 2010; Loutfy et al., 2007; Easton et al., 2002). POPs are able to accumulate in fish by feeding cultured fish with fishery products and by-products that can potentially increase the concentration of POPs in fish. Furthermore the unconsumed feed and feces from fish will be available for consumption of benthic organisms underneath the farms which will be eaten by higher trophic predators (Russell et al., 2011). To date to best of our knowledge, there is no available data on the levels and profile of PAHs in fish feed used in aquaculture in Malaysia. In fact from worldwide perspective, only a handful reports available in providing information of levels and profile of PAHs in fish feed (Tsapakis et al., 2010; Loutfy et al., 2007; Easton et al., 2002). The primary focus of this study is on contamination of PAHs in cultivated organism namely oysters and fish. Only limited data available in the risk assessment of PAHs to human (Mirsadeghi et al., 2011; Sim et al., 2010). This study also serves as a preliminary report on human health risk assessment of daily intake of PAHs due to ingestion of cultivated fish and shellfish in Malaysia.

The environmental impact of the aquaculture especially mariculture is well documented in temperate region. Aquaculture has been documented as the point source for micro organic chemical PAHs contamination of coastal marine sediments (Tsapakis et al., 2010). However limited information is available in tropical region like Malaysia (Gao et al., 2005; Wu, 1995). Furthermore in temperate ecosystem the use of commercial feed has been well assimilated and the environmental impact has been well described. This is not the case for tropical and subtropical zone where trash fish is still the desirable choice mainly due to cheap cost. The environmental impact of trash fish especially organic enrichment is expected to be severe compared to commercial feed owing to the local environmental conditions (Gao et al., 2005; Wu, 1995). The enrichment of organic matters and PAHs in aquaculture cages has not been studied in Malaysia tropical climate so far.

## 1.3 Significance of study

Marine aquaculture is concentrated at coastal areas which are abundant not only with in natural resources but also pollution because they are hotspot for human activities. The organisms cultivated here being exposed to many types and loads of pollutions from water and sediments surrounding the farms. The organisms being cultivated are 100% used as source of food by local and international consumers. As such there is concern in terms of safety for human health. Likewise, aquaculture activities are also known to cause pollution to the environment (Martinez-Porchas and Martinez-Cordova, 2012; Tsapakis et al., 2010; Cole et al., 2009; Carroll et al., 2003).

The extend of pollution depends on the physical, chemical and biological characteristics of local environment and the management practices such as feeding habits and fish stocking (Gao et al., 2005; Carroll et al., 2003). Obviously aquaculture add to environmental stress along with pollution from land-based aquaculture and run-off from other land-based industries (McKinnon et al., 2010). In northern Europe, guidelines, monitoring procedures and environmental quality standards for salmon farming are established. In Norway, a national standard for monitoring of fish farms was established containing criteria for nutrient impacts, organic matter, micro pollutants, fecal bacteria, and water quality for various coastal uses (Carroll et al., 2003). In Malaysia, currently there is no guideline or practices for monitoring of environmental effects of aquaculture activities even though some form of monitoring of micro pollutants such as trace metals and pesticides is ongoing. There is also no guideline or standards for safe levels of PAHs in edible biota, sediment and water.

This study can be deemed as a pioneering scientific investigation into the extend of PAHs pollution in aquaculture eco-system. This study correlates enrichment of PAHs in fish farms in Malaysia, caused by loading of organic matters. The results of this study can be used as a benchmark for future policy guidelines when determining quality criteria for the aquaculture activities in Malaysia. This refers to concerns regarding sedimentation related to intensive aquatic animal culture. This study is further enhanced by investigating the health risk associated with the consumption of contaminated fish and seafood by humans. It has far-reaching benefits for the protection of ecosystem health, environmental conservation of ecologically sensitive areas and for future protection of human health.

## 1.4 Objectives of study

The overall aim of this study is to evaluate the status and trends of PAHs contamination in aquaculture farms and the risk of consuming selected cultured seafood by the population. The specific objectives of this study are:

1. To determine the distribution, characterization and source apportionment of PAHs in sediments and biota (fish, oysters and fish feed) from selected aquaculture farms in Peninsular Malaysia.
2. To determine the enrichment of total organic carbons, nitrogen and PAHs in sediment of selected fish farms.
3. To evaluate the relationship between the PAHs levels in the sediments with the biota, level of bioaccumulation and contributing sources in selected aquaculture farms.
4. To assess the cancer and noncancer risk of the PAHs in aquaculture products for human health.

## 1.5 Study hypothesis

This study initiated to address a few hypotheses as listed below:

1. There are significant correlations between the PAHs level in the sediment with the biota.
2. There are significant differences in PAHs level in sediments and fish between locations.
3. There are significant differences in PAHs level in oyster between location and time.

This thesis has been divided into 5 chapters. Chapter 2 is about review of literatures related to this research topic. Chapter 3 describes the material and method covering sampling, analytical procedure and quality control. The extraction, cleanup and instrumental analysis of target PAHs analytes were elaborated. Methods for determination of total organic carbons, lipid contents and statistical methods also have been described. Detail explanation on human health risk assessment due to exposure to PAHs introduced in this chapter. Chapter 4 is about results and discussion. This chapter is divided into four parts. Part I explores the status of PAHs contamination in surface sediment of aquaculture farms. The spatial distribution and trends were described with help of forensic and robust chemometric methods. Source identification and source apportionment of PAHs in aquaculture sediments were discussed. The enrichment of organic matters and PAHs were identified due to fish farm activities were discussed in part II. The spatial and temporal trends of PAHs in oysters and edible tissue of fish and the source of contamination were discussed in part III. Meanwhile part IV is dedicated to human health risk assessment due to exposure of PAHs in sediment and consumption of contaminated oyster and fish from aquaculture farms. Each matrix was discussed separately to see the potential risk of exposure to PAHs. Chapter 5 is conclusion made from this study and recommendation and future direction of this research (Figure 1.1).

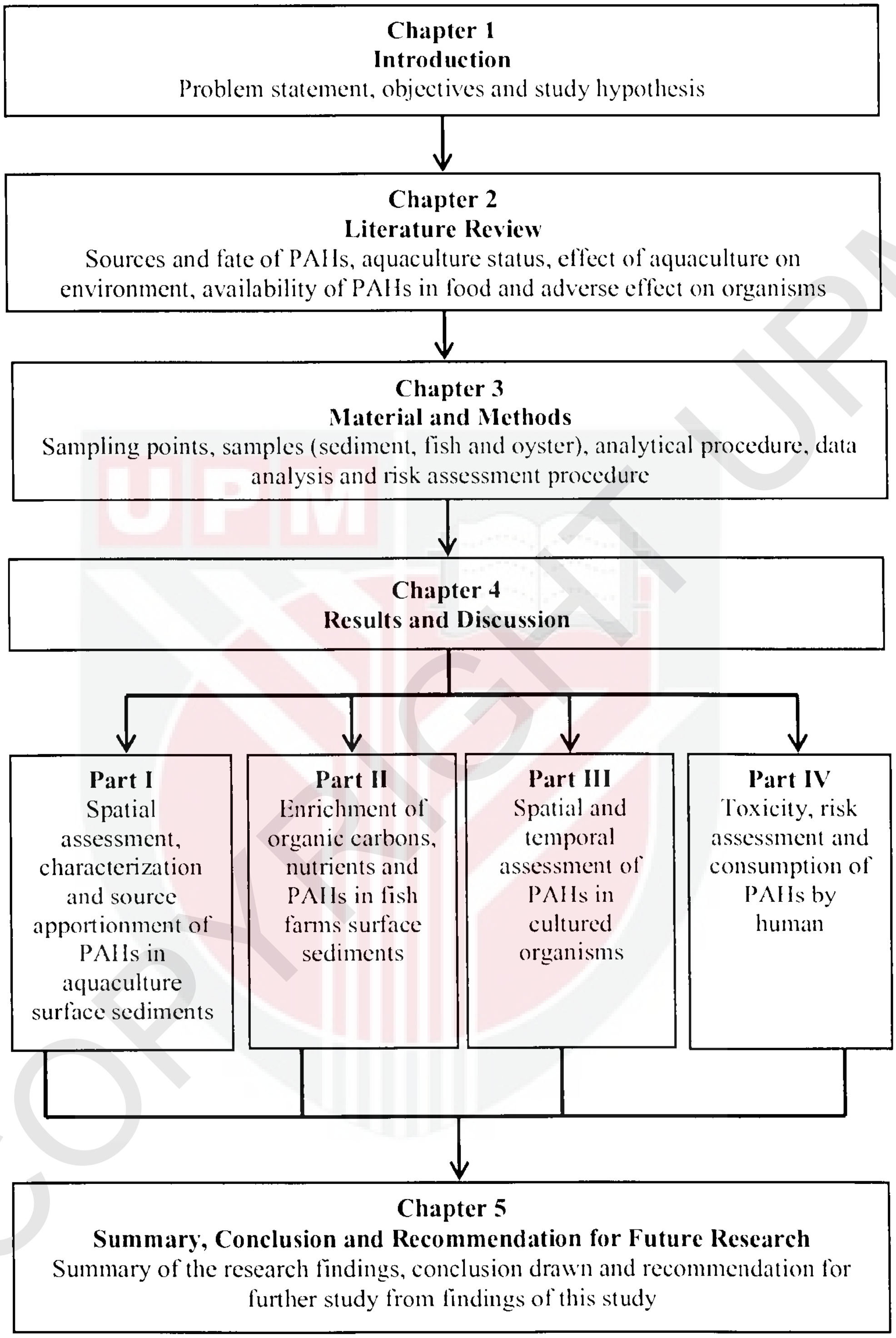


**Figure 1.1. Flowchart of the thesis outline**

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