DISTRIBUTION OF METALS AND PERSISTENT ORGANIC POLLUTANTS IN SELECTED SHRIMP SPECIES FROM FIVE FISH LANDING SITES IN PENINSULAR MALAYSIA

NORANIS BINTI ISMAIL

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

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NORANIS BINTI ISMAIL

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ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **NORANIS BINTI ISMAIL**

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DISTRIBUTION OF METALS AND PERSISTENT ORGANIC POLLUTANTS IN SELECTED SHRIMP SPECIES FROM FIVE FISH LANDING SITES IN PENINSULAR MALAYSIA

ABSTRACT

The concentration of contaminants such as metals and persistent organic pollutants (POPs) in the marine ecosystem has become a major concern since it can accumulate within the food chain of marine life. In general, this study aimed to analyse the abundance of selected metals (Hg, As, Cu, Pb and Cd) and presence of selected POPs (dichlorodiphenyltri-chloroethanes and hexa-chlorocyclohexanes) in selected shrimp samples collected from fish landing sites in Peninsular Malaysia. This study also aimed to assess the quality of shrimp by comparing with the FAO/WHO Standard and Malaysian Food Act and Food Regulations 1983 (MFA). The concentration of metals in shrimp samples were used to calculate the estimated daily intake (EDI) of metals, target hazard quotients (THQ) and target cancer risk (TR) for locals. In this study, three selected shrimp species obtained from five fish landing sites located along the East and West Coast of Peninsular Malaysia were analysed using Inductively Couple Plasma-Mass Spectrophotometer (ICP-MS) for heavy metals while the analysis for POPs were performed using Gas Chromatography Electron-Capture Detector (GC-ECD). The highest concentration of As, Hg and Pb were detected in shrimp samples collected from Perak, which are *P. merguiensis* (328.93 ± 0.78 µg/g), *P. merguiensis* (1012.83 ± 0.78 µg/g) and *P. monodon* (4.07 ± 1.31 µg/g), respectively. The highest concentration of Cu was found in *L. vannamei* (7.56 ± 0.51 µg/g) collected from Malacca, whereas for Cd was found higher in *L. vannamei* (0.66 ± 0.38 µg/g) collected from Johor. Significant difference was obtained between the accumulation of Pb in *L. vannamei* and Cd concentration in *P. monodon* for all sampling locations ($p<0.05$). Most of the values were higher than the permitted limit set by FAO/WHO 2010, as well as, the Food Act 1983 and Food Regulations 1985. From this study, it can be clearly seen that shrimps collected along the west coast of Peninsular Malaysia are more contaminated because of more industries and denser population, thus more contaminants were discharged. As for POPs, dichlorodiphenyltri-chloroethanes (DDT) is more abundant in the shrimp species compared to hexa-chlorocyclohexanes (HCH). It probably resulted from the excessive application of pesticides and disease control agents that contain DDT. It can be concluded that shrimp samples collected from Peninsular Malaysia are contaminated with selected metals and POPs.

Keywords: marine, shrimp, metals, DDT and HCH.

TABURAN LOGAM DAN PENCEMAR ORGANIK KEKAL DI DALAM SPESIS UDANG TERPILIH DARIPADA LIMA TAPAK PENDARATAN IKAN DI SEMENANJUNG MALAYSIA

ABSTRAK

Tahap bahan cemar seperti logam dan bahan pencemar organik berterusan (POPs) dalam ekosistem marin telah menjadi kebimbangan utama kerana ia boleh berkumpul dalam rantaian makanan hidupan laut. Secara umum, kajian ini bertujuan untuk menganalisis kepekatan logam terpilih (Hg, As, Cu, Pb dan Cd) dan kehadiran POPs terpilih (diklorodifeniltri-kloroethana dan hexa-klorosikloheksana) di dalam sampel udang dipilih yang dikumpulkan dari tapak pendaratan ikan di Semenanjung Malaysia. Selain itu, kajian ini bertujuan untuk menilai kualiti udang dengan membandingkan dengan Piawai FAO / WHO dan Akta Makanan Malaysia dan Peraturan-Peraturan Makanan 1983 (MFA). Kepekatan logam dalam sampel udang digunakan untuk mengira anggaran pengambilan harian (EDI) logam, nilai sasaran bahaya (THQ) dan risiko kanser sasaran (TR) untuk penduduk tempatan. Dalam kajian ini, tiga spesies udang terpilih yang diperoleh daripada lima tapak pendaratan ikan yang terletak di sepanjang Pantai Timur dan Barat Semenanjung Malaysia telah dianalisis menggunakan Plasma Gandingan Aruhan Spektrometer Jisim (ICP-MS) bagi logam manakala analisis untuk POPs telah dijalankan menggunakan Kromatografi Gas Pengesan Penangkap Elektron (GC-ECD). Kepekatan tertinggi As, Hg dan Pb dikesan dalam sampel udang yang dikumpul dari Perak, iaitu *P. merguiensis* (328.93 ± 0.78 µg/g), *P. merguiensis* (1012.83 ± 0.78 µg/g) dan *P. monodon* (4.07 ± 1.31 µg/g). Kepekatan tertinggi Cu didapati pada *L. vannamei* (7.56 ± 0.51 µg/g) yang dikumpul dari Melaka, manakala untuk Cd didapati lebih tinggi dalam *L. vannamei* (0.66 ± 0.38 µg/g) yang dikutip dari Johor. Perbezaan ketara antara kedua pengumpulan Pb dalam *L. vannamei* dan kepekatan

Cd dalam *P. monodon* untuk semua lokasi persampelan ($p < 0.05$). Kebanyakan nilai adalah lebih tinggi daripada had yang dibenarkan yang ditetapkan oleh FAO / WHO 2010, dan juga, Akta Makanan 1983 dan Peraturan-Peraturan Makanan 1985. Dari kajian ini, ia boleh dilihat dengan jelas bahawa udang yang dikumpul sepanjang pantai barat Semenanjung Malaysia adalah lebih tercemar kerana mempunyai lebih banyak industri dan penduduk lebih padat yang menyebabkan lebih banyak bahan cemar telah dilepaskan. Bagi POPs, diklorodifeniltri-kloroetana (DDT) adalah lebih banyak dalam spesies udang berbanding heksa-klorosikloheksana (HCH). Ia mungkin berpunca daripada penggunaan yang berlebihan racun perosak dan agen kawalan penyakit yang mengandungi DDT. Dapat disimpulkan bahawa sampel udang dikumpul dari Semenanjung Malaysia tercemar dengan logam dan POPs terpilih.

Katakunci: laut, udang, logam, DDT dan HCH.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA : Analyses of Variance

ATSDR : Agency for Toxic Substances and Disease Registry

DDD : 1,1-dichloro-2,2bis (p-chlorophenyl)ethane

DDE : Dichloro diphenyl dichloroethane

DDT : Dichlorodiphenyltrichloroethane

EDI : Estimated Daily Intake

ENHIS : Environment and Health Information System

EU : European Union

GCE-CD : Gas Chromatograph Equipped with an Electron-Capture Detector

HC : Hexachlorobenzene

HCHs : Hexachlorocyclohexanes

HUFA : Highly Unsaturated Fatty Acids

ICP-MS : Inductively Couple Plasma-Mass Spectrophotometer

LD50 : Lethal dose for 50 percent of the animals tested

MARS-5 : Microwave accelerated reaction system

MRL : Minimal Risk Level

NYSDOH : New York State Department of Health

OCPs : Organochlorine Pesticides

OPP : Organophosphate pesticide

PAH : Polycyclic aromatic hydrocarbon

POPs	:	Persistent Organic Pollutants
THQ	:	Target Hazard Quotient
TR	:	Target Cancer Risk
USEPA	:	United States Environmental Protection Agency
UNEP	:	United Nations Environment Programme
WHO	:	World Health Organization

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Seafood as protein sources

Nutritious food are necessary to maintain human health. Seafood sources such as fish, bivalves and crustaceans are an important food source. Sea products are one of the main resources of food industry necessary to meets the world's food requirements (Food and Agriculture Organization, 2010). Shrimp contains nutrients and protein that are essential for human health, thus offers an alternative to protein from chicken, mutton and beef. The analysis of nutrient in shrimp muscles (Table 1.1) recorded lower lipid content, which is around 1.15g/100g as compared to other meaty food (Vasagam *et al.*, 2013).

According to Gates (2010), shrimps contain a high source of vitamin B12, selenium, highly unsaturated fatty acids (HUFA) and astaxanthin, which is a potent natural antioxidant. In addition, shrimps contain protein and minerals such as calcium and iron (Baboli et al., 2013).

Penaeus monodon, Penaeus merguiensis and *Litopenaeus vannamei* are the shrimp species that are most commonly consumed in Malaysia due to its high supply and affordable prices. Other than being sold at the wet market, shrimps can be processed into other products such as frozen shrimp, tempura and sushi. According to Alina *et al*. (2012) shrimp contributed about 15% of the total value for traded fisheries products in the world in 2010. Although shrimp farming generate production and continuous supply of shrimp industry, mangrove ecosystem are being destroyed. The environmental pollution within this area has cause imbalances to the biodiversity. Thus, many consumers prefer to take wild-caught products as compared to aquafarm products.

Table 1.1: Average analysed nutrient profile of 100g edible shrimp muscles* (Vasagam, 2013)

Citation report table is derived from Current Science Association, Copyright Current Science.

Nutrient	Value
Protein (g)	19.4±0.56
Lipid (g)	1.15±0.19
Water (g)	76.3±0.57
Energy (kcal)	89.0±1.12
Essential amino acids (mg)	
Isoleucine	930.7 ± 8.10
Leucine	1463.9 ± 22.30
Lysine	1480.1 ± 27.57
Methionine + cysteine	668.1 ± 16.57
Phenylalanine + tyrosine	1389.2 ± 19.27
Threonine	756.0 ± 8.89
Tryptophan	223.3 ± 2.90
Valine	935.7 ± 5.89
Lipid composition	
ΣSFA (mg)	257.5 ± 3.71
ΣMUFA (mg)	163.5 ± 7.90
ΣPUFA (mg)	321.0 ± 5.23
Eicosapentaenoic (mg)	112.0 ± 3.02
Docosahexaenoic (mg)	75.5 ± 1.43
Σ*n*-3 PUFA (mg)	204.5 ± 2.23
Σ*n*-6 PUFA (mg)	106.0 ± 2.31
n-6/*n*-3 PUFA	0.5 ± 0.01
PUFA/SFA	1.3 ± 0.05
Cholesterol (mg)	173 ± 6.93
Macro minerals (mg)	
Calcium	107.3 ± 1.96
Magnesium	58.5 ± 1.38
Phosphorus	303.4 ± 3.22
Potassium	259.6 ± 3.25
Sodium	176.1 ± 3.04
Micro minerals (µg)	
Copper	918 ± 4.62
Iron	2196.5 ± 16.61
Manganese	50.5 ± 1.64
Selenium	44 ± 1.06
Zinc	1403.5 ± 5.43

*The average values (*n* = 60) with nutrients from the data obtained for tiger shrimp (*Penaeus monodon*) and Indian white shrimp (*Fenneropenaeus indicus*) from both wild caught (10–20 g) and cultured (20–25 g). The average proximate composition (%) of feeds used is 9.55, 38.32, 6.30, 3.93, 12.51 and 29.39 for moisture, crude protein, ether extract, crude fibre, total ash and nitrogen-free extract respectively.

Wild caught shrimps accumulate to approximately 45% of the global shrimp supply and are the source of income for about 900,000 fishermen worldwide (Banks & Macfadyen, 2011). However, wild-caught shrimps are also exposed to contamination from various sources. Examples of these contamination are pollution to coastal and ocean area by marine debris, introduction of chemicals from industry and oil spill from marine activities.

According to Gesamp (1991), marine pollution can be defined as "the introduction by means directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for usage of seawater and reduction of amenities".

Marine pollution occurs as a consequence from the increase of industrial, agricultural and urban activities. There are three main types of pollutants into the marine environment, namely wastewater, runoff and contaminants that are released from the atmosphere (Fartoosi, 2013). Marine pollution could influence the contamination of seafood sources as the ocean acts as the final receiver of pollutants. The following sections discuss the contamination of products from the marine environment.

1.2 Contamination in seafood

Pollutants from anthropogenic activities could accumulate in seafood sources and transfer along the food chain, causing negative impacts to the environment. Pollutants such as anthropogenic chemicals could affect marine life and contaminate human food sources. The abundance of chemical compounds in the food sources are a significant concern to the public due to the ill effects towards human health.

The contamination of food sources especially seafood were reported by several studies. Methylmercury consumption from seafood was recorded where nearby sea was contaminated with mercury discharge from various sources (Chen *et al*., 2012). Rahouma *et al*. (2012) reported that the concentration of zinc (Zn) in *Acetes indicus* collected from Malacca and Kedah were 45.79 ppm and 45.08 ppm, respectively which was below the World Health Organization (WHO) limits. However, the concentration of manganese (Mn) in *Acetes indicus* from Malacca and Kedah was 6.10 ppm and 6.95 ppm, respectively that exceed the permissible limit (Rahouma *et al.*, 2012).

Marine organisms like bivalves and crustaceans have the ability to increase the level of metals concentration through filter feeding habits (Akankali & Elenwo, 2015). According to Hu, Jin and Kavan (2014), metals are not biodegradable and can be biomagnified in the food chain, thus finally can enter the human body when consumed. The ability for the bioaccumulation of the contaminant in marine environment resulted in high risk to human health. The harmful effects of metals to human health due to over consumption could be neurotoxic, carcinogenic, mutagenic or teratogenic (Verma & Dwivedi, 2013).

Besides metals, sea products are also susceptible to persistent organic pollutants (POPs). POPs have the ability to transfer to the environment through water, soil and air as they are semi-volatile. According to Naso *et al.* (2005), POPs that are used in industries have properties like chemical stability and hydrophobicity, that give them the ability to bioaccumulate easily in biota. The accumulation of these contaminants depend on the lipids content of the organisms and other environmental conditions.

POPs like organochlorine pesticides (OCPs) are highly persistent in the environment. This chemicals were used to control malaria and typhus, but it was banned in most advanced countries (Aktar *et al*., 2009). Besides that, it is used widely in insecticides and may escape from agricultural land to rivers and ocean by run off. Although pesticides have been applied to the target organisms, nevertheless non target species could affected seriously.

OCPs like hexachlorocyclohexanes (HCHs) were found in Baiyangdian Lake, North China (Hu *et al.*, 2010). This situation could result in a constant risk to the ecosystem. Due to the ability of sea animals to accumulate these contaminants from the environment, they are widely used as a biomonitoring agent. This agent acts as the indicator of the level of contamination.

1.3 Problem Statement

In June 2008, European Union (EU) banned Malaysian seafood products due to health concern where the seafood industries in the country failed to meet the standards set by the agriculture audit authorities of the EU (Retnam & Zakaria, 2010). The bioaccumulation of metals and POPs in commercial shrimps have become a major concern since these contaminants can enter the food web.

Information or research about the accumulation of these contaminants in the aquatic life, especially on commercial shrimps are very rare. Therefore, more studies on the status of the distribution of metals and POPs in marine organisms should be conducted.

It is necessary to understand the level of pollutants within a specific area to monitor the accumulation of contaminants. This study is important to ensure the safety of public health and to provide information on the status of contamination in shrimps from different fish landing sites in Malaysia.

1.4 Objectives of research

In general, this study is aimed to analyse the presence of metals and OCPs in selected shrimp samples from selected fish landing sites in Malaysia. Specifically, this study aimed:

a) To determine metals (Cu, As, Cd, Hg and Pb) concentration in shrimp species obtained from selected fish landing sites in Peninsular Malaysia.

b) To investigate selected OCPs (DDT and HCH) distribution in shrimp species taken from selected fish landing sites in Peninsular Malaysia.

c) To assess the quality of shrimp for the human consumption according to Food and Agriculture Organization/World Health Organization, 2010 and Malaysian Food Act and Food Regulations, 1983.

1.5 Outline of the thesis

Chapter 1: Introduction

This chapter presents a brief introduction and overview about seafood as protein sources and contamination in seafood. The problem statement and objectives of this research are addressed.

Chapter 2: Literature review

Chapter 2 discusses the background of shrimps production in Malaysia and types of shrimp species in this study, which are *Penaeus monodon*, *Penaeus merguiensis* and *Litopenaeus vannamei.* Apart from that, food security especially on seafood products, marine contamination and types of marine contamination are elaborated. There are two types of marine contamination that are focused in this study, which are metals contamination and persistent organic pollutants contamination.

Chapter 3: Materials and method

In Chapter 3, the chemicals, instruments, study area and procedure for the analysis of metals and OCPs are explained. Besides that, statistical analysis and health risk assessment by United States Environmental Protection Agency (USEPA) procedures namely Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Target Cancer Risk (TR) are included.

Chapter 4: Results and Discussion

In this chapter, the results of metals analysis and distribution of OCPs in shrimp species between selected sampling locations are presented and discussed. Comparison of metals accumulation in shrimp species and its correlation between each other are also discussed. Statistical analysis with one way analyses of variance (ANOVA) for metals concentration in shrimp samples between the sampling locations and health risk assessment are presented.

Chapter 5: Conclusion

The conclusion on accumulation of metals and distribution of OCPs in shrimp samples between different species and sampling sites are elaborated in this chapter.

CHAPTER 2: LITERATURE REVIEW

2.1 Shrimps production in Malaysia

Important states for fishing are located along the west coast of Peninsular Malaysian (Perak and Selangor), while the top two shrimp aquaculture states of Sabah and Penang are situated in eastern Borneo and in the northwest portion of the Malaysian Peninsula, respectively (Figure 2.1) (Portley, 2016). Nowadays there is an increasing number of aquaculture farms in Malaysia that breed and produce shrimps in order to fulfil consumers' demand. However, annual production shows that wild shrimp has exceeded farmed shrimp production in Malaysia (Figure 2.2) (Portley, 2016).

Figure 2.1: Map of Malaysia with the major shrimp aquaculture states of Sabah and Penang indicated with circles and the main states for wild harvest, Perak and Selangor, indicated with a square (Portley, 2016)

Citation report graphic is derived from Sustainable Fish, Copyright Sustainable Fisheries Partnership.

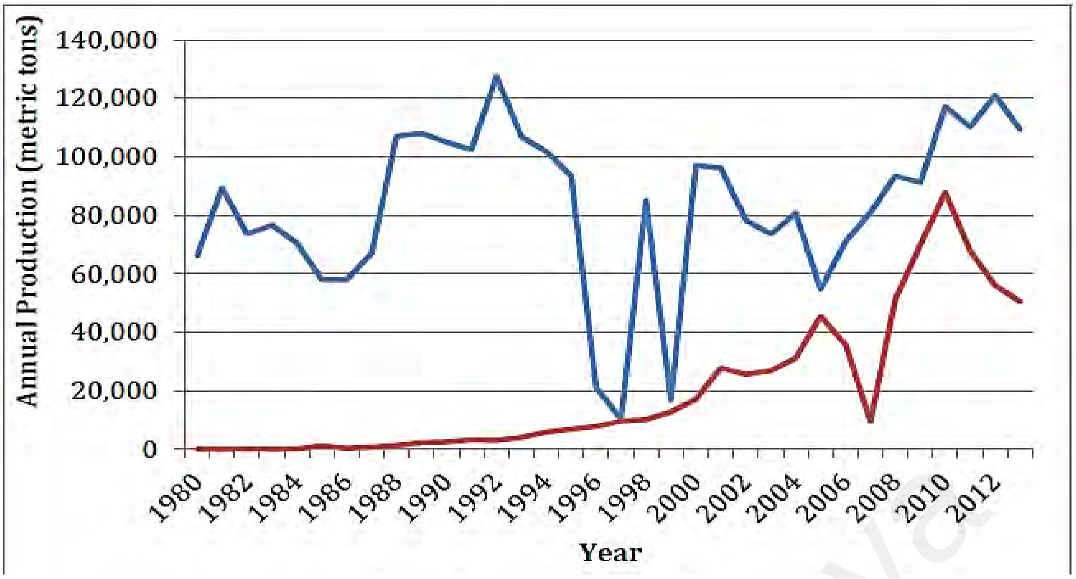

Figure 2.2: Malaysian wild shrimp (blue) and farmed shrimp (red) annual production in metric tons, 1980-2013 (Portley, 2016)

Citation report graphic is derived from Sustainable Fish, Copyright Sustainable Fisheries Partnership.

According to Portley (2016), farmed shrimp has experienced low production due to disease namely white spot syndrome, gill-associated virus, Taura syndrome virus, Monodon Baculo virus and Hepatopancreatic parvo virus. The spread of diseases to farmed shrimp could be due to the lack of water treatment system. Percentage of wild and farmed shrimp species harvested in Malaysia are shown in Table 2.1. The next sections discuss the types of shrimps commonly consumed worldwide.

Table 2.1: Percentage of wild and farmed shrimp species harvested in Malaysia (Portley, 2016)

Citation report table is derived from Sustainable Fish, Copyright Sustainable Fisheries Partnership.

Wild target species	**Percentage of national harvest (%)**
Banana shrimp	10
Giant tiger shrimp	1
Flower shrimp	89
Geragau shrimp	
Parapenaeopsis spp.	
Pink shrimp	
Farmed target species	**Percentage of national harvest (%)**
Whiteleg shrimp	89
Giant tiger shrimp	10
Giant freshwater shrimp	1

2.2 Types of shrimps in this study

2.2.1 *Penaeus monodon*

Penaeus monodon, which is also known as giant tiger prawn, could be found along the coasts of Australia, South East Asia, South Asia and East Africa (Food & Agriculture Organization, 2006). Figure 2.3 shows the main producing countries of *P.monodon*.

Figure 2.3: Main producer countries of *P.monodon* (Food & Agriculture Organization, 2006)

Citation report graphic is derived from Fisheries and Aquaculture Department, Copyright Food and Agriculture Organization of the United Nations.

According to Food and Agriculture Organization (2006), *P.monodon* matures and breeds in tropical marine habitats, and undergo their production cycle in coastal estuaries, lagoons or mangrove areas. Table 2.2 shows the life history phases of *P.monodon* and the diagram of its life history is illustrated in Figure 2.4.

Table 2.2 : Life history phases of *P.monodon* (Motoh, 1985)

Citation report table is derived from SEAFDEC/AQD Institutional Repository (SAIR), Copyright SEAFDEC Aquaculture Department.

Phase	**Begins at**	**Duration**	**Carapace length (mm)**		**Mode of life**	**Habitat**
			Male	**Female**		
Embryo	Fertilization	12 hours	0.29[a]		Planktonic	Outer littoral area
Larvae	Hatching	20 days	0.5-2.2		Planktonic	Outer/ inner littoral area
Juvenile	Completion of gill system	15 days	2.2-11.0		Benthic	Estuarine area
Adolescent	Stability of body proportion, development of outer genitalia	4 months	11-30[b]	11-37[c]	Benthic	Estuarine area
Subadult	Commencement of sexual maturity, first copulation	4 months	30-37[d]	37-47[e]	Benthic	Inner/ outer littoral area
Adult	Completion of sexual maturity	10 months	37-71[f]	47-81[f]	Benthic	Outer littoral area

[a] Egg diameter, [b] Minimum size with joined petasma, [c] Minimum size with adult-like thelycum, [d] Miminum size with spermatozoa in terminal ampoules, [e] Minimum size with spermatozoa in thelycum, [f] Maximum size ever found.

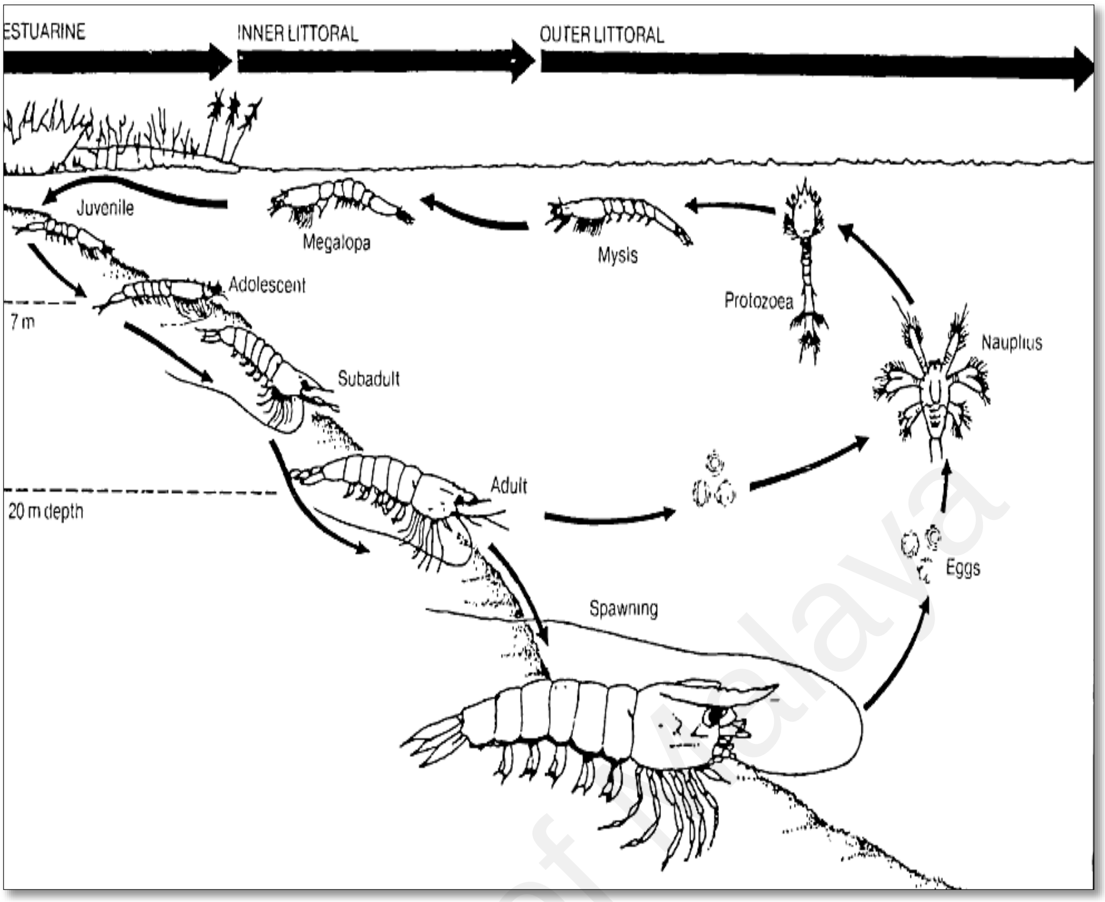

Figure 2.4: Diagrammatic representation of life history of *P.monodon* (Motoh, 1985)

Citation report graphic is derived from SEAFDEC/AQD Institutional Repository (SAIR), Copyright SEAFDEC Aquaculture Department.

The various types of product from *P.monodon* has raised its demand in the market, namely frozen shrimp, tempura shrimp, sushi and shrimp balls. In order to fulfil the high demand on *P.monodon*, aquaculture production has been introduced in several Asian countries.

2.2.2 *Penaeus merguiensis*

Penaeus merguiensis which is also known as banana prawn can be found at the sea of Persian Gulf, India, Malaysia, Thailand, Philippines, Indonesia, and Australia (Food & Agriculture Organization, 2015). Its abundance was influenced by food availability, characteristics of mangrove habitat, existence of predators, and competitors (Mulya *et al.*, 2012). Production of *P.merguiensis* through wild capture are insufficient to supply world population demand, thus requires other alternatives including aquaculture farming. Figures 2.5 and 2.6 show the global production of *P.merguiensis* from wild capture and aquaculture farming, respectively.

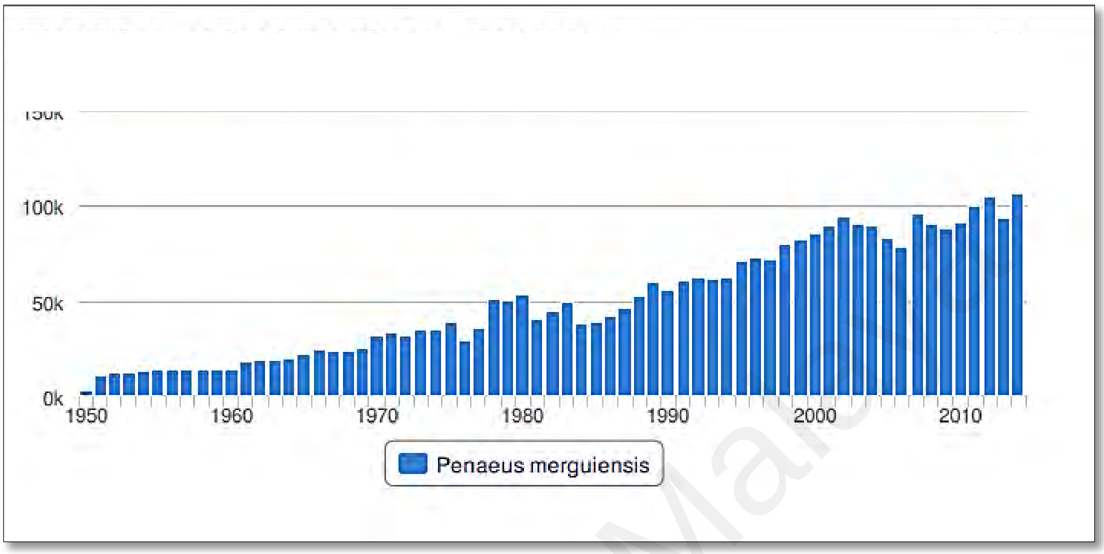

Figure 2.5: Global wild capture production for species (tonnes) (Food & Agriculture Organization, 2015)

Citation report graphic is derived from Fisheries and Aquaculture Department, Copyright Food and Agriculture Organization of the United Nations.

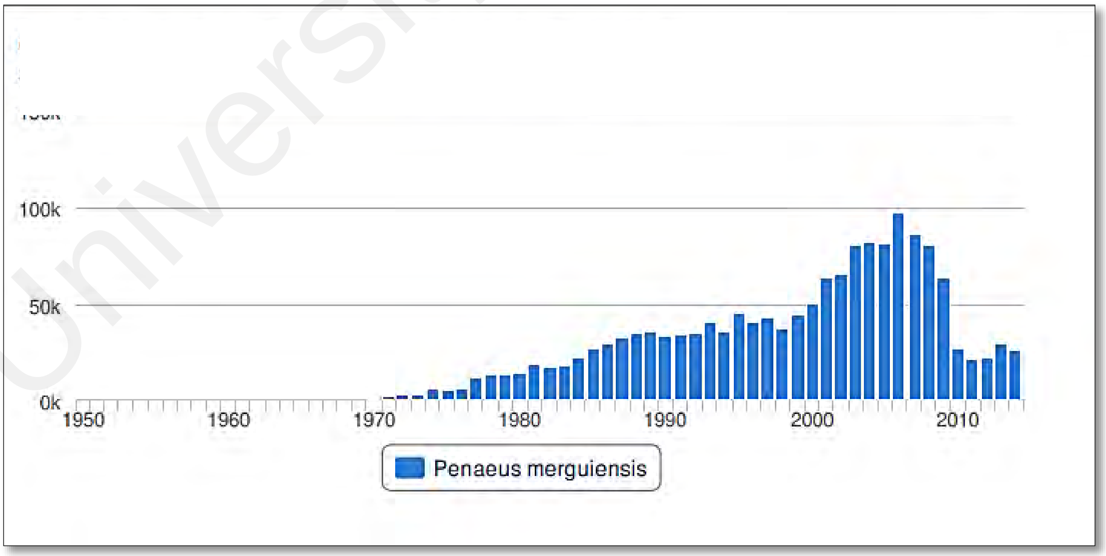

Figure 2.6: Global aquaculture production for species (tonnes) (Food & Agriculture Organization, 2015)

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Total wild capture production of *P.merguiensis* increased gradually from 1950 to 2014. For aquaculture farmed, the production of *P.merguiensis* has been on increase from 1970 to 2007 and decline in 2008 due to infections of diseases.

2.2.3 *Litopenaeus vannamei*

Litopenaeus vannamei or white shrimp inhabits tropical marine habitats. According to Food and Agriculture Organization (2015), adults shrimp inhabits and spawn in open ocean, while post larvae transfer to coastal to spend their juvenile, adolescent and sub-adult stages live in coastal estuaries, lagoons or mangrove areas. Larger *L. vannamei* feeds close to the bottom of the sea on actively moving organisms (Varadharajan & Pushparajan, 2013).

The main producing countries of white shrimp are China, Thailand, Indonesia, Brazil, Ecuador, Mexico, Venezuela, Honduras, Guatemala, Nicaragua, Belize, Vietnam, Malaysia, Taiwan, Pacific Islands, Peru, Colombia, Costa Rica, Panama, El Salvador, United States of America, India, Philippines, Cambodia, Suriname, Saint Kitts, Jamaica, Cuba and Dominican Republic (Food and Agriculture Organization, 2015) (Figure 2.7). *L. vannamei* has high potential to be grown in aquaculture industries to boost international market. However, culture of *L. vannamei* could suffer from disease. Thus, it is important to increase food security in seafood products in order to maintain its availability to consumers. Following sections discuss about food security in seafood products.

Figure 2.7: Main producing countries of *L. vannamei* (Food and Agriculture Organization, 2015)

Citation report graphic is derived from Fisheries and Aquaculture Department, Copyright Food and Agriculture Organization of the United Nations.

2.3 Food security on seafood product

Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (Food & Agriculture Organization, 2006).

Figure 2.8 shows the dimensions of food security. There are four dimensions of food security namely food availability, food access, utilization and stability (Food & Agriculture Organisation, 2006). The availability of food with adequate quantities and quality is important in food security. Study by Loring *et al*. (2013) reported that many people in Alaska especially those with lower income agree with the improved of food security as they could access to local seafood.

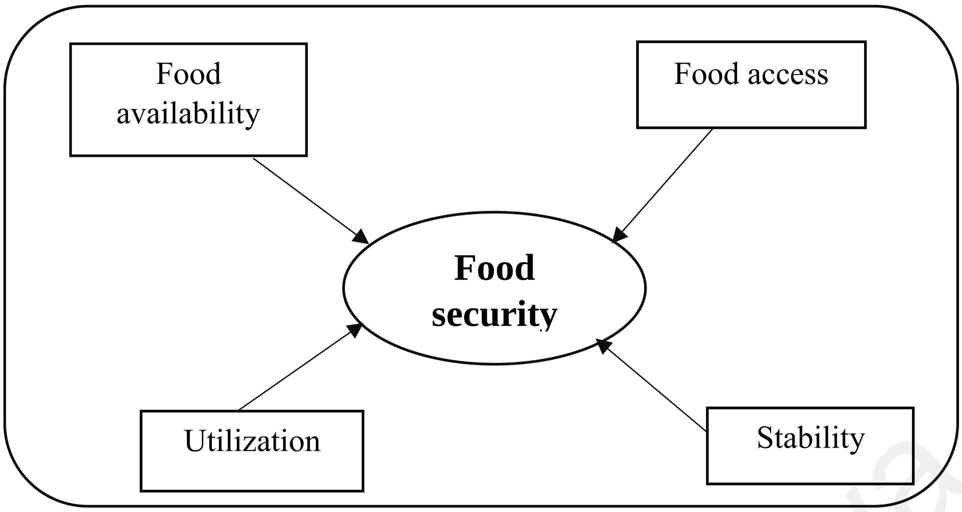


Figure 2.8: Dimensions of food security (Food & Agriculture Organisation, 2006)

Citation report graphic is derived from Fisheries and Aquaculture Department, Copyright Food and Agriculture Organization of the United Nations.

However, the increase in demands of ocean resources, marine contamination and climate change pose a problems to supply sufficient food sources especially seafood to world population. It could create food-insecurity to the low income level group within the affected area. Besides that, increase in population growth and economic problems also contribute to food insecurity. Naylor (2016) reported that more than two billion people are food-insecure towards calorie, micronutrient deficiencies, food supplies and prices.

Therefore, the introduction of sustainable aquaculture industry become major contributor in order to increase food security in global seafood. The growth of aquaculture production could increase the accessibility of seafood with good quality. According to Naylor (2016) aquaculture is expected to supply majority of global seafood demand as a consequences of the rise in population and deficiency of wild fisheries worldwide.

Besides that, the introduction of new technology and modern device for wild capture of seafood at the ocean could increase food security. However, the rise in pollution and ocean demand has caused marine contamination, thus affecting seafood supply and quality. The next section discusses more about marine contamination.

2.4 Marine contamination

Marine environment in Malaysia has very rich biodiversity and resources with various species of flora and fauna. Its biodiversity are highly complex that generate various interactions within the ecosystems. Figure 2.9 shows the demands of coastal and marine resources by humans and environment. This includes fisheries, aquaculture, recreation, industrial, military, shipping and navigation.

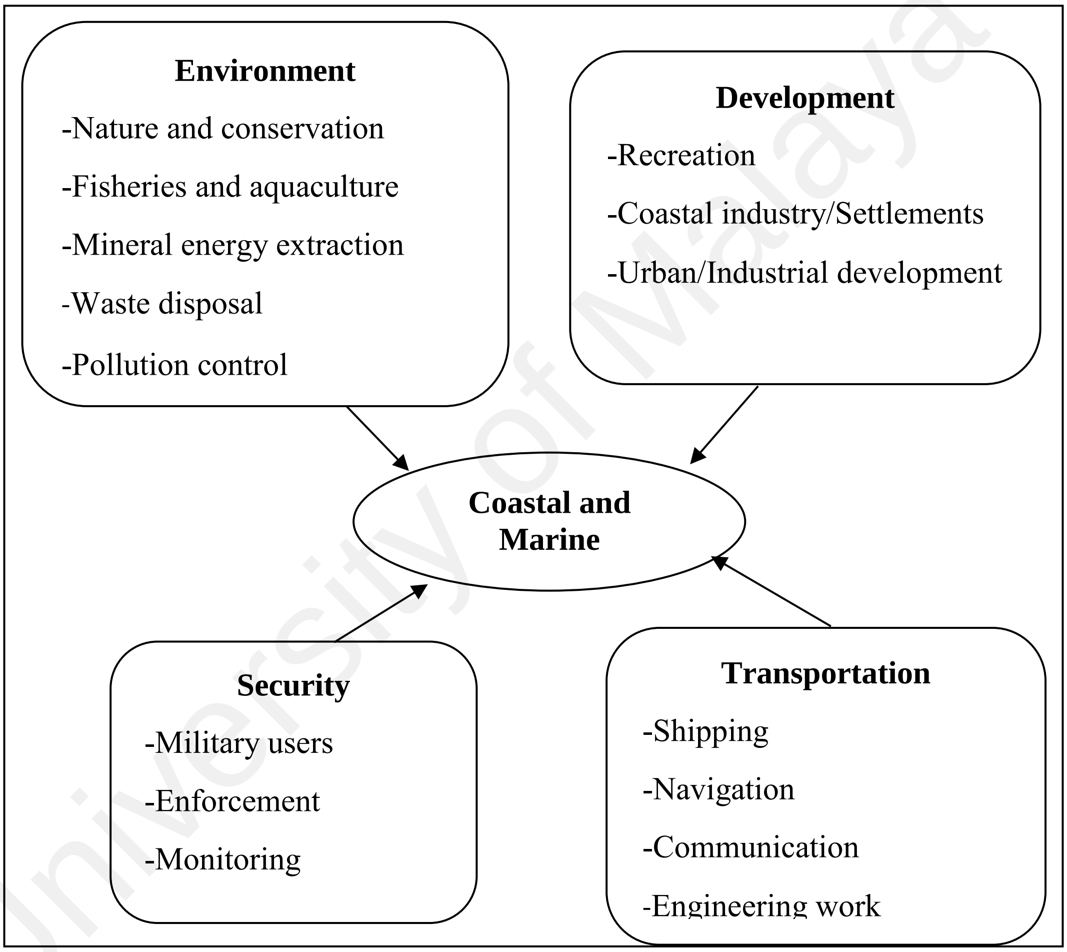


Figure 2.9: Demands for coastal and marine resources (Boateng, 2006)

Citation report graphic is derived from International Federation of Surveyors, Copyright International Federation of Surveyors.

One of the worst marine issues nowadays is marine pollution involving the contamination of ocean and river water quality by unwanted and native matters. Contamination to the marine environment occurs when input from human activities increases the substances ocean above the natural background level (Clark, 2001).

Marine contamination mainly are resulted from atmospheric, land and sea-based activities. According to Gesamp (1991), marine pollution were created by human, either directly or indirectly, and by the discharge of substances into marine environment that cause impacts to humans, plants, animals and marine activities. Anthropogenic activities influences the quality of marine ecosystems and are responsible for the harm to the environment.

2.5 Types of marine contaminations

2.5.1 Metals contaminations

Metals can be defined as elements with metallic properties such as ductility, conductivity, stability as cations, and ligand specificity (Umamaheswari *et al.*, 2011), for example mercury (Hg), lead (Pb), copper (Cu), arsenic (As), cadmium (Cd) and chromium (Cr). It is toxic in nature, poorly soluble in water and non-biodegradable. Some of the metals required by living organisms is shown in Table 2.3. Metals are introduced into the water system either directly or indirectly, originated from natural sources and human activities.

Table 2.3: Roles of metals on animal bodies (Khayatzadeh & Abbasi, 2010)

Citation report table is derived from International Applied Geological Congress, Copyright Geological Congress.

Metals	Roles on animal bodies
As	non specific growth stimulation
Co	constituent of vitamin B12
Cr	regulator of metabolism of glucose and cholesterol
Fe	essential to almost all organisms (in haemoglobin for respiration, cytochromes, catalyses, peroxides)
Mn	a transition metal
Mo	baldheaded and xanthenes oxidases formation of fatty acids and uric acid respectively

Besides natural processes, the other causes of metals pollution in the ocean could be from anthropogenic pollutants such as discharges from industrial waste and agriculture, leachate that contains metals, disposal of batteries, pollution from petroleum industrial activities and urban sewage. Clark *et al*. (1997) found that Pb, Zn, Ni and Cu are the most common metal pollutants from anthropogenic activities (Table 2.4). Table 2.5 shows the maximum contamination levels for metals in water.

Table 2.4: The world wide emission of metals to the atmosphere by natural sources and anthropogenic sources Clark *et al.* (1997)

Citation report table is derived from National Centre for Biotechnology Information, Copyright National Library of Medicine.

Metals	**Natural sources (thousands of tonnes per year)**	**Anthropogenic sources (thousands of tonnes per year)**
Ni	26	47
Pb	19	450
Cu	19	56
As	7.8	24
Zn	4	320
Cd	1.0	7.5
Se	0.4	1.1

Table 2.5: Maximum contamination levels for metals in water (United States Environmental Protection Agency, 1987)

Citation report table is derived from National Service Centre for Environmental Publications, Copyright Environmental Protection Agency.

Metals	**Max. conc. in drinking water (mg/l)**	**Max .conc. in H_2O supporting aquatic life (mg/l or ppm)**
Cadmium	0.005	0.008
Lead	0.01	0.0058
Zinc	5.00	0.0766
Mercury	0.002	0.05
Calcium	50	Tolerable >50
Arsenic	0.01	-

Industrial activity has introduced high concentrations of metals into the environment. For example, ash generated by coal burning power plants are enriched with metals and this holds ecological importance because of their toxicity, persistence, and bio-accumulation (Lubna *et al*., 2012). Soft metal ions like Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Au^{3+}, Pt^{4+}, and Ti^{+} are highly toxic and present in a variety of waste streams that could contaminate the environment (Umamaheswari *et al.*, 2011).

A part from that, metals pollution has become the environmental issues in developed and developing countries. Marine environment have the tendency to be exposed to contaminants such as metals, which remain for a long time as they are not easily decomposed. As a result, it will effect marine life and other organisms in the food chain. Even at low concentration metals give impact to living organisms (Jakimska *et al*., 2011). Therefore, studies on metals contamination in marine organisms are necessary to protect human health.

Metals can be adsorbed onto suspended particulate matter in the sea and affect marine organisms (Jakimska *et al.*, 2011). Metals have the ability to accumulate in living organisms any time they are taken up and stored more rapidly than being metabolised or excreted (Zeitoun *et al.*, 2014). According to Khayatzadeh and Abbasi (2010), metals can cause toxicity to organisms as it cause oxidative stress by formation of free radicals and could replace essential metals in pigments or enzymes and disrupt its function.

Golovanova (2008) reported that metals such as mercury, cadmium, copper and zinc are considered as the most dangerous in the eco-toxicological aspect which can accumulate in many important organs. Marine organisms contamination by metals raise public concern as it effect human health. Silva *et al.* (2016), reported the following ranges of metal elements in the tissue of *L.vannamei* collected from Bahia, Brazil in µg g^{-1}: Al: 13.4-886.5, Cd: 0.93-1.80; Cu: 24.8-152; Fe: 3.2-410.9; Mn: 0.36-24.4; Se: 0.094-9.81 and Zn: 20.3-109.4.

The toxic effect of certain metals caused it to interfere with the environmental processes and enter the food chain to effect human health (Mitra & Zaman, 2014). Metals could be absorbed into human body through ingestion of marine organisms like fish and crustaceans. Therefore, analysis for metals can be done by assessing the quality of seafood for human consumption based on limit allowed by Food and Agriculture Organization and World Health Organization (Codex Alimentarius Commision, 2010) and standard set by Ministry of Health Malaysia as stated in the Malaysian Food Act (1983) (Table 2.6). Metals may undergo biotransformation and metabolism, and it can excreted from human body without giving any toxic effects, depending on its chemical characteristics and dosage (Zeitoun *et al.*, 2014). This contaminants have the ability to bind to vital cellular components like structural proteins, enzymes and nucleic acids, and interfere with their biological function after a long term exposure and carcinogenic (Rajeswari & Sailaja, 2014).

Table 2.6: Permitted level of metals in seafood

Metals	**FAO/WHO (Codex Alimentarius Commision, 2010) (µg/g)**	**Malaysian Food Act (1983) (µg/g)**
Cu	-	-
As	0.1	1
Cd	2	1
Hg	0.5	0.5
Pb	0.3	1

Apart from that, metals contaminant could resulted in dermatological diseases, skin cancer, internal organs cancers, cardiovascular disease, diabetes, as well as, reproductive, immunological and neurological effects in human body (Kaoud & EL-Dahshan, 2010). Zeitoun *et al.* (2014) reported that metals toxicity due to long-term exposure can cause muscular and neurological degenerative processes that imitate Alzheimer's and Parkinson's disease.

2.5.1.1 Background of selected metals

i) Mercury (Hg)

Hg could be emitted to the environment through natural and anthropogenic sources (Barbara *et al.,* 2016). Figure 2.10 shows the natural sources of Hg emission to the environment. The anthropogenic sources of Hg emission mainly include acid rain, coal burning and mining. Besides that, Hg is a metals that has been used in fluorescent lamps, thermal power plants and thermometer.

In zero oxidation state, Hg^0 exists as vapour or as liquid metal, Hg_2^{2+} exists as inorganic salts and Hg^{2+} may form either inorganic salts or organomercury compounds (Rajeswari & Sailaja, 2014). This pollutant occurs in several forms which can result in toxic effects. Hg is present in marine organisms mostly as methylmercury (Pia *et al.*, 2007).

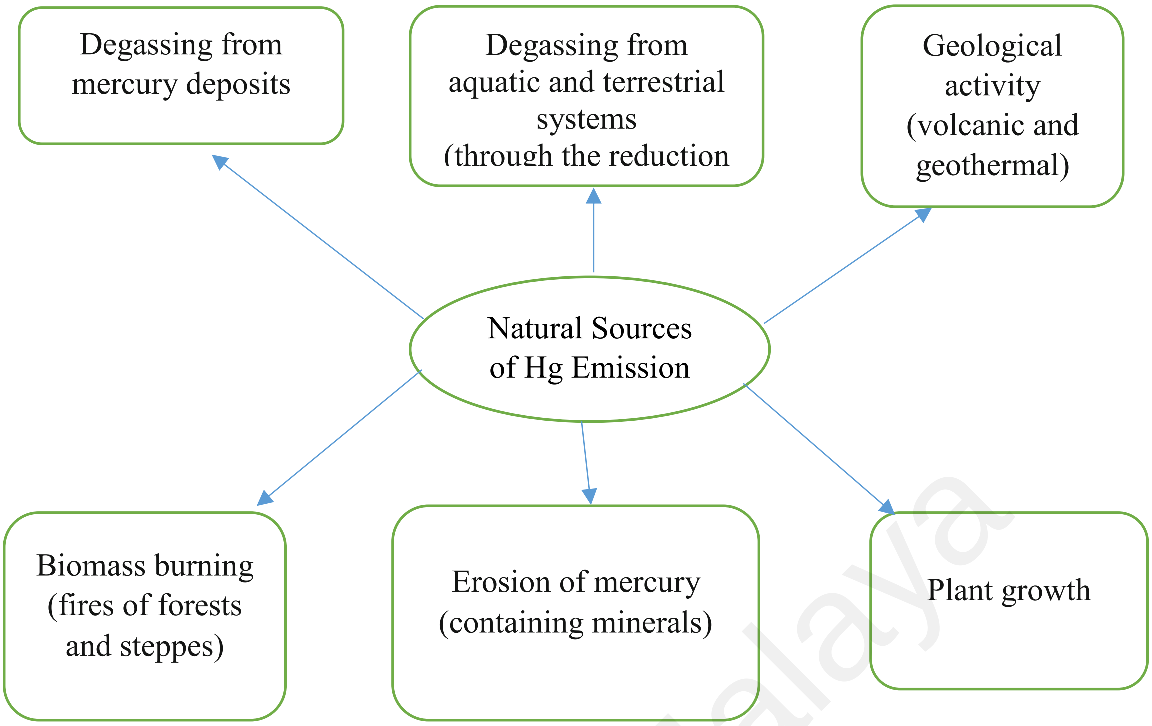


Figure 2.10: Natural sources of Hg emission to the environment

Hg poisoning could cause several diseases like acrodynia (pink disease), Hunter-Russell syndrome and Minamata disease (Rajeswari & Sailaja, 2014). According to Clarkson *et al.* (2003), the Minamata Tragedy in Japan in the 1950s was due to methyl mercury contamination on fishes from the discharge of Hg into ocean. In human, it resulted in the increased risk of coronary heart disease. Table 2.7 shows the concentration of Hg in seafood collected from west Peninsular Malaysia.

Table 2.7: Hg concentration (µg/g) in seafood from west Peninsular Malaysia (Anual *et al.*, (2018)

Citation report table is derived from Microchemical Journal, Copyright The Authors.

Groups/family/species	Common name	Hg concentration (µg/g)
Scrombidae		
Rastrelliger kanagurta	Indian mackerel	0.059
Rastrelliger brachysoma	Indo-Pacific mackerel	0.025
Scomberomorus guttatus	Indo-Pacific king mackerel	0.051
Scomberomorus commerson	Narrowbarred Spanish mackerel	0.108
Rastreliger faughni	Faughni mackerel	0.058
Scomber australasicus	Slimy mackerel	0.098
Gymnosarda unicolor	Dogtooth tuna	0.360
Thunnus tonggol	Longtail tuna	0.160
Penaeidae		
Metapenaeus barbata	Sand velvet shrimp	0.025
Metapenaeus affinis	Pink shrimp	0.076
Metapenaeus brevicornis	Yellow shrimp	0.021
Parapenaeopsis sculptilis	Rainbow shrimp	0.053
Parapenaeopsis hardwickii	Spear shrimp	0.060
Penaeus indicus	Indian white prawn	0.024
Penaeus merguiensis	Banana prawn	0.064
Penaeus monodon	Giant Tiger Prawn	0.043
Loliginidae		
Loligo duvaucelli	Indian squid	0.036
Loligo sibogae	Sibogae squid	0.056
Loligo uyii	Little squid	0.063
Loligo chinensis	Mitre squid	0.024
Loligo edulis	Sword tip squid	0.041
Sciaenidae		
Nibea soldado	Soldier croaker	0.144
Otolithes ruber	Tigertooth croaker	0.097
Otolithoides biauritus	Bronze croaker	0.187

ii) Lead (Pb)

Pb is the most widespread metals contaminant and being used extensively in gasoline during the 1930s-1970s (Rajeswari & Sailaja, 2014). Besides that, it has been widely used in batteries, thermal power plants, mining industry, smelting, paints, ceramics and water pipes. There are several symptoms for acute Pb poisoning such as headache, irritability and abdominal pain (Quaterman, 1986). According to Ferrer *et al*. (2006) Pb is the most toxic metal and it can be absorbed through ingestion of food, water, and inhalation where in the body, Pb inhibit the synthesis of haemoglobin and cause dysfunctions to kidneys and joints.

World Health Organization (2010) reported that Pb is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Research done by Agoes *et al.* (2009) revealed that, 0.022 µg/g of Pb were found in *P. merguiensis* collected from Indonesia. A part from that, research done by Hossain and Khan (2001) found Pb concentration in a range of 0.8 to 1.3 µg/g in *P. monodon* collected from Bangladesh.

iii) Arsenic (As)

The most toxic forms of As are the inorganic As (III) and (V) that are well known as rat poison (Codex Alimentarius Commision, 2010). In the marine environment As is often found in high concentrations in organic forms, up to 50 mg/kg of As on a wet weight basis can be detected in some seafood including seaweed, fish, shellfish and crustaceans (Codex Alimentarius Commision, 2010). Intake of As lead to toxicity in large quantities to gastrointestinal tract, central nervous systems and death. Gomez-Caminero *et al.* (2001) reported that exposure to As via drinking water can cause cancer in the lungs, kidneys, urinary bladder and skin. Piyawat *et al.* (2012) reported 0.010 µg/g of As concentration in *Macrobrachium rosenbergii* collected from Thailand.

iv) Copper (Cu)

Cu were used in smelting operations, mining, electroplating industry for pipes, alloys and coating and commercial uses. Cu is distributed in food sources like liver, shellfish and crustaceans, grains, cereal products and potatoes, which contribute to about 65% of total dietary intake (Codex Alimentarius Commision, 2010). However, Cu does not appear to be mutagenic nor carcinogenic to affect the reproduction and cumulative toxic hazard for man. Yet, this is an exception for individuals that suffer from Wilson's diseases (Codex Alimentarius Commision, 2010). Ip *et al.* (2005), found 28 μg/g of Cu concentration in *Metapenaeus ensis* collected from China.

v) Cadmium (Cd)

Cd has been found as a highly toxic element to human being (Storelli & Barone, 2013). Cd has the ability to accumulate and retained in human body. Exposure to Cd can caused lung diseases, and bone defects in humans and animals (Rajeswari & Sailaja, 2014). Solidum *et al.* (2013) reported that, Cd is toxic to kidney and resulted in bone demineralization. It has been recognized as a toxic metal that have the ability to interrupt the biological systems. Cd gives toxicity effects to the central nervous system and parenchymatous organs even at very small concentrations over a long period of exposure (Michael *et al.,* 1993). It is highly toxic that it can cause pulmonary effects during the inhalation of cadmium in atmosphere, renal damage, skeletal damage, cardiovascular disease and cause of death. High exposure of Cd through inhaled dusts and fumes resulted in lung disease and cadmium pneumonitis, and bone defects like osteomalacia and osteoporosis (Verma & Dwivedi, 2013). Sofia (2005) reported Cd concentration in *Penaeus coromandelica* ranged from 0.01 to 0.06 μg/g.

2.5.2 Persistent Organic Pollutants (POPs) contaminations

POPs are the contaminants for environment that are persistent and resistant to biodegradation. It could be characterized to have low water solubility and high lipid solubility, which resulted in the bioaccumulation in the fatty tissues, and semi-volatile. Some of the chemical structures of POPs are shown in Figure 2.11.

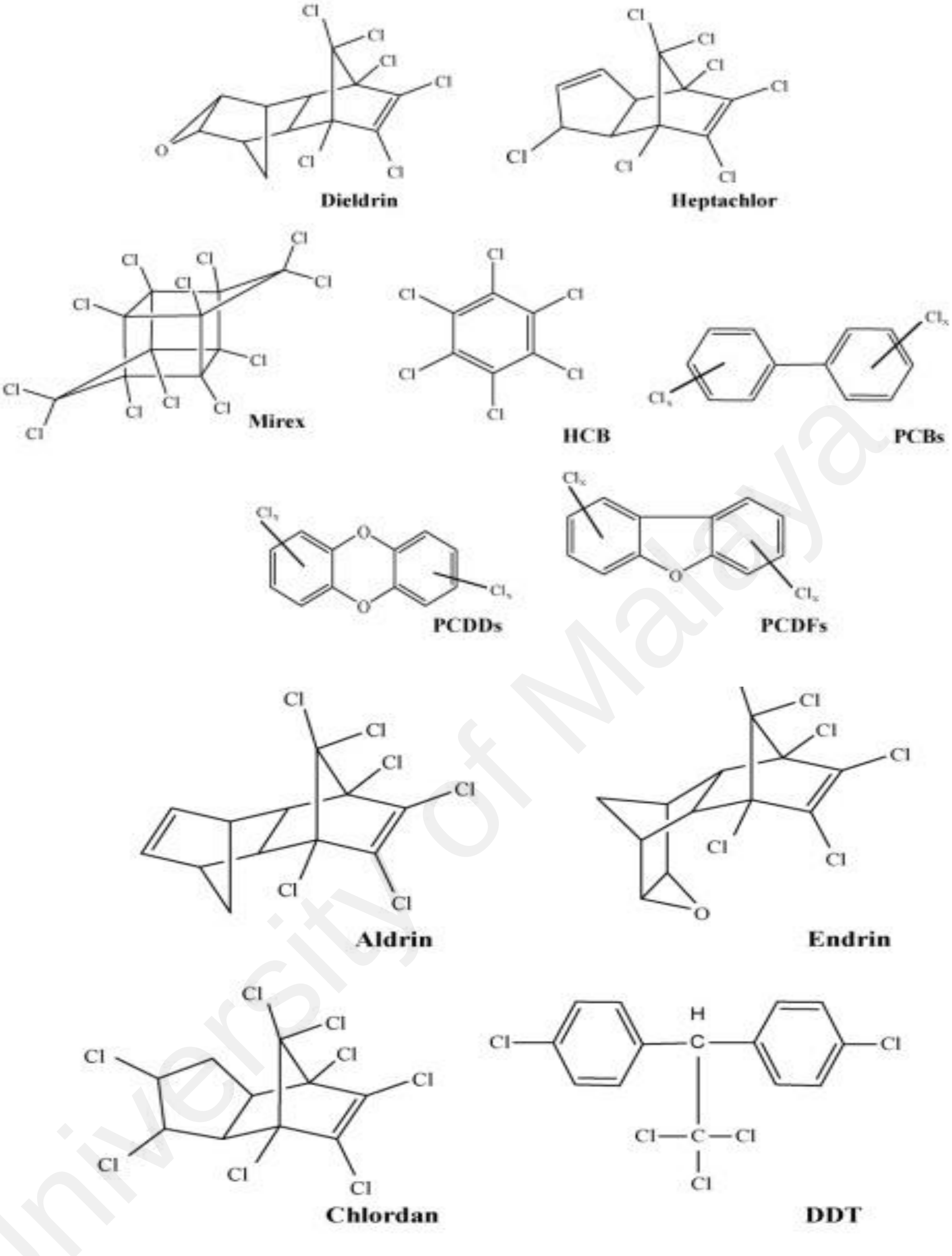


Figure 2.11: Selected chemical structures of POPs (El-Shahawi *et al*., 2010) Citation report graphic is derived from Talanta, Copyright Elsevier.

Stockholm Convention (2004) have identified 12 Dirty Dozen POPs namely, aldrin (a pesticides), chlordane (insecticides), DDT (avoid malaria, typhus), dieldrin (for textile pests and insects), dioxins and furans (produce from the manufacturing of chemicals), endrin (insecticides and pesticides), heptachlor (used as insecticides), hexachlorobenzene

(HCB) (fungicides), mirex (insecticides and fire retardant), PCBs (used in manufacturing industry) and toxaphene (insecticides).

The use of this pollutant has been forbidden in developed countries. However some developing countries still depend on them due to their low cost in industry, agriculture, and public health (Hu *et al.*, 2010). Table 2.8 shows the status of POPs in Malaysia. According to El-Shahawi *et al.* (2010), this pollutants have been banned in United States and Canada, yet they were found in the Arctic of Alaska since it can be transported towards colder area and sink.

Table 2.8: Status of POPs in Malaysia (Ricardo *et al.*, 2002)

Citation report table is derived from National Centre for Biotechnology Information, Copyright National Library of Medicine.

POPs	Status
Aldrin	Banned in 1994
Chlordane	Banned in 1998
DDT	Banned in 1999
Dieldrin	Banned in 1994
Endrin	Withdrawn by company
Heptachlor	Banned in 1990
HCB	Never registered
Mirex	Banned
Toxaphene	Banned

The invertebrates may acts as an important agent in the accumulation of POPs in the food web. This pollutant has the ability to bioaccumulate by increasing its concentration in tissues of organisms at higher level of the food chain. Table 2.9 shows the value of Minimal Risk Level (MRL) for organophosphate pesticide (OPP), polycyclic aromatic hydrocarbon (PAH) and organochlorine pesticides (OCP) intake in food.

Table 2.9: Minimal Risk Level (MRL) values for OPP, PAH and OCP intake in food (Agency for Toxic Substances and Disease Registry, 2010)

Citation report table is derived from Joint Research Centre, Copyright European Union.

OPP	**Minimal Risk Levels (mg/kg/day)**
Chlorpyrifos	0.003
Dichlorvos	0.004
Disulfoton	0.001
Parathion methyl	0.0007
PAH	**Minimal Risk Levels (mg/kg/day)**
Acenaphthene	0.6
Anthracene	10
Fluoranthene	0.4
Fluorene	0.4
Naphthalene	0.6
OCP	**Minimal Risk Levels (mg/kg/day)**
Aldrin	0.002
Chlordane	0.001
p,p'-DDT	0.0005
Dieldrin	0.0001
Endosulfan	0.005
Endrin	0.002
Heptachlor	0.0001
Hexachlorobenzene	0.008
Alpha-HCH	0.008
Beta-HCH	0.05
Gamma-HCH	0.003
Methoxychlor	0.005

2.5.2.1 Organochlorine pesticides (OCPs)

OCPs can be classified based on the functional groups of their molecular structures or specific function on the target species. This include insecticides, fungicides and herbicides (Van der Hoff & Van Zoonen, 1999). OCPs was used to prevent, control and reduce pests. It is used excessively to increase crop production in order to meet the high demand from consumers. The basic characteristics of OCPs include highly persistence, low aqueous solubility, low polarity and have high lipid solubility (Ravindran *et al.,* 2016). Pesticides could leave residues on crops and it decreased over time. However, some pesticides residues remain on the crops. Table 2.10 and 2.11 shows the uses of OCPs and properties for OCPs, respectively.

Table 2.10: Uses of OCPs (Ricardo *et al.*, 2002)

Citation report table is derived from National Library of Medicine, Copyright Dove Medical Press Limited.

OCPs	**Use/Source**
Aldrin and dieldrin	Insecticides used on crops such as corn and cotton; also used for termite control
Chlordane	Insecticide used on crops, including vegetables, small grains, potatoes, sugarcane, sugar beets, fruits, nuts, citrus and cotton. Used on home lawn and garden pests. Also used extensively to control termites
DDT	Insecticide used on agricultural crops, primarily cotton and insects that carry diseases such as malaria and typhus
1,1-dichloro-2,2bis (p-chlorophenyl)ethane (DDD)	Insecticides
Dichloro diphenyl dichloroethane (DDE)	Insecticides
Endrin	Insecticide used on crops such as cotton and grains; also used to control rodents
Dicofol	Acaricide
Methoxychlor	Insecticides
Lindane	Acaricide Insecticides Rodenticide
Endosulfan	Insecticides
Isodrin	Insecticides
Isobenzan	Insecticides
Mirex	Insecticides
Pentachlorophenol	Fungicide Herbicide Insecticides

Table 2.11: Properties of OCPs (United Nations Environment Programme, 2003)

Citation report is derived from UNEP Annual Evaluation Report, Copyright United Nations Environment Programme.

Name of compounds	**Half live (years)**	**Toxicity in rats (oral) LD50 (mg/kg)**	**Food and Agriculture Organization/WHO tolerance limit (mg/kg/day)**
HCHs	>1 – 2	60 – 250	0.1 – 0.5
Heptachlor	0.75 – 2	40 – 119	0.2
Aldrin	0.05 - 1.6	67	0.2
Endosulfan	0.1 – 0.4	18 – 160	0.2
DDT	15	113 – 118	5.0
DDD	5 – 10	4000	-
DDE	10	800 – 1240	-
Chlordane	10	200 – 700	-
Isodrin	0.5 – 6	8.8	-
Dieldrin	3 – 4	40 – 70	0.2
Endrin	<12	3 – 43	0.05
Isobenzan	2.8	4.8	-
Mirex	10	600 – 740	-

LD50: Lethal dose for 50 percent of the animals tested

OCPs contamination in the environment is a huge concern since it involved issues on food safety. Besides pests, human were also exposed to the mutagenic and carcinogenic effects of this pollutants. OCPs like hexa-chlorocyclohexanes (HCHs) and dichlorodiphenyltri-chloroethanes (DDTs) were applied intensively to control insects to increase crop yields after World War II (Hu *et al.*, 2010). HCHs and DDTs are anthropogenic contaminants that are persistent where they accumulate in the food web to give potential risks to the ecosystem and human health (Hu *et al.*, 2010).

Generally, human were exposed to OCPs through food ingestion, besides environmental exposure such as domestic and public health uses. Tan and Vijayaletchumy (1994), reported that HCH, DDT, heptachlor and dieldrin were found in rivers flowing through paddy field in Malaysia. Mohamad *et al*. (2015) reported the presence of DDT, heptachlor and endrin in *P. monodon* collected from B'hat Sadar, Bangladesh. Table 2.12 shows the biochemical effects of OCPs.

Table 2.12: Biochemical effects of selected OCPs

OCPs	Organism	Biochemical effects	References
Aldrin and Dieldrin	Human	Neurotoxic, reproductive, developmental,immunological, genotoxic, tumerogenic effects and nausea	USEPA (2003)
	Mouse, rat, guniea pig, rabbit and dog	Convulsions, loss in body weight, depression, increased irritability, salivation and hyperexitability	
Chlordane	Human	Convulsions, tremor, mental confusion and incoordination	Kajiwara *et al.* (2001)
	Mice	Reduced fertility, liver cancer	
	Seals	Cancer, trauma, meningocephalitis	
DDE	Human	Cyst in hands, itching, psoriasis, eczema, leucoderma, skin rashes	Subramaniam and Solomon (2006)
DDT	Mice	Liver tumors, liver changes including hepatocellular hypertrophy, margination and formation of lipospheres	WHO (1979)
	Birds	Egg shell thinning	
	Fish	Affects membrane function and enzymes	
	Salmons	Impaired behavioral development	
	Human	Dark or blurred vision, anxiety and restlessness, as well as psychiatric symptoms such as depression, memory loss, and confusion and acute pancreatitis.	USEPA (2000)

Table 2.12, continued.

OCPs	**Organism**	**Biochemical effects**	**References**
Endosulfan	Human	Decreases the white blood cell count and macrophage migration, adverse effects on humoral and cell-mediated immune system. Affects semen quality, sperm count, spermatogonial cells, sperm morphology and other defects in male sex hormones, DNA damage and mutation	Pratap & Vandana (2007)
Lindane	Human	Damage human liver, kidney, neural and immune systems, and induces birth defects cancer, cause neurotoxicity, reproductive toxicity and hepatotoxicity	Vijaya *et al.* (2011)

CHAPTER 3: METHODOLOGY

3.1 Chemicals and stock solutions

Reagents and chemicals used were all analytical grade: nitric acid (65%), sulphuric acid, anhydrous Na_2SO_4, dichloromethane and hexane. Deionised water was used for sample dilution and rinsing of glassware. All glassware were soaked in 2% nitric acid for 24 hours and rinsed with deionised water before use.

A multi-element standard solution IV for Inductively Couple Plasma-Mass Spectrophotometer (ICP-MS Agilent 7500a, Japan) was used to prepare the series of standard solutions of Hg, As, Pb, Cd and Cu, by dilution with 5% nitric acid. The concentration of Hg, As, Pb, Cd and Cu were 0, 10, 30, 50, 75 and 100 µg L^{-1}, respectively.

3.2 Instrumentation

Digestion was carried out using microwave accelerated reaction system (MARS-5, Australia), based on the parameters shows in Table 3.1.

Table 3.1: Microwave digestion operating conditions (Rasdi *et al.,* 2013)

Citation report is derived from Creative Common Attribution, Copyright International Journal of Molecular Sciences.

Step	Power (W)	% max	Time (min) to raise temperature	Temperature (°C)	Running time (min)
1	400	100	15	200	5
2	400	100	1	210	5
3	400	100	1	220	5

ICP-MS (Agilent 7500a, Japan) was used to analyse the concentration of metals in shrimp samples. The operating parameters are shown in Table 3.2.

Table 3.2: ICP-MS operating conditions

ICP-MS Systems	Parameter
RF Power	1550 watts
RF Matching	1.55 V
Reflected Power	0 W
Sample Uptake Time	30 sec
Sample Uptake Rate	0.4 r sec^{-1}
Sample Depth	5.0–5.5 mm
Coolant Argon Flow Rate	15 L min^{-1}
Carrier Gas Flow Rate	1.2 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Water RF/TP Flow Rate	2.4 L min^{-1}
Water RF/TP Temperature	20 °C

For the determination of the presence of DDTs and HCH isomers, the cleaned extracts were analysed using a Gas Chromatograph Equipped with an Electron-Capture Detector (GC-ECD 7890A, USA). A 60 m x 0.25 mm I.D. DB-5 column coated with 5%

diphenylpolydimethylsiloxane (film thickness 0.25 µm) were used for the separation. The oven temperature was programmed to increase from 90°C (holding time of 2 minutes) to 130°C at a rate of 15°C per min and finally to 290°C at 4°C per min, holding the final temperature for 20 minutes. The injector and detector temperatures were programmed at 280°C and 320°C, respectively. Injection were performed in splitless mode and helium was used as carrier gas (30.5 psi).

3.3 Study Area

Fresh shrimp samples were collected from five fish landing sites from selected states (Figure 3.1) namely Perak, Pahang and Johor at West Coast of Peninsular, and Kelantan and Pahang at East Coast of Peninsular Malaysia.

Figure 3.1: Location of sampling sites

Table 3.3: Location and coordinates of sampling sites

Location	**Coordinates**
Matang, Perak	4.8471° N, 100.6944° E
Serkam, Melaka	2.1395° N, 102.3766° E
Pasir Puteh, Kelantan	5.8362° N, 102.4077° E
Cherating, Pahang	4.1296° N, 103.3867° E
Tanjung Piai, Johor	1.2836° N, 103.5076° E

3.4 Sampling Procedure and Storage of Samples

Three shrimp species namely, *Penaeus monodon, Penaeus merguiensis* and *Litopenaeus vannamei* were collected from Perak, Malacca, Johor, Kelantan and Pahang (Figure 3.2, 3.3 and 3.4). These shrimps were chosen because they are popular food source and likely to be among the most consumed shrimp in Malaysia.

Figure 3.2: *Penaeus monodon*

Figure 3.3: *Penaeus merguiensis*

Figure 3.4: *Litopenaeus vannamei*

Samples were collected four times on alternate months between May - November 2015. The shrimp samples were placed in insulated boxes containing ice and brought to the laboratory for analysis. Upon arrival at the laboratory, 20 shrimp samples were chosen randomly and washed with deionised water and its muscle tissues were taken. The muscle tissue were homogenised using a food processor and kept in polythene bags at -20^{o}C for further analysis (Alina *et al.*, 2012). Muscle tissues were used instead of exoskeleton or viscera because of it is widely consumed by human.

3.5 Sample Preparation and Analysis of Metals

The metals in the shrimp muscles were analysed using the method described by Alina *et al.*(2012) with several modification. 0.5 g of homogenized muscle tissues of each shrimp species were put into a Teflon vessel where 10 mL of 65% nitric acid were added. The samples were then digested with a microwave (MARS-5).

After cooling, the mixture were filtered using filter paper and diluted to 50 mL with deionised water. ICP-MS (Agilent 7500a, Japan) was used to analyse target element (Cu, As, Cd, Hg and Pb). The analyses were done in triplicates.

3.6 Sample Preparation and Qualitative Analysis of OCPs

The extraction of DDT and HCH and instrumental analysis were performed as described in Frimpong *et al.*(2012). Muscle tissues of shrimp samples were ground with anhydrous Na_2SO_4 and then extracted with set-up soxhlet using dichloromethane and hexane at the ratio of 4:1. The cleaned extract were purified with sulphuric acid and concentrated by evaporation. It was analysed using GC-ECD (7890A, USA).

3.7 Statistical Analysis

One way analyses of variance (ANOVA) for metals concentration in shrimp samples between the sampling locations was analysed using SPSS 16. The value of $p<0.05$ will indicate a significant difference between the variables.

3.8 Health Risk Assessment by USEPA Procedures

The concentration of metals in shrimp samples were used to calculate the estimated daily intake (EDI) of metals, target hazard quotients (THQ) and target cancer risk (TR) for locals.

3.8.1 Estimated Daily Intake (EDI)

EDI was determined by the following equation:

$$EDI = \frac{MC\ X\ IR}{\mathrm{BW}} \qquad (3.1)$$

Where *MC* is the metal concentration in the samples (mg/kg wet weight), and *IR* is the ingestion rate, which is taken as 19.5×10^{-3} kg/day (Little *et al.*, 2002; Speedy, 2003); this consumption rate was used in the health-risk assessment. BW is the average body weight, which is 62.65 kg as carried out by Malaysian Adults Nutrition Survey (MANS) (Azmi *et al.,* 2009). *EDI* was compared with the recommended reference doses (*RfD*) (mg kg^{-1} day^{-1}) as stated in the following equation:

Hazard Ratio, $HR = EDI/RfD$ (3.2)

RfD values for metal are Cd (0.001), Cu (0.04), Pb (0.0035), As (0.0003), and Hg (0.0001) (USEPA, 2010). HR values more than one indicates there is a potential risk to human health, whereas a result of less than one indicates no risk.

3.8.2 Target Hazard Quotient (THQ)

THQ was used to the risk level (non-carcinogenic) due to pollutant exposure. To estimate the human health risk from consuming metal-contaminated fish and crustacean, THQ was calculated as per USEPA Region III Risk-based Concentration Table (USEPA, 2011).

The equation used for estimating THQ are,

$$THQ = \frac{EF\ x\ EDI\ x\ IR\ x\ Cf\ x\ CM}{WAB\ x\ ATn\ x\ RfD}\ \text{X}\ 10^{-3} \qquad (3.3)$$

Where *EF* is the exposure frequency (365 days/year), *EDI* is the exposure duration (30 years for non-cancer risk, as used by USEPA (2011), *IR* is the ingestion rate (49.5 g/person/day) (USEPA, 2011), *Cf* is the conversion factor (0.0208) to convert fresh weight (FW) to dry weight (DW) given that 79% is the moisture content in fish, *CM* is the metals concentration in shrimps (mg/kg w/w), *WAB* is the average body weight (bw) (62.65 kg) and *ATn* is the average exposure time for non-carcinogens (EF × ED) (365 days/year for 30 years (ATn = 10,950 days), as used in characterising no cancer risk (USEPA, 2011). The oral reference dose (*RfD*) of the metal (an estimate of the daily exposure to which the human population may be continuously exposed over a lifetime without an appreciable risk of deleterious effects) and RfD mg kg^{-1} day^{-1} were used for analysis according to USEPA (2016).

3.8.3 Target Cancer Risk (TR)

TR was used to indicate carcinogenic risks. The method to estimate TR is also provided in USEPA Region III Risk-Based Concentration Table (USEPA, 2011). The model for estimating TR is as follows:

$$TR = \frac{EF\ x\ ED\ x\ IR\ x\ Cf\ x\ CM\ x\ CPSo}{WAB\ x\ ATc}\ \text{x}\ 10^{-3} \qquad (3.4)$$

Where *CM* is the metal concentration in fish (µg/g), *IR* is the ingestion rate (g/day), *CPSo* is the carcinogenic potency slope for oral route (mg/kg bw/day), and *ATc* is the averaging time of carcinogens (365 days/year for 70 years), as used by USEPA (2011). US EPA recommended that CPSo values were used for As, Cd and Pb to determine the *TR* of each metal in shrimp samples.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Metals Analysis

This study investigated metals concentration in *Penaeus monodon, Penaeus merguiensis* and *Litopenaeus vannamei* and the results obtained can be used for future studies in Malaysia and other countries in Asia. The levels of metals contamination in shrimp samples were compared with the permitted level that were recommended by FAO/WHO, 2010 and standard set by Ministry of Health Malaysia as stated in Malaysian Food Act 1983 and were expressed in µg/g wet weight samples.

One-way ANOVA was performed to evaluate significant differences between metals concentration in shrimp samples and the sampling locations showed that there are significant differences between the sampling locations ($p<0.05$) in the concentration of Pb and Cd. Other metals concentration in shrimp samples have no significant differences among the different sampling locations.

Table 4.1: One way ANOVA between Pb concentration in shrimp samples and sampling locations

ANOVA

Pb concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.534	4	.883	12.987	.002
Within Groups	.476	7	.068		
Total	4.010	11			

Table 4.2: One way ANOVA between Cd concentration in shrimp samples and sampling locations

ANOVA

Cd concentration

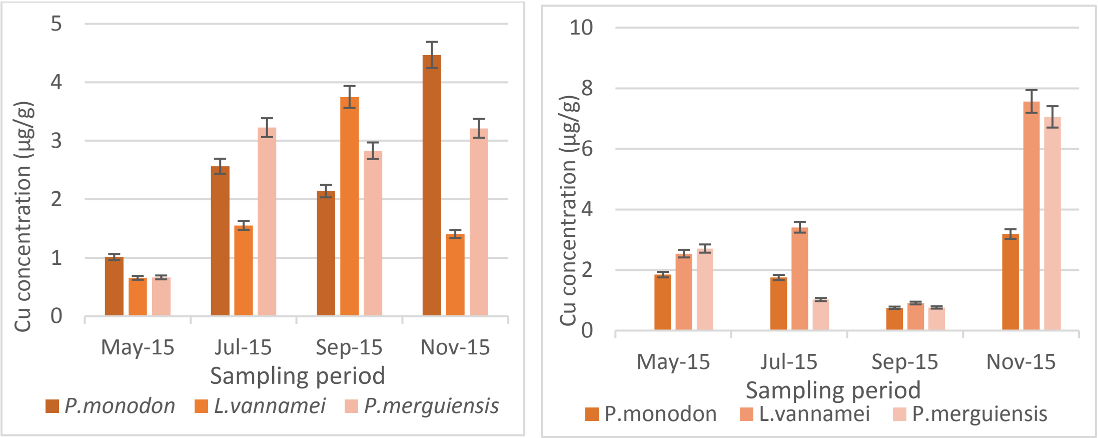
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24368.401	4	6092.100	3.746	.026
Within Groups	24391.780	15	1626.119		
Total	48760.181	19			

4.1.1 Concentration of Cu in shrimp species between selected sampling locations

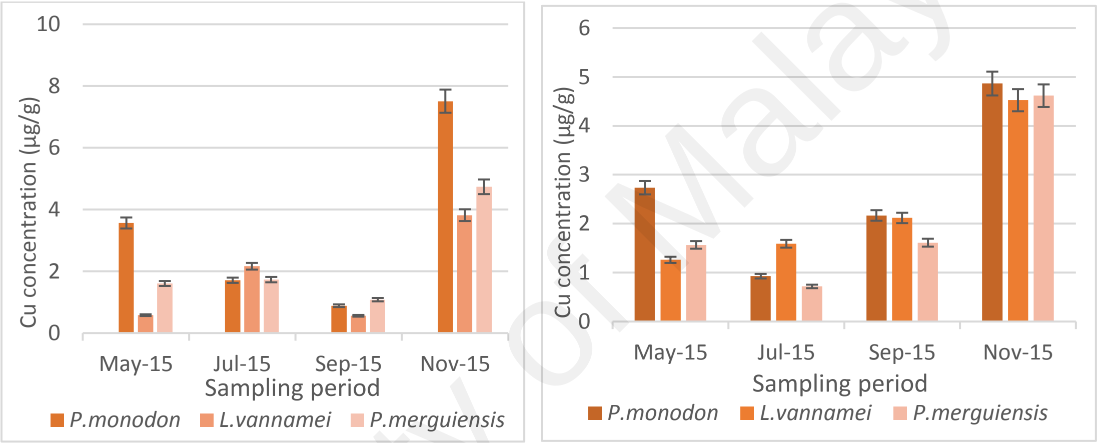
Figure 4.1 represents Cu concentration in three shrimp species collected from Perak, Malacca, Kelantan, Pahang and Johor. The highest Cu concentration was found in *L. vannamei* collected from Malacca (7.56 ± 0.51 µg/g), followed by *P. monodon* from Johor (7.51 ± 1.06 µg/g) and *P. merguiensis* from Malacca (7.05 ± 0.2 µg/g).

The differences in Cu concentration between shrimp species probably due to the dissimilarity in their feeding habits and biological characteristics. The contamination levels of metals are strongly related to feeding habits and lifestyle of the shrimp species. Similar results have been reported by Olgunoglu (2015), with different metal concentration in tissues of shrimp species (*Plesionika martia*, *Plesionika edwardsii*, and *Aristeus antennatus*) collected from northeast Mediterranean Sea, Turkey.

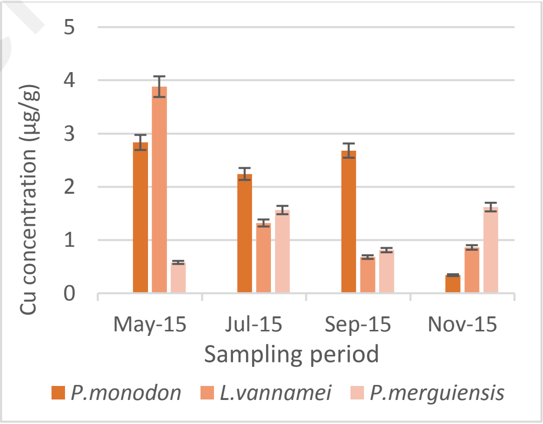
The differences in the level of Cu recorded among shrimp species might also be influenced by the environmental conditions like pH and temperature of the seawater. This is supported by Mitra and Zaman, (2014), who reported that the difference in metal concentration among shrimp species studied was due to the variations in environmental conditions.



(A) Perak (B) Malacca



(C) Johor (D) Pahang



(E) Kelantan

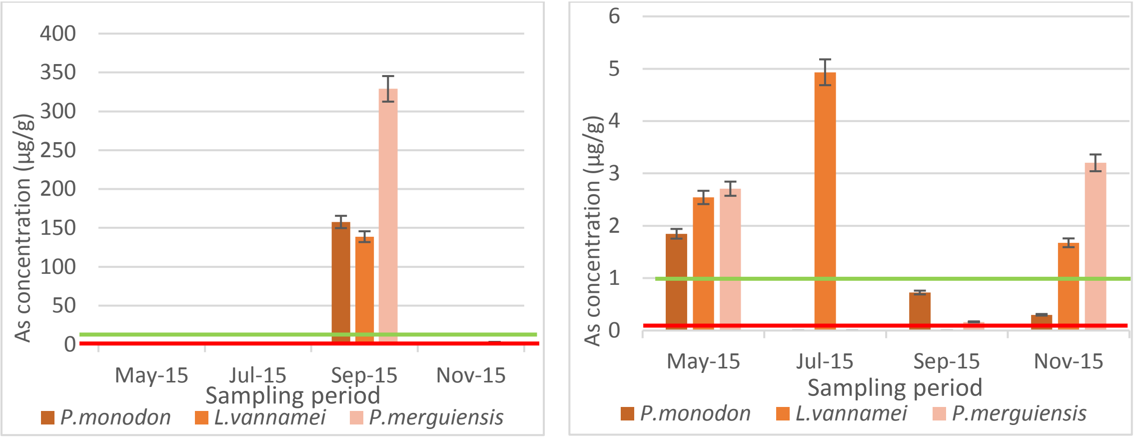
Figure 4.1: Concentration of Cu in shrimp samples collected from selected sampling locations

Anthropogenic activities within the sampling area like agriculture and shipping probably released some of the elements that eventually caused Cu to accumulate into surface water and reflected in shrimp samples. A part from that, the highest Cu concentration in shrimp samples were detected in Malacca and Johor. It might be due to these locations had receive various pollutants from extreme boat traffic for fishing and tourisms purposes. The highest Cu concentration in shrimp samples collected from Perak, Malacca, Johor and Pahang were detected during November 2015, while for Kelantan, in May 2015. This probably due to release of Cu that are used in antifouling paint from extreme boat traffic in Kelantan during May 2015, which is before monsoon, thus affecting shrimp samples through dietary intake.

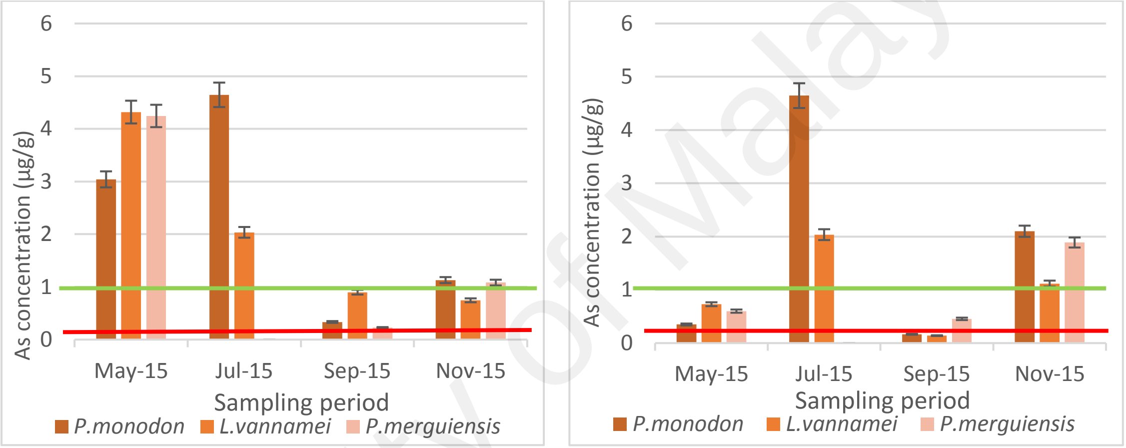
High detection in November 2015 could be due to these sampling locations had received huge amount of rain water throughout the rainy season, and caused Cu to enter river and ocean through the release of fossil fuels. Cu could settle from air during rainfall and bind to sediment in surface water (Rahman *et al.,* 2013), hence being accumulated in shrimp samples and other marine organisms through consumption of sediments. Cu that have been used in pesticide and fungicide might also washed away during rainy seasons, thus released to the environment and contaminate shrimp samples through feeding habit. However, the concentrations of Cu in all shrimp species in this study was below the acceptable limits set by FAO/WHO (Codex Alimentarius Commision, 2010) (10 µg/g) and Malaysia Food Act and Food Regulations (1983) (30 µg/g).

4.1.2 Concentration of As in shrimp species between selected sampling locations

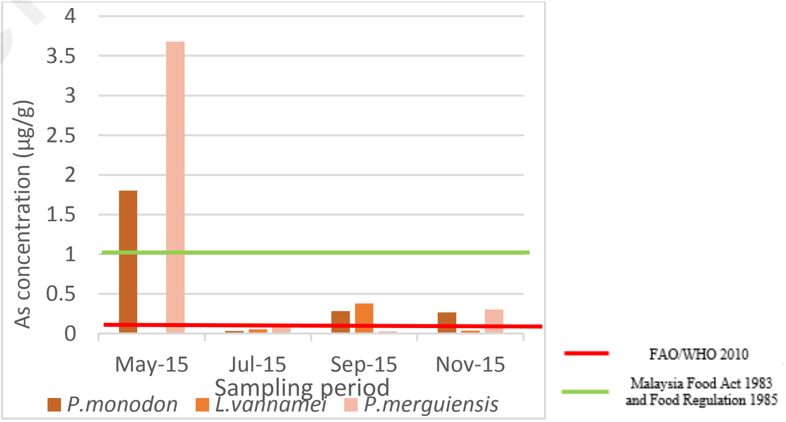
Besides that, the highest As concentration were detected in all shrimp samples collected from Perak, probably due to waste discharged from agricultural applications and metal work industries located in Matang, Perak, whereby effluents are discharged into the river and sea. This contaminants possibly affect the marine organisms through dietary intake.



(A) Perak (B) Malacca



(C) Johor (D) Pahang



(E) Kelantan

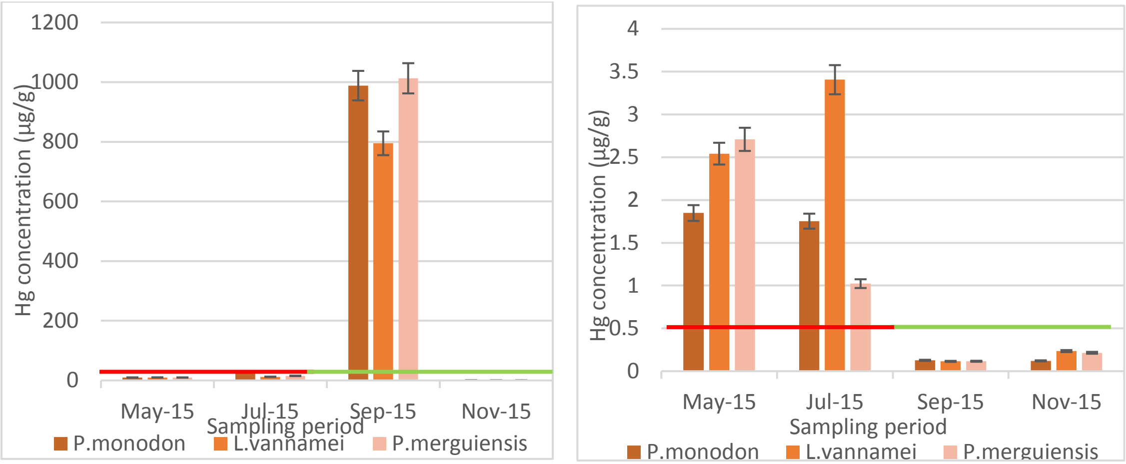
Figure 4.2: Concentration of As in shrimp samples collected from selected sampling locations

As seen in Figure 4.2, the highest As concentration in shrimp samples collected from Malacca, Johor, Pahang and Kelantan were detected in July and May, while for Perak, in September 2015. This could be due to heavy rainwater in Perak during September 2015, which is during monsoon, that caused runoff of As from agricultural land, wood preservative, paint and metals that finally flow to the ocean, therefore reflected in shrimp samples.

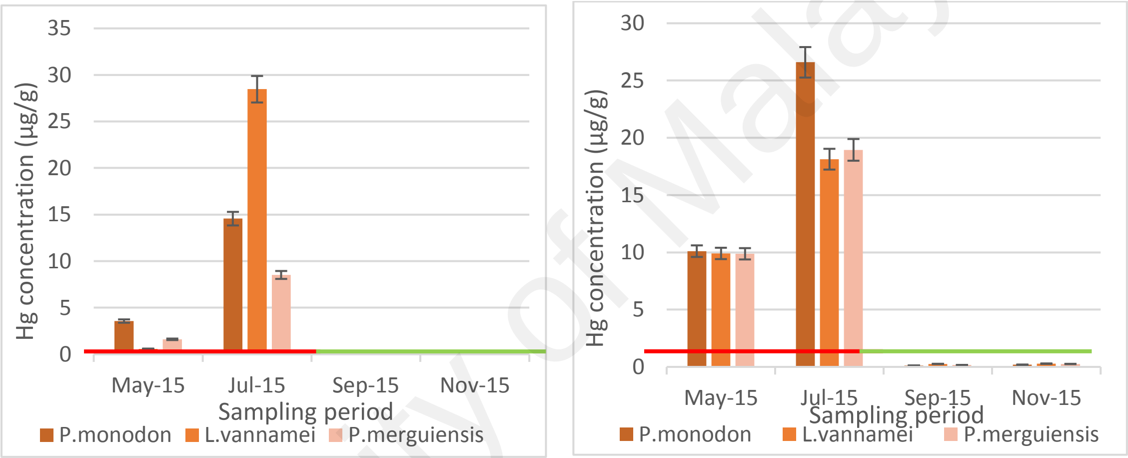
The three highest concentrations of As, which is *P. merguiensis* (328.93 ± 0.78 µg/g), *P. monodon* (157.7± 5.86 µg/g) and *L. vannamei* (138.77 ± 0.99 µg/g), indicated higher value compared to allowed limits set by FAO/WHO (Codex Alimentarius Commision, 2010) (0.1 µg/g) and Malaysia Food Act and Food Regulations (1983) (1 µg/g). Almost all species indicates higher value than allowed limits but at different sampling period.

4.1.3 Concentration of Hg in shrimp species between selected sampling locations

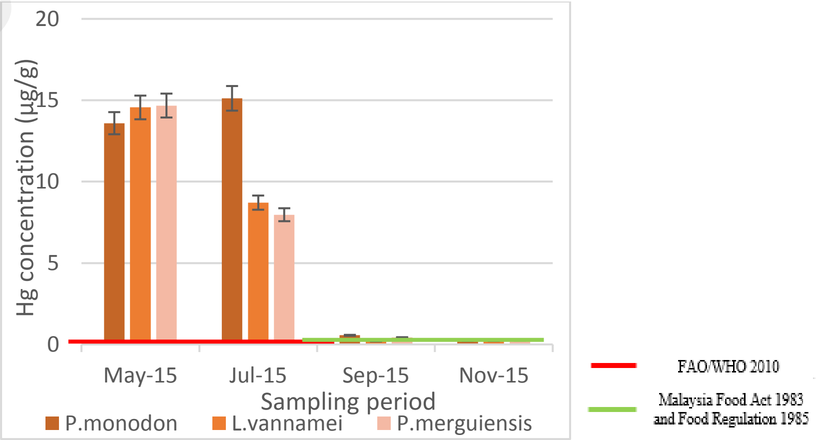
Figure 4.3 represents Hg concentration in three shrimp species collected from Perak, Malacca, Kelantan, Pahang and Johor. In this study, the highest Hg concentration was detected in all shrimp muscles collected from Perak, which were *P. merguiensis* (1012.83 ± 0.78 µg/g), followed by *P. monodon* (988.27 ± 5.86 µg/g) and *L. vannamei* (794.93± 0.99 µg/g). Hg was highest in *P. merguiensis* probably due to its habitat at the bottom of ocean which allow the sedimentation of higher density metal sediment compared to other species. Apart from that, this results happened probably because of the direct or unintentional discharge of waste from domestic and industries at sampling location that contain Hg, then will be trapped in sediments which acts as pollutant reservoir and taken by shrimp species as their food. Besides that, marine organisms like shrimp can be contaminated with Hg from acid rain through filter feeding.



(A) Perak (B) Malacca



(C)Johor (D) Pahang



(E) Kelantan

Figure 4.3: Concentration of Hg in shrimp samples collected from selected sampling locations

This is supported by Baboli and Velayatzadeh (2013), that marine organisms have the ability to filter their food from marine water thus resulted in accumulation of Hg and its most toxic organic compounds.

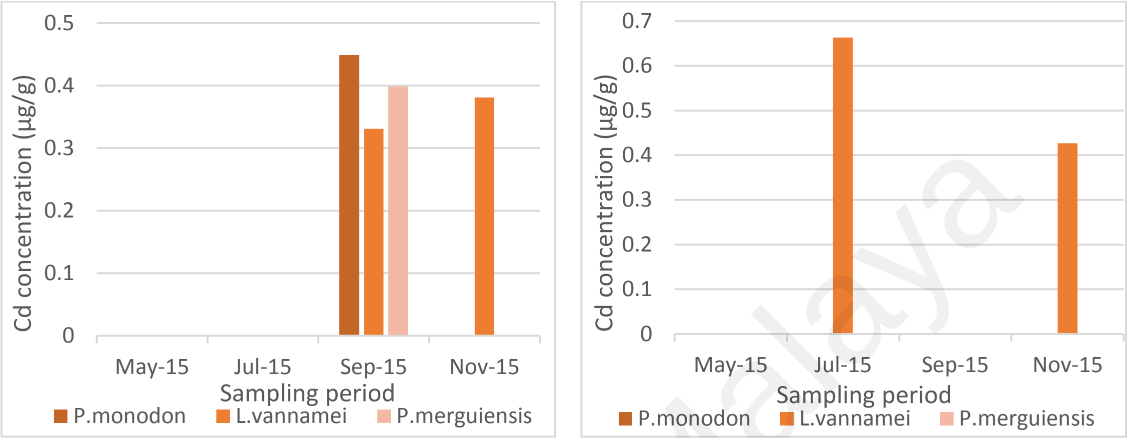
As seen in Figure 4.3, the highest concentration of Hg in shrimp samples collected from Malacca, Johor, Pahang and Kelantan were detected during July, while for Perak, in September 2015, which is in rainy season. This might be due to heavy rainwater that caused runoff of Hg originate from burning of fossil fuels and cement production near sampling location that flow into the ocean. This contaminant could distributed in aquatic environment and being uptake by shrimp samples.

Comparison with the study by Zurahanim *et al.* (2018) the concentration of Hg in muscles of crustaceans from west coast of Peninsular Malaysia ranged from 0.012 to 0.119 µg/g which is much lower than the recorded in this study. A study by Strorelli (2008) conducted in Italy reported Hg concentration in *Penaeus affinis* was 0.09 - 0.69 µg/g and was lower than this study. All the values in this study exceed the allowable limits set by FAO/WHO (Codex Alimentarius Commision, 2010) and Malaysia Food Act, which is 0.5 µg/g.

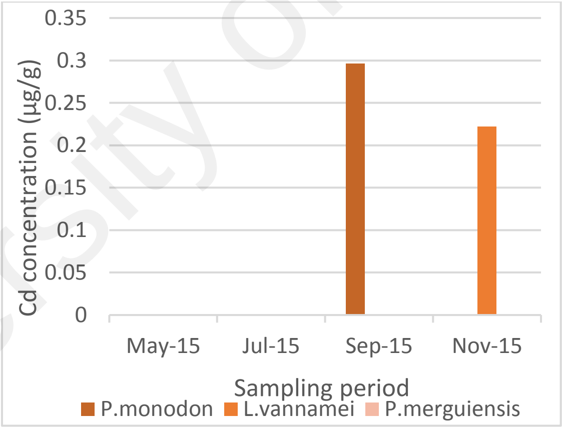
4.1.4 Concentration of Cd in shrimp species between selected sampling locations

Figure 4.4 shows Cd concentration in three shrimp species collected from Perak, Johor and Kelantan. The highest Cd concentration were detected in *L. vannamei* (0.66 ± 0.38 µg/g) collected from Johor, followed by *P. monodon* (0.45 ± 0.04 µg/g) collected from Perak and *L. vannamei* (0.43 ± 0.11 µg/g) collected from Johor. The accumulation of Cd were different between shrimp species could be due to variation in dietary uptake.

The presence of high Cd in shrimp samples collected from Johor might be due to the releases of municipal wastewater and incineration fumes from Tanjung Bin Power Plant into the sea. Besides that, the release of untreated wastewater into Sungai Kelantan, mainly from batik industry that contain Cd could flow into the ocean. This is agreed by Slamet *et al.* (2017), that colouring process in batik industry use synthetic dye contain metals like Cd. Eventually, this will contaminate the marine food chain including the shrimp groups.



(A) Perak (B) Johor



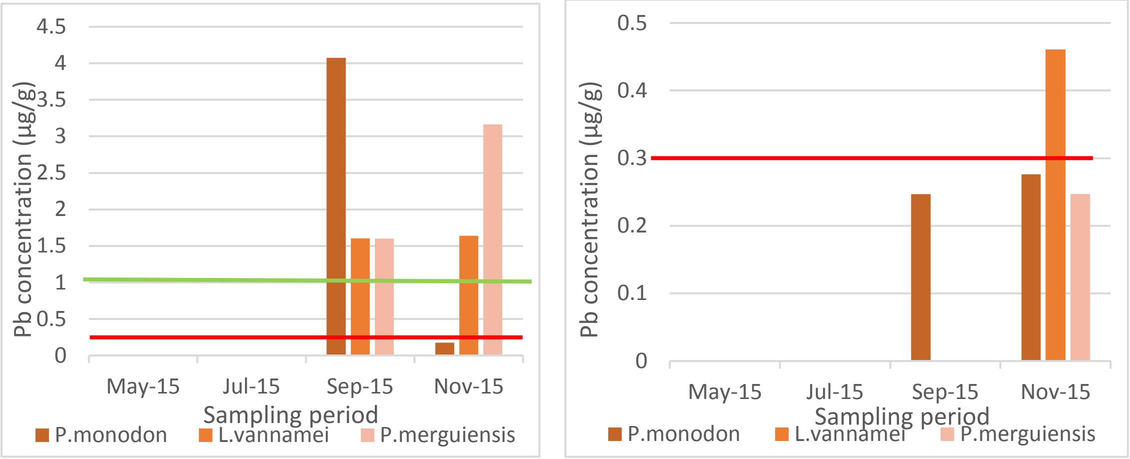
(C) Kelantan

Figure 4.4: Concentration of Cd in shrimp samples collected from selected sampling locations

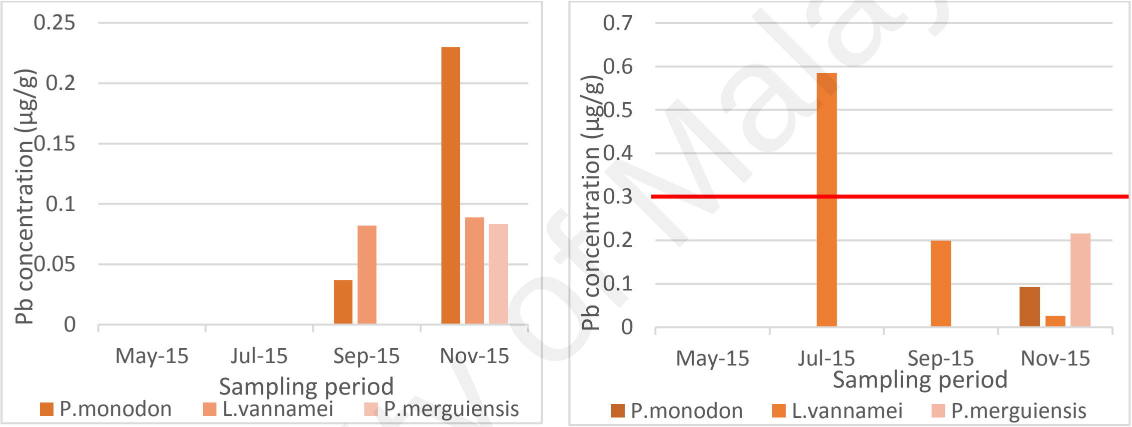
As seen in Figure 4.4, a few shrimp species collected from Perak, Johor and Kelantan indicates below detection of Cd concentration. This could be due to shrimp samples have high tolerance toward Cd. Apart from that, all samples collected from Malacca and Pahang have Cd concentration below the instrument detection limit. This might probably due to the absence of Cd source within this area. From Figure 4.4, the highest Cd concentration were detected in July 2015, which is before monsoon, while the second and third highest Cd concentration were detected in September and November 2015, which is during monsoon. This could be due to during monsoon there is an increase in river runoff that originate from agricultural land, wastewater discharged from domestic and industries like electroplating and paper pulp that finally flow to the ocean, thus reflected in high concentration of Cd in shrimp samples. Research done by Rahouma *et al.* (2013) indicates higher concentration of Cd (0.76 µg/g) in *Acetes intermedius* collected from Terengganu, compared to this study. However, all Cd concentration detected in shrimp samples were below the acceptable limits set by FAO/WHO (Codex Alimentarius Commision, 2010) (2 µg/g) and Malaysian Food Act 1983 (1 µg/g).

4.1.5 Concentration of Pb in shrimp species between selected sampling locations

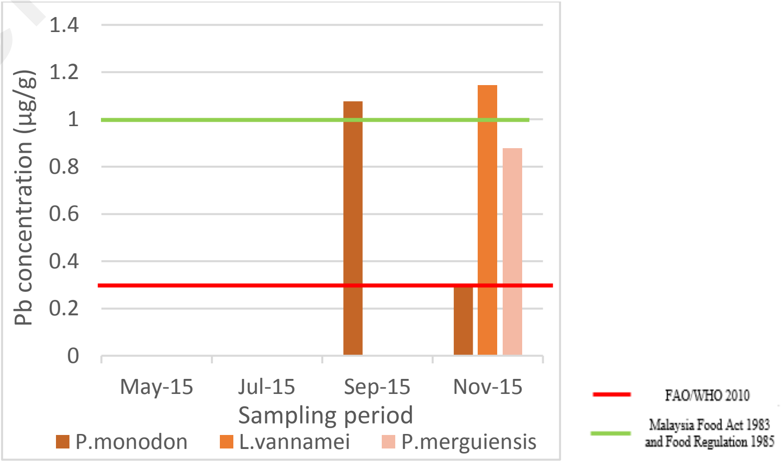
The concentration of Pb in muscle of shrimp species collected from Perak, Malacca, Johor, Pahang and Kelantan were shows in Figure 4.5. The highest Pb concentration were detected in shrimp samples collected from Perak, which is *P. monodon* (4.07 ± 1.31 µg/g), followed by *P. merguiensis* (3.16 ± 0.26 µg/g) and *L. vannamei* (1.64 ± 0.11 µg/g). It can be seen that the concentration of Pb were different among the shrimp species. This probably because of variation in their ability to bioconcentrate Pb in their muscles. Pb concentration detected in all shrimp species might originate from paint, emission from industries and vehicles from nearby areas. Besides that, all the highest Pb concentration in shrimp samples were detected from Perak. This perhaps due to fishing village and agriculture activity located near to sampling location that release wastewater contain Pb into marine environment. Pb could be deposited on sediment and being digest by marine organisms, thus affecting shrimp samples.



(A) Perak (B) Malacca



(C) Johor (D) Pahang



(E) Kelantan

Figure 4.5: Concentration of Pb in shrimp samples collected from selected sampling locations

Apart from that, there is a lack of solid waste collection system at sampling location where people who live near to river or fishing village tend to dump their waste into the river and ocean, thus deposited in sediments and affecting aquatic organisms like shrimps. However, the concentration of Pb in a few shrimp samples were below the detection of ICP-MS. This could be due to shrimp samples have high tolerance toward Pb.

From Figure 4.5, the three highest Pb concentration were detected in September and November 2015, which is during monsoon. The concentration of Pb in shrimp samples collected during monsoon was high probably due to heavy rainwater that caused runoff of Pb from building applied with old paint, batteries, oil spill, mining and smelting sites.

All the highest Pb concentration in this study are contradicted with study done by Rahouma *et al*. (2012) that found lower concentration of Pb (1.29 ± 0.85 µg/g) in *Acetes indicus* collected from West Coast of Peninsular Malaysia. This might be due to lack of anthropogenic sources of pollution exist along the West Coast of Peninsular Malaysia during previous study compared to this study. Some shrimp samples have exceed permissible limits set by FAO/WHO (Codex Alimentarius Commision, 2010) (0.3 µg/g) and Malaysian Food Act 1983 (1 µg/g).

In general, the highest concentration of As, Hg and Pb were detected in shrimp samples collected from Perak, which are *P. merguiensis* (328.93 ± 0.78 µg/g), *P. merguiensis* (1012.83 ± 0.78 µg/g) and *P. monodon* (4.07 ± 1.31 µg/g), respectively. The highest concentration of Cu was found in *L. vannamei* (7.56 ± 0.51 µg/g) collected from Malacca, whereas for Cd was found higher in *L. vannamei* (0.66 ± 0.38 µg/g) collected from Johor. Besides that, the lowest concentration for As, Cd and Cu were detected in shrimp samples collected from Kelantan, which are *P. merguiensis* (0.03 ± 0.01 µg/g), *L. vannamei* (0.22 ± 0.10 µg/g) and *P. monodon* (0.34 ± 0.09 µg/g), respectively. The lowest concentration for Hg was found in *L. vannamei* (0.11 ± 0.06 µg/g) collected from Malacca, whereas Pb was found lowest in *L. vannamei* (0.03 ± 0.02 µg/g) collected from Pahang.

4.1.6 Comparison of metals accumulation in shrimp species

Table 4.1 shows several studies on the patterns of trace metals accumulation in muscles of shrimp species. In this study, the distribution of metals in muscles of all selected shrimp species collected from Perak and Kelantan followed the order of Hg>As>Cu>Pb>Cd, while Malacca, Johor and Pahang followed the order of Hg>Cu>As>Pb>Cd. The high accumulation of Hg and As in all selected shrimp species could be related to high level of pollution in their habitat from various industries, thus biomagnified in their tissues.

From Table 4.1, it can be seen the order of Cu>Pb>Cd in shrimp samples from this study were almost same with other study. The presence of relatively high concentration of Cu in all shrimp species in this study and other researches probably due to Cu essentiality for biological functions. It is agreed by Mitra *et al.* (2012) that Cu are needed for growth and cell metabolism of crustaceans. Gokoglu *et al.* (2008) have reported the same finding for shrimp species (*Penaeus semisulcatus, Parapenaeus longirostris* and *Paleomon serratus*) collected from Mediterranean, Gulf of Antalya, Turkey.

The presence of relatively low level of Pb and Cd in shrimp species in this study and other researches might be due to the fact that these elements is needed in trace amounts in living tissues. Pb and Cd are non-crucial elements for living organisms (Mitra *et al*., 2012). Apart from that, low accumulation of Pb and Cd in shrimp species are due to presence of developed systems to excrete toxic metals in their body (Pourang *et al.*, 2004). This is in agreement with research done by Mine (2015), that Pb and Cd are not detected in tissue of shrimp species.

Table 4.3: Patterns of metals in muscle of shrimp species

Species	Tissue	Order	Reference	Location
P. monodon	Muscle	Hg>As>Cu>Pb>Cd	This study	Malaysia
P. merguiensis	Muscle	Hg>As>Cu>Pb>Cd	This study	Malaysia
L. vannamei	Muscle	Hg>As>Cu>Pb>Cd	This study	Malaysia
Penaeus californiensis	Muscle	Zn>Fe>Cu>Mn	Paez-Osuna and Tron-Mayen (1995)	Mexico
P. monodon	Muscle	Zn>Fe>Pb>Cd	Guhathakurta and Kaviraj (2000)	India
Penaeus semisulcatus	Muscle	As>Pb>Hg>Cd	Madany *et al.* (1996)	Bahrain, Arabian Gulf
P. merguiensis	Muscle	Zn>Cu>Fe>Cr>Ni>Mn>Cd	Pourang and Amini (2001)	Persian Gulf
Penaeus Semisulcatus	Muscle	Zn > Cu> Cd	Pourang *et al.* (2005)	Persian Gulf
Litopenaeus stylirostris	Muscle	Zn > Cu> Pb> Cd	Frias-Espericueta *et al.* (2007)	Gulf of California
P. monodon	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2011)	River Ganga
P. monodon	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2012)	India Sundarbans
Penaeus indicus	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2012)	India Sundarbans
Penaeus semisulcatus	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2012)	India Sundarbans
P.merguensis	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2012)	India Sundarbans
Metapenaeus brevicornis	Muscle	Zn > Cu> Pb> Cd	Mitra *et al.* (2012)	India Sundarbans

4.1.7 Correlation between concentrations of metals

The correlation coefficient between metals concentration were analysed and shown in Figure 4.6 and 4.7. In this study, positive correlation are observed for Hg and As (r^2 = 0.8209) and Pb and As (r^2 = 0.747) for all the shrimp samples collected from the five fish landing sites. This results show that metal pairs of Hg and As, and Pb and As probably were discharged from the same sources and location for example industrial wastewater, mining, electroplating, extreme boat and shipping, hence accumulate in the shrimp samples. Poor correlations between other metal pairs in shrimp samples might be due to complexity in the accumulation process. This is comparable with study done by Silva *et al.* (2016) that found positive correlation in muscles of *L. vannamei* from Bahia, Brazil between pairs of Fe-Zn, Mn-Cu, Se-Cu and Se-Mn.

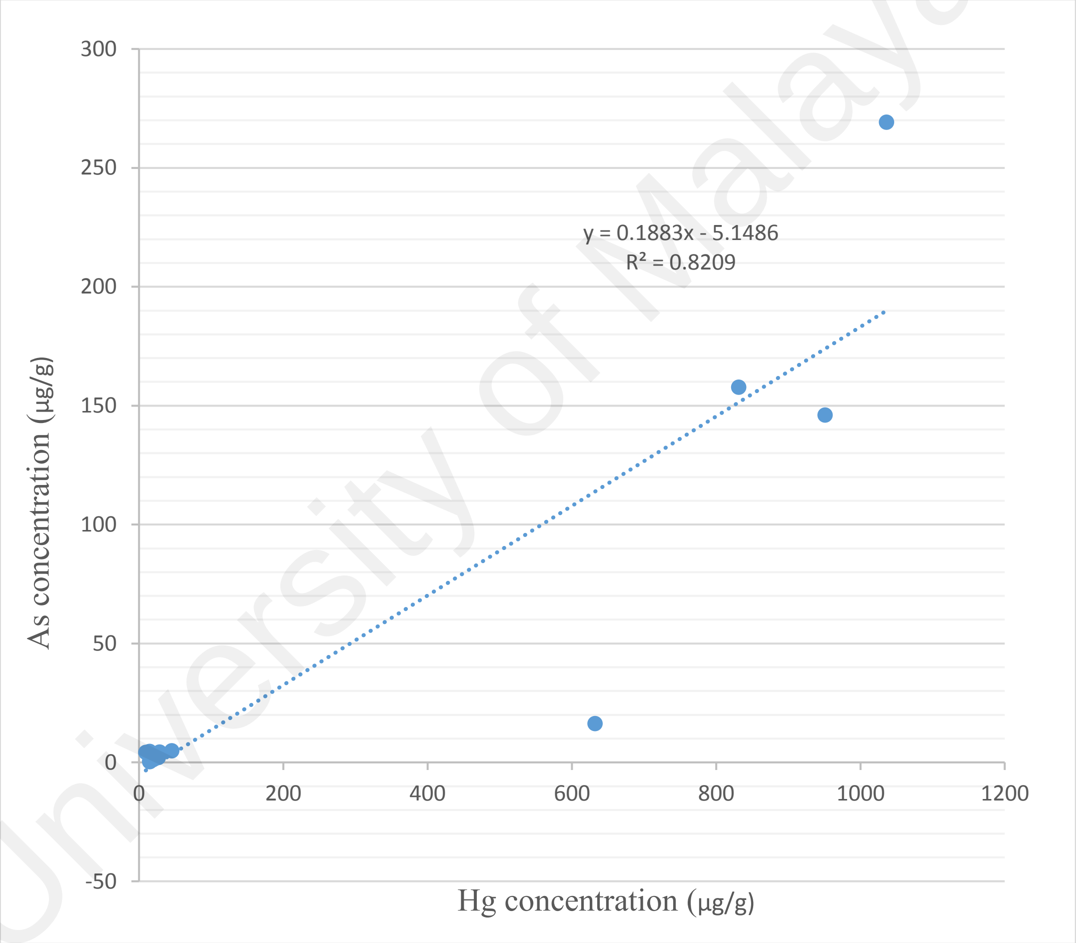


Figure 4.6: Correlation of Hg and As concentration in shrimp samples

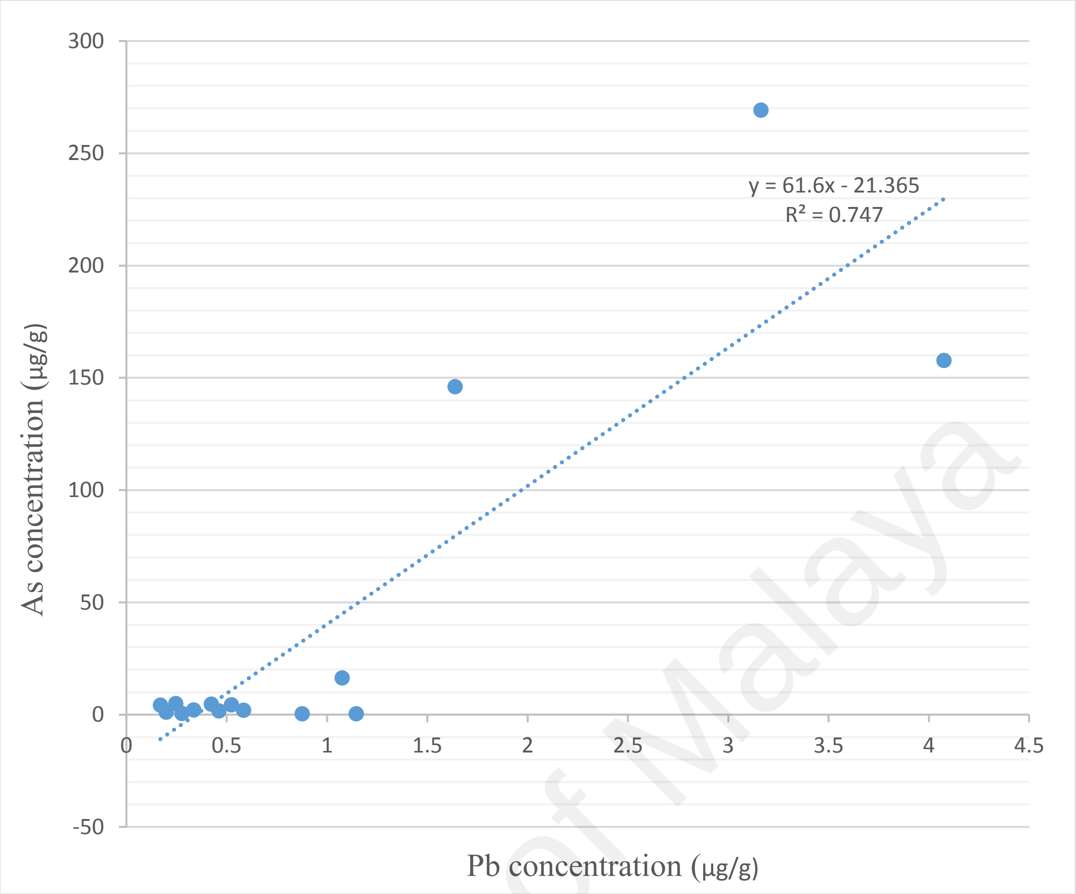


Figure 4.7: Correlation of Pb and As concentration in shrimp samples

4.1.8 Correlation of metals accumulation between shrimp species

The correlation coefficient of metals accumulation between shrimp species were analysed. Positive correlations were only obtained for the concentration of As and Hg, in *P. merguiensis* and *L. vannamei* as shown in Figure 4.8 and 4.9. These positive correlations are possibly because of similarity in dietary uptake, metabolic rates of shrimp species, habitat and other environmental factors that influence the same bioavailability of toxic metals in their muscles tissue (Silva *et al*., 2016). This finding is agreeable to the research by Fazel *et al.* (2015) who found positive correlation of some metals in *J. belangerii* and *M. affinis* collected from northwest Persian Gulf.

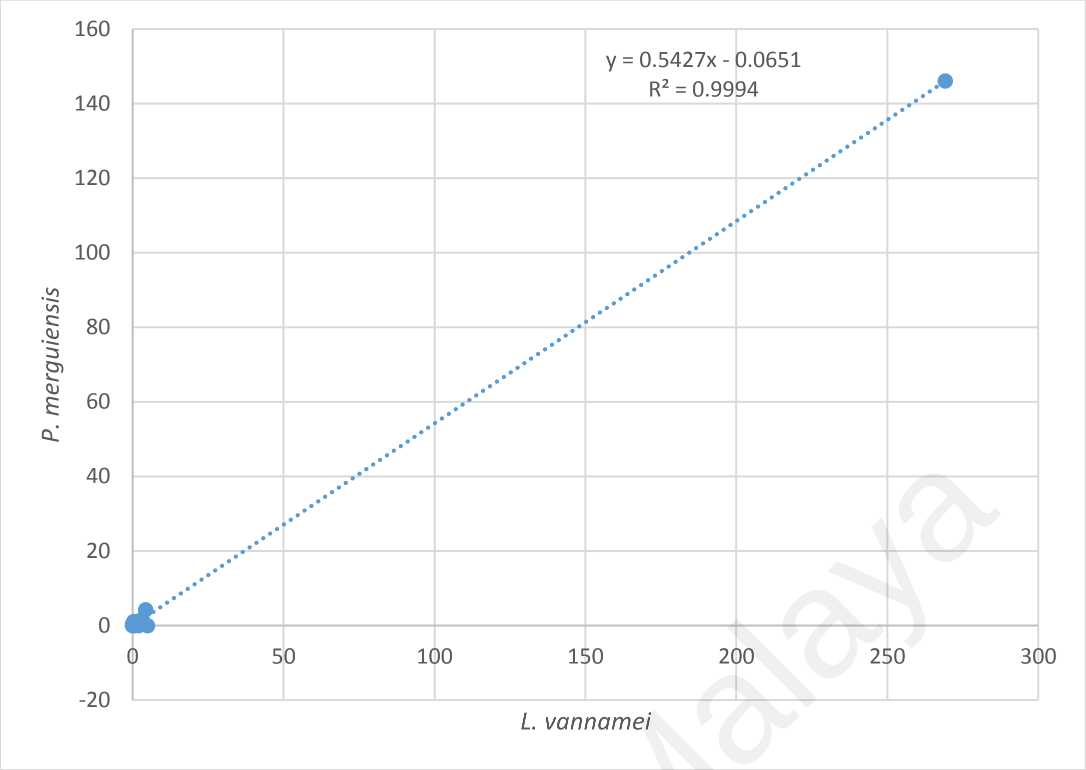


Figure 4.8: Correlation of As concentration between *P. merguiensis* and *L. vannamei*

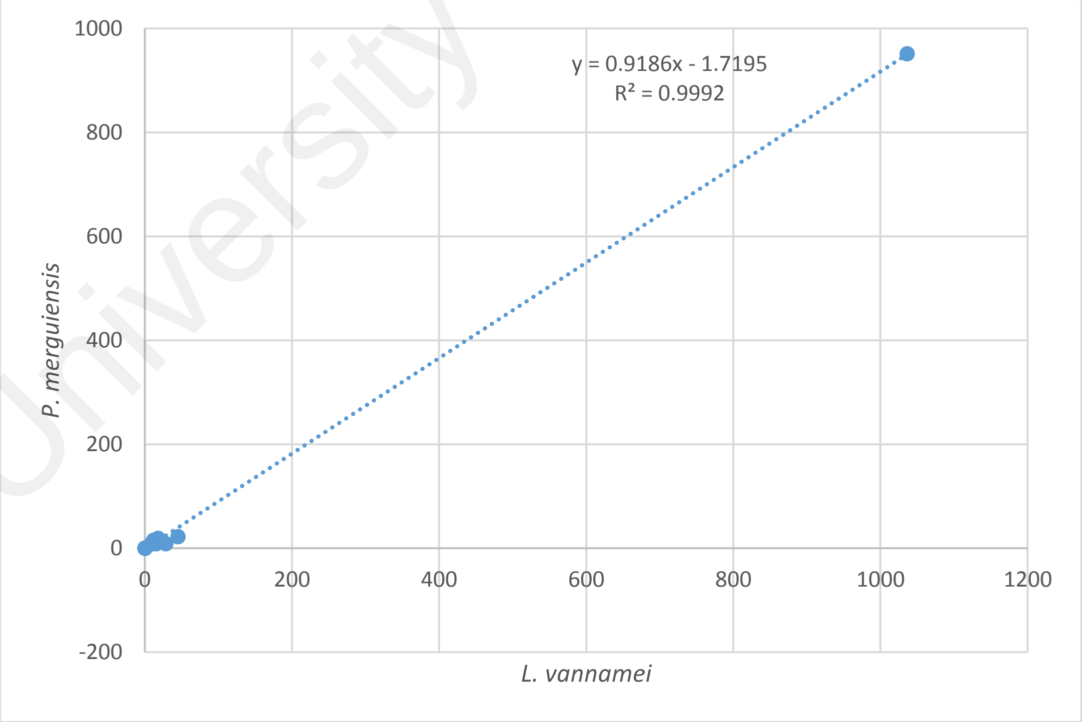


Figure 4.9: Correlation of Hg concentration between *P. merguiensis* and *L. vannamei*

4.1.9 Health Risk Assessment

4.1.9.1 Estimated Daily Intake (EDI)

The EDI (mg/kg body-weight/day) of Hg, As, Pb, Cd and Cu from selected shrimp species consumption by the average adults of Malaysian is presented in Table 4.2, whereas Table 4.3 shows the HR values. EDI values of Hg in selected shrimp species ranged from 3 x 10^{-4} to 0.08; 3.6 x 10^{-5} to 0.03 in As; 9.6 x 10^{-5} to 6.3 x 10^{-3} in Pb; 1.7 x 10^{-5} to 8.5 x 10^{-5} in Cd and 3.6 x 10^{-4} to 1.1 x 10^{-3} in Cu.

HR values in case of Cd and Cu did not exceed one for all shrimp species collected from all sampling locations. However, in case of Hg, HR values in all shrimp samples from five sampling locations exceeded one that ranged between 3 to 800. For As, all shrimp samples shows HR values more than one, except for *P. monodon* (0.73) from Malacca, *P. merguiensis* (0.76) from Pahang and *L. vannamei* (0.12) from Kelantan.

In case of Pb, all samples shows HR values more than one, except for *P. monodon* (6.57 x 10^{-3}) from Kelantan and *L. vannamei* collected from Perak, Johor and Kelantan, with HR values 0.03, 0.02 and 4.9 x 10^{-3}, respectively. HR values that not exceed one in the samples indicates that the average consumption of the shrimp species from the sampling area would not cause health risk, whereas a values more than one indicates there is a potential risk according to New York State, Department of Health (2007).

EDI and HR revealed higher consumption of some toxic metals like Hg, As and Pb through shrimp samples could give potential hazard with the greater risks for its end consumers. Results from this study were in comparable with research done by Jiang *et al.* (2016) and Raknuzzaman *et al*. (2016) in China and Bangladesh, respectively.

Table 4.4: Estimated Daily Intake (EDI) (mg/kg body-weight/day) from selected shrimp species; (BDL = Below detection limit)

Sampling area	Shrimp species	Metals				
		Hg	**As**	**Pb**	**Cd**	**Cu**
Perak	*P. monodon*	0.08	3.1×10^{-3}	6.3×10^{-3}	3.5×10^{-5}	7.9×10^{-4}
	P. merguiensis	0.08	0.03	9.1×10^{-3}	2.6×10^{-5}	7.7×10^{-4}
	L. vannamei	0.06	0.01	9.6×10^{-5}	5.5×10^{-5}	5.7×10^{-4}
Malacca	*P. monodon*	3×10^{-4}	2.2×10^{-4}	BDL	BDL	5.9×10^{-4}
	P. merguiensis	3.2×10^{-4}	4.7×10^{-4}	BDL	BDL	8.9×10^{-4}
	L. vannamei	4.9×10^{-4}	7.1×10^{-4}	BDL	BDL	1.1×10^{-3}
Johor	*P. monodon*	1.4×10^{-3}	7.1×10^{-4}	BDL	BDL	1.1×10^{-3}
	P. merguiensis	8.2×10^{-4}	4.3×10^{-4}	BDL	BDL	6.8×10^{-4}
	L. vannamei	2.3×10^{-3}	6.2×10^{-4}	8.5×10^{-5}	8.5×10^{-5}	5.5×10^{-4}
Pahang	*P. monodon*	2.8×10^{-2}	5.6×10^{-4}	BDL	BDL	8.3×10^{-4}
	P. merguiensis	2.3×10^{-3}	2.3×10^{-4}	BDL	BDL	6.6×10^{-4}
	L. vannamei	2.2×10^{-3}	3.1×10^{-4}	BDL	BDL	7.4×10^{-4}
Kelantan	*P. monodon*	0.04	0.01	2.3×10^{-5}	2.3×10^{-5}	6.3×10^{-4}
	P. merguiensis	1.8×10^{-3}	3.2×10^{-4}	BDL	BDL	3.6×10^{-4}
	L. vannamei	1.8×10^{-3}	3.6×10^{-5}	1.7×10^{-5}	1.7×10^{-5}	5.2×10^{-4}

Table 4.5: Hazard ratio (HR) from selected shrimp species; (BDL = Below detection limit)

Sampling area	Shrimp species	Metals				
		Hg	As	Pb	Cd	Cu
Perak	*P. monodon*	800	10.3	1.8	0.035	0.02
	P. merguiensis	800	100	2.6	0.026	0.02
	L. vannamei	600	33	0.03	0.055	0.01
Malacca	*P. monodon*	3	0.73	BDL	BDL	0.01
	P. merguiensis	3.2	1.56	BDL	BDL	0.02
	L. vannamei	4.9	2.36	BDL	BDL	0.03
Johor	*P. monodon*	14	2.36	BDL	BDL	0.03
	P. merguiensis	8.2	1.43	BDL	BDL	0.02
	L. vannamei	23	2.06	0.02	0.085	0.01
Pahang	*P. monodon*	280	1.86	BDL	BDL	0.02
	P. merguiensis	23	0.76	BDL	BDL	0.02
	L. vannamei	22	1.03	BDL	BDL	0.02
Kelantan	*P. monodon*	400	33	6.57×10^{-3}	0.023	0.02
	P. merguiensis	18	1.06	BDL	BDL	9×10^{-3}
	L. vannamei	18	0.12	4.9×10^{-3}	0.017	0.013

4.1.9.2 Target Hazard Quotient (THQ)

THQ for metals through consumption of shrimp species are presented in Table 4.4. The result shows that, THQ values of Pb, Cd and Cu in all species collected from the five states were all below one. However, THQ values of Hg in all species collected from five sampling locations were all higher than one. In case of As, the THQ values in all samples were all below one, except for all shrimp samples collected from Perak ; *P. monodon* (21.77), *P. merguiensis* (45.64) and *L. vannamei* (19.28), Malacca; *L. vannamei* (1.25), Johor; *P. monodon* (1.25) and *L. vannamei* (1.1) and Kelantan *P. monodon* (17.84). THQ values of metals in samples that below one indicate no potential hazard and health risk from the consumption of a single metal through the ingestion of these shrimp species (Mohammad *et al.*, 2018). Besides that, THQ values above one shows that the exposed population through ingestion of contaminated foods have deleterious effects (Mohammad *et al.*, 2018).

Table 4.6: Target Hazard Quotient (THQ) of selected shrimp species; (BDL = Below detection limit)

Sampling area	**Shrimp species**	**Metals**				
		Hg	**As**	**Pb**	**Cd**	**Cu**
Perak	*P. monodon*	421.9	21.77	0.05	0.02	0.01
	P. merguiensis	426.5	45.64	0.06	0.03	6.9 x 10^{-3}
	L. vannamei	335.9	19.28	0.04	0.02	7.6 x 10^{-3}
Malacca	*P. monodon*	1.6	0.394	6.1 x 10^{-3}	BDL	7.7 x 10^{-3}
	P. merguiensis	1.7	0.83	2.9 x 10^{-3}	BDL	0.01
	L. vannamei	2.6	1.25	5.4 x 10^{-3}	BDL	0.01
Johor	*P. monodon*	7.6	1.25	3.1 x 10^{-3}	BDL	0.01
	P. merguiensis	4.3	0.76	9.7 x 10^{-4}	BDL	9.4 x 10^{-3}
	L. vannamei	12.1	1.1	2 x 10^{-3}	0.04	7.3 x 10^{-3}
Pahang	*P. monodon*	15.2	0.99	1.1 x 10^{-3}	BDL	0.01
	P. merguiensis	12	0.4	2.5 x 10^{-3}	BDL	8.7 x 10^{-3}
	L. vannamei	11.74	0.55	9.5 x 10^{-3}	BDL	9.7 x 10^{-3}
Kelantan	*P. monodon*	198.14	17.84	0.02	0.01	8.3 x 10^{-3}
	P. merguiensis	9.6	0.56	0.01	BDL	4.7 x 10^{-3}
	L. vannamei	9.7	0.06	0.01	9.1 x 10^{-3}	6.9 x 10^{-3}

4.1.9.3 Target Cancer Risk (TR)

The carcinogenic potency slope factor only available for As, Pb and Cd. Therefore, the TR values for As, Pb and Cd due to exposure from shrimp samples were listed in Table 4.5. TR values for As, Pb and Cd ranged from 7.8 x 10^{-5} to 3.4 x 10^{-3}, 7.8 x 10^{-5} to 1.2 x 10^{-4} and 7.3 x 10^{-5} to 1.5 x 10^{-5}. The excess cancer risk lower than 10^{-6} is considered to be negligible, the cancer risk above 10^{-4} is considered unacceptable, and risks that lie between 10^{-6} and 10^{-4} are considered acceptable (USEPA, 2010).

From this study, the carcinogenic risk for As was close to the acceptable range in all shrimp species collected from Malacca, Johor, Kelantan and Pahang, except for all shrimp species collected from Perak that indicated unacceptable range. In the case of Pb, TR values for all shrimp species from all sampling locations lies between 10^{-6} and 10^{-4} that shows an acceptable range.

For Cd, a few shrimp species have below detection limit, except for all species collected from Perak, *L. vannamei* (7.3 x 10^{-5}) collected from Johor, *P. monodon* (1.9 x 10^{-5}) and *L. vannamei* (1.5 x 10^{-5}) collected from Kelantan, that lies within acceptable range. Results from this study are comparable with research done by Mohammad *et al.* (2018) in Bangladesh.

Table 4.7: Target Cancer Risk (TR) of shrimp samples. (BDL = Below detection limit)

Sampling area	Shrimp species	Metals		
		As	Pb	Cd
Perak	*P. monodon*	4.2×10^{-3}	6.4×10^{-4}	3×10^{-5}
	P. merguiensis	8.8×10^{-3}	7.1×10^{-4}	2.7×10^{-5}
	L. vannamei	3.7×10^{-3}	4.9×10^{-4}	4.8×10^{-5}
Malacca	*P. monodon*	7.6×10^{-5}	7.8×10^{-5}	BDL
	P. merguiensis	1.6×10^{-4}	3.7×10^{-5}	BDL
	L. vannamei	2.4×10^{-4}	6.9×10^{-5}	BDL
Johor	*P. monodon*	2.4×10^{-4}	4×10^{-5}	BDL
	P. merguiensis	1.5×10^{-4}	1.2×10^{-5}	BDL
	L. vannamei	2.1×10^{-4}	2.6×10^{-5}	7.3×10^{-5}
Pahang	*P. monodon*	1.9×10^{-4}	1.4×10^{-5}	BDL
	P. merguiensis	7.8×10^{-5}	3.2×10^{-5}	BDL
	L. vannamei	1.1×10^{-4}	1.2×10^{-4}	BDL
Kelantan	*P. monodon*	3.4×10^{-3}	2.1×10^{-4}	1.9×10^{-5}
	P. merguiensis	1.1×10^{-4}	1.3×10^{-4}	BDL
	L. vannamei	1.2×10^{-5}	1.7×10^{-4}	1.5×10^{-5}

4.2 Qualitative Analysis of Organochlorine Pesticides (OCPs)

The distribution of DDT and HCH were investigated in selected shrimp species collected from Perak, Pahang, Johor, Malacca and Kelantan.

4.2.1 Distribution of OCPs in shrimp samples between sampling locations

Table 4.8 shows the distribution of DDT and HCH in shrimp samples collected from Perak, Malacca, Johor, Kelantan and Pahang.

Table 4.8: Distribution of DDT and HCH in shrimp samples

Sampling locations	**Shrimp species**	**Sampling period**							
		May 2015		**July 2015**		**September 2015**		**November 2015**	
		DDT	**HCH**	**DDT**	**HCH**	**DDT**	**HCH**	**DDT**	**HCH**
Perak	*P. monodon*	+	-	+	-	+	+	+	-
	P. merguiensis	+	+	+	-	-	-	+	-
	L. vannamei	+	-	+	-	+	-	+	-
Malacca	*P. monodon*	+	-	-	+	+	-	+	-
	P. merguiensis	+	-	+	-	+	-	+	-
	L. vannamei	+	-	+	-	+	+	+	-
Johor	*P. monodon*	+	-	+	+	+	-	+	-
	P. merguiensis	-	+	-	+	+	-	-	+
	L. vannamei	+	-	+	-	+	-	+	-
Pahang	*P. monodon*	+	-	+	-	-	-	+	+
	P. merguiensis	+	-	+	-	+	-	+	-
	L. vannamei	+	-	+	-	+	-	+	-
Kelantan	*P. monodon*	+	+	+	-	+	-	+	-
	P. merguiensis	+	-	+	-	+	-	+	-
	L. vannamei	+	-	+	-	+	-	+	+

+ Presence of DDT or HCH, - Absence of DDT or HCH

Besides that, the usage of agrochemicals like pesticides and fungicides on vegetable, fruit and tea faming, especially in Cameron Highland, Pahang are the common practice to control pests and disease (Mispan *et al.*, 2015). The runoff from these farm that contain DDT and HCH might flow into the river and ocean, thus pose threat to aquatic environment like shrimps.

These pollutants are commonly found in chemicals to control pests in palm oil plantations too. Although these POPs are banned, the residues of the contaminants can still be found in the environment. Residues from extreme usage of DDT and HCH in runoff that flow into the river and marine environment might cause contamination to sediments, thus accumulate in shrimp muscles through ingestion. This is because sediments represent an important potential exposure pathway for POPs to aquatic organisms (Van Ael *et al.*, 2012).

The results from this study are agreeable to the findings by Lee *et al.* (2003), where the residue level of organochlorine insecticides were higher in river or marine water near to agricultural areas as compared to areas that are not involved in paddy or vegetable farming (Lee *et al.,* 2003). Besides that, distribution of POPs in this study are comparable with research done by Tan (2001) that found the presence of DDT and absence of HCH at nine stations along Perak River.

4.2.2 Distribution of OCPs between shrimp species.

Several studies had been reported on the distribution of POPs in seawater and marine organisms in Malaysia environments. However, the study about distribution of POPs in shrimp species collected from Malaysian seawater is very limited. Thus, this study could be used as a reference research related to the distribution of POPs in *L. vannamei*, *P. monodon* and *P. merguiensis.*

In this study, DDT were highly distributed in *L. vannamei* followed by *P. monodon* and *P. merguiensis*. HCH were found to highly distribute in *P. merguiensis*, followed by *L. vannamei* and *P. monodon.* This is because every species of marine organisms have different habitat and feeding habit that influences the transport and accumulation of POPs in their tissue muscles. This is agreeable to the research done by Sani (2007), that found HCH would accumulate better in bottom feeder species like *Perna viridis*, *Metapenaeus monoceros* and the blood cockles.

4.2.3 Distribution of OCPs in shrimp samples before and during monsoon.

From this study, DDT was found to dominate almost all shrimp samples collected before (May and July 2015) and during monsoon (September and November 2015), as compared to HCH. It might be due to the climate change that give effect on the distribution of DDT in shrimp samples. The increase in temperature during sunny days at sampling locations before monsoon, probably influences volatility of DDT, hence reflected in its distribution in shrimp samples during this period. Previous study done by Lamon *et al* (2009), found the same results where the increase in atmospheric temperature from 10^0C to 15^0C resulted in doubles of vapour pressure of PCB-153.

In addition, high distribution of DDT during monsoon could be due to higher wind speeds that caused faster atmospheric transport of DDT into water and sediments of marine environment, thus accumulate in shrimp species. Apart from that, heavy rainfall during monsoon might allow DDT that bound on soil particles leach into rivers and seawater, hence reach the marine organisms.

This is agreeable to the study done by Wilken *et al.* (1994) and Hilscherova *et al.* (2007) that found flooding events resulted in redistribution of POPs which were previously stored in sediment and soils. The result in this study are comparable to the research by Abdullah *et al.* (2015) that detect the highest distribution of HCH and DDT during rainy season.

Table 4.9 shows the comparison between POPs distribution in shrimp species from this study and other studies. It can be clearly seen that the distribution of POPs varied among species of marine organisms.

Table 4.9: Presence of POPs in several marine life species

Species	**Type of POPs present**	**References**	**Location**
P. monodon	DDT, HCH	This study	Malaysia
P. merguiensis	DDT, HCH	This study	Malaysia
L. vannamei	DDT, HCH	This study	Malaysia
Anadara granosa	Aldrin, DDT, Dieldrin, Endosulfans, Endrins, HCH	Hossain, 2001	Bay of Bengal
Arius sp.	Aldrin, DDT, Dieldrin, Endosulfans, Endrins, HCH	Hossain, 2001	Bay of Bengal
Perna viridis	Aldrin, DDT, Dieldrin, Endosulfans, Endrins, HCH	Hossain, 2001	Bay of Bengal

CHAPTER 5: CONCLUSION

The accumulation of metals in shrimp sample was found to vary with different species. Metal contamination by Pb, As and Hg was found to be highest in shrimp samples collected from Perak, while Cu and Cd accumulation was the highest in shrimp collected from Malacca and Johor, respectively. In this study, the levels of Hg, Pb and As in shrimp samples were more than the acceptable level prescribed by Food and Agriculture Organization/WHO (2010) and Malaysian Food Act.

Between the geographical regions, shrimp samples collected from the West Coast are more polluted than those of the East Coast. In this study, the distribution of metals in muscles of all selected shrimp species collected from Perak and Kelantan followed the order of Hg>As>Cu>Pb>Cd, while Malacca, Johor and Pahang followed the order of Hg>Cu>As>Pb>Cd. This findings also showed positive correlation between metal pairs Hg-As and Pb-As. Besides that, there is positive correlation between the concentration of As and Hg in *P. merguiensis* and *L. vannamei.*

EDI and HR revealed higher consumption of Hg, As and Pb through shrimp samples could give potential hazard. THQ values of Pb, Cd and Cu in all species collected from the five states were all below one. However, THQ values of Hg in all species collected from five sampling locations were all higher than one. In the case of As, the THQ values in all samples were all below one, except for all shrimp samples collected from Perak ; *P. monodon* (21.77), *P. merguiensis* (45.64) and *L. vannamei* (19.28), Malacca; *L. vannamei* (1.25), Johor; *P. monodon* (1.25) and *L. vannamei* (1.1) and Kelantan *P. monodon* (17.84). TR values for As was near to the acceptable range in all shrimp species collected from Malacca, Johor, Kelantan and Pahang, except for all shrimp species collected from Perak that indicated an unacceptable range. In the case of Pb, TR values for all shrimp species from all sampling locations was between 10^{-6} and 10^{-4} that shows an acceptable range.

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