EVALUATION OF HABITAT QUALITY AND ALLERGENIC CHARACTERIZATION OF MUD CRAB (*Scylla olivacea*) FROM SUNGAI MERBOK, KEDAH

HAIDR MSAHIR ATESHAN

UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

This study aims to evaluate the habitat quality and allergenic characterization of mud crab, *Scylla olivacea* from Merbok River, Kedah. The habitat quality variables, biological contaminants and levels of heavy metals in both water and *S. olivacea* were analyzed using Ion chromatography, Coupled Plasma Mass Spectrometry (ICP-MS) and biological standard tests. Meanwhile, the allergenic characteristics of this crab were identified by proteomics methods including SDS-PAGE, immunoblotting, 2-DE and mass spectrometry analysis. The correlation of habitat quality and the crab allergenicity was determined by bivariate correlation. Most of the habitat quality parameters, biological contaminants and level of heavy metal present in concentrations exceeded the reference standard limits in both river water and *S. olivacea*, indicating pollution of the river water. The raw mix crab extracts have 26 to 33 protein bands, and 21 of them were allergenic. Four major allergens of *S. olivacea* were identified. The allergens of 20, 36, 43 and 75 kDa were identified as actin, tropomyosin, arginine kinase and hemocyanin, respectively. A majority of the allergenic proteins were detected in all crab parts but at various frequencies. Some of these major and minor allergens were stable to boiling and acid treatments. Strong positive correlations between Cr-Zn, Cr-As, Cr-Pb, Mn-As, Mn-Pb, Fe-Ni, Zn-As and Zn-Pb. It should be noted that Cr, As, Pb and Fe are strongly negatively correlated with total protein concentration and number of protein bands in raw mix parts of *Scylla olivacea*, suggesting that a relationship exists between habitat quality and the protein profiles of *S. olivacea*. This study could facilitate the improvement of crab allergic patients' management as high contamination from the surrounding environment of *S. olivacea* might result in health risks to consumers including allergic patients.

PENILAIAN KUALITI HABITAT DAN PENCIRIAN ALERGEN KETAM LUMPUR (*Scylla olivacea*) DARI SUNGAI MERBOK, KEDAH

ABSTRAK

Kajian ini bertujuan untuk menilai kualiti habitat dan ciri alergen ketam lumpur, *Scylla olivacea* dari Sungai Merbok, Kedah. Pembolehubah kualiti habitat, bahan cemar biologi dan tahap logam berat di dalam air dan *S. olivacea* dianalisis menggunakan kromatografi Ion, Spektrometri Massa Plasma Berpasangan (ICP-MS) dan ujian biologi piawai. Sementara itu, ciri alergen pada ketam ini dikenal pasti dengan kaedah proteomik termasuk SDS-PAGE, imunoblotting, 2-DE dan analisis spektrometri jisim. Korelasi kualiti habitat dan alergenisiti ketam ditentukan oleh korelasi bivariat. Sebilangan besar parameter kualiti habitat, bahan cemar biologi dan tahap logam berat terdapat dalam kepekatan melebihi had piawai rujukan di kedua-dua air sungai dan *S. olivacea*, yang menunjukkan pencemaran air sungai. Ekstrak ketam campuran mentah mempunyai 26 hingga 33 jalur protein, dan 21 daripadanya adalah alergenik. Empat alergen major *S. olivacea* dikenal pasti. Alergen 20, 36, 43 and 75 kDa dikenali sebagai aktin, tropomiosin, arginin kinase dan hemocianin, masing-masing. Majoriti protein alergenik dikesan di semua bahagian ketam namun pada pelbagai kekerapan. Sebahagian daripada alergen major dan minor ini stabil untuk mendidih dan rawatan asid. Hubungan positif yang kuat antara Cr-Zn, Cr-As, Cr-Pb, Mn-As, Mn-Pb, Fe-Ni, Zn-As dan Zn-Pb. Harus diingatkan bahawa Cr, As, Pb dan Fe sangat berkorelasi secara negatif dengan kepekatan protein total dan bilangan jalur protein pada campuran bahagian badan *Scylla olivacea* mentah, menunjukkan bahawa terdapat hubungan antara kualiti habitat dan profil protein *S. olivacea*. Kajian ini dapat membantu kepada peningkatan pengurusan pesakit alergi ketam kerana pencemaran yang tinggi daripada persekitaran *S. olivacea* boleh mengakibatkan risiko kesihatan kepada pengguna termasuk kepada pesakit alergi.

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LIST OF ABBREVIATIONS

AIRC	Allergy and Immunology Research Centre
AK	Arginine kinase
As	Arsenic
BOD	Biochemical Oxygen Demand
Br	Bromide
BSA	Bovine Serum Albumin
Ca	Calcium
Cd	Cadmium
Cl	Chloride
COD	Chemical Oxygen Demand
Cr	Chromium
CR	Cross reactivity
CW	Carapace Width
DBPCFC	Double-blind placebo-controlled Oral Food Challenge
DO	Dissolved Oxygen
DOE	Department of Environment
F	Fluoride
FAO	Food and Agriculture Organization of United Nations
Fe	Iron
HDM	House Dust Mites
HKL	Hospital Kuala Lumpur
IC	Ion-Chromatography
ICMSF	International Commission on Microbiological Specifications for Foods
IgE	Immunoglobulin E
INWQS	Interim National Water Quality Standard

K	Potassium
kDa	KiloDalton
Li	Lithium
MALDI-TOF	Matrix-Assisted Laser Desorption-ionization Time of Flight
Mg	Magnesium
MLC	Myosin light chain
Mn	Manganese
MPN	Most Probable Number
MREC	Medical Research and Ethics Committee
MS	Mass Spectrometry
MW	Molecular weight
Na	Sodium
NH_4	Ammonium
Ni	Nickel
NIAID	National Institute of Allergy and Infectious Diseases
NO_2	Nitrite
NO_3	Nitrate
Pb	Lead
PBS	Phosphate buffered saline
PCA	Principal Component Analysis
PO_4	Phosphate
SCP	Sarcoplasmic calcium-binding protein
SDS	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SIgE	Specific IgE
SO_4	Sulphate
SPT	Skin Prick Test
TBS	Tris-buffered saline

TDS	Total Dissolved Solid
TM	Tropomyosin
TSS	Total Suspended Solids
TTBS	Tween 20-Tris-buffered saline
WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Malaysia is rich with aquatic systems that provide crucial supplies for the growth of the country and the community both socially and economically (Department of Environment Malaysia, 2015). These supplies consist of water, food, medicine, transport, energy, and commercial aquatic resources (Yusoff, Shariff & Gopinath, 2006). Industrialization and other human activities, which have resulted in the contamination of water bodies such as rivers and seas and subsequently reducing fishery commodities, lowering aesthetic appeal in water recreation zones, and decreasing biodiversity, all of which indicate that the aquatic environments are being disregarded (Wolfe, Vidal & Dove, 2018; Baliarsingh, Lotliker & Sudheesh, 2018; DOE, 2015).

Over the past two decades, numerous studies have been carried out to investigate the quality of water and the most important pollutants that enter the water sources and their impact on aquatic life (Cardinale, 2012; Al-Badaii, 2011; Chen & Zhang, 2007). These studies concluded that pollutants can be divided into two main categories according to origin or source natural and non-natural or human-induced activity substances. Floods, soil erosion, and other natural sources can lead to a significant impact on water pollution. Besides that, precipitation can also be a natural factor that might increase water pollution due to the solubility of rocks, salts, and many of the elements that could negatively affect the surface water bodies (Berger et al., 2017). On the other hand, anthropogenic as non-natural activities play a wide role in the increase of threats of pollutants in the rivers, ponds, and other water bodies (Al-Badaii, 2011). According to the Department of Environment, Malaysia, anthropogenic activities play a substantial role in contributing to the pollution of river water among other substances (DOE, 2014). The higher prevalence of anthropogenic activities have led to the buildup of harmful chemicals including heavy metals (Ahmad Ismail, 2006), and biological contaminants such as algae, bacteria, protozoan, or viruses in the river water (Islam & Tanaka, 2004).

Heavy metals have been identified as among the most harmful contaminants threatening marine life (Al-Yousuf et al., 2000). Aquatic organisms take up these metals via their bare tissues and during feeding (Fukunaga et al., 2011; Chapman et al., 1998). Evidence has shown that marine life accrues heavy metals from the water and their food intake along the food chain (Ndome et al., 2010). Such high levels of heavy metal present in seafood may harm human health when consumed (Gabr et al., 2008; Stanciu et al., 2005). Among others, arsenic (As) can lead to the development of skin cancer

(Järup, 2003), plumbum (Pb) may impair blood flow (Kamaruzzaman et al., 2011) whilst zinc (Zn) in excessive amounts can lead to electrolyte imbalance and fatigue (Chinnaraja et al., 2011).

Another major global concern is water pollution caused by pathogens and the resulting diseases that come with it (Pandey, Kass, Soupir, Biswas & Singh, 2014). Sewage effluent flowing into the water ecosystem may contain a variety of biological contaminants including viruses and bacterial pathogens (Islam & Tanaka, 2004). It has been reported that a lot of bacteria dwelling in the waters may carry the possibility of transferring the pathogens to the aquatic organisms which can be transferred to the human consumer after consuming the shellfish. Thus, it can bring harm and risk to human health (Krishna et al., 2016; Baker-Austin et al., 2010; Finkelstein, 1996; Faghri et al., 1984; Tison & Seidler, 1983; Oliver et al. 1982; Tamplin et al., 1982). Shellfish such as crabs normally have a high amount of microbes on their body parts due to the water, sediments and their food consumption (Farhana et al., 2015). Therefore, to indicate the contamination of shellfish with human pathogens, indicator organisms such as Coliforms have been applied (Faghri et al., 1984; Erkenbrecher, 1981). These shellfish can accumulate the toxins and pass them on to the consumers that may cause public health diseases, including food allergies and water-borne diseases such as diarrhea and gastrointestinal illness (Faghri et al., 1984).

Moreover, the warm temperate tropical areas worldwide normally have many species of mud crabs, *Scylla* spp., which are usually abundant in the subtidal muddy environment, nearby intertidal, and mangrove swamps (Azra & Ikhwanuddin, 2016). Four species of mud crab have been described, i.e., *Scylla serrata, Scylla* *paramamosain, Scylla tranquebarica* and *Scylla olivacea* (Varadharajan & Soundarapandian, 2014; Keenan et al., 1998). These crabs inhabit brackish waters throughout the Pacific and Indian Oceans, from Tahiti, Australia, and Japan to southern Africa (Akpaniteaku, 2014; Dai & Yang, 1991). These crabs are an important fishery resource in Australia, Japan, Taiwan, Indonesia, Malaysia and the Philippines where they are used as aquaculture as well due to their valuable contents (Azra & Ikhwanuddin, 2016; Ma et al., 2015; Shelly & Lovatelli, 2011; Watanabe et al., 1996; Watanabe & Sulistiono, 1993). Additionally, mud crabs have a consistently high demand in the trade markets every year (Bain & Mandal, 2017). However, their high amount of consumption has exposed them more to the toxic substances which are biomagnified through their food chain. They are also suggested to be a biomonitor species in the Reef Water Quality Protective Plan (RWQPP) Marine Monitoring Program due to their ability to accumulate various kinds of contaminants as well as their significance for commercial and recreational fisheries.

Among the mud crabs, *S. olivacea* or the orange mud crab is a large swimming crab that can be found in the mangrove areas from Southeast Asia and Indo-Pacific regions to southeastern and eastern Africa (Jirapunpipat et al., 2009; Klinbunga et al., 2000; Fuseya & Watanabe, 1996). In the Indo-West Pacific region, *S. olivacea* has a significant commercial value and is highly-priced for the export and local markets (Keenan, 2004). The production of *S. olivacea* in saline water millponds is common in the northern part of Peninsular of Malaysia, especially in Kedah, Penang, Perak and Perlis (Rosly et al., 2013).

Nearly 1.3 million tonnes of crabs including the mud crab are produced annually and are considered to be among the most common crustacean food items (FAO, 2010). However, according to the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) reports, the ingestion of shellfish including crabs is listed as among the eight main causes of food allergens and is usually found in the coastal zones (Huang et al., 2010; Liang et al., 2008).

Food allergy is delineated as the inverse reaction to food which comprises an irregular immune system response to particular proteins in foods (Fei et al., 2016). It has been reported that food allergy has serious effects on young children with 6% and about 3 to 4% of adults in human communities (Sicherer & Sampson, 2010). In many Asian countries, crabs are among the shellfish that are highly demanded and consumed. Crab allergy is also highlighted as among the major forms of food allergy in this region which affects approximately 1 to 2% of young children (Pedrosa et al., 2015). According to other studies, shellfish including the crab impact about 16.2% of food allergy among adults in Singapore (Thong et al., 2007) and 44% among patients with rhinitis and asthma in Malaysia (Shahnaz et al., 2001). In fact, workplaces are also the main sources of crab allergy due to various activities such as cleaning and scrubbing, aerosols inhalation during cutting, boiling, drying and cooking (Thomassen et al., 2016; Kamath et al., 2014; Jeebhay & Lopata, 2012).

Various symptoms occur after the ingestion of polluted shellfish and the number of toxins being consumed determines the extent of the symptoms (Woo & Bahna, 2011). Hence, it is essential to differentiate between poisonous shellfish and allergic shellfish. Studies on the symptoms were conducted to determine the cause of food allergies. It was discovered that several matters need to be considered such as the background of each type of shellfish, the digested amount, time of onset, symptoms in other people who had consumed the same meal, and the type of symptoms (Roberts et al., 2016; Howarth et al., 2016; Woo & Bahna, 2011). Therefore, there is a valid prerequisite for understanding the antiquity of the specifically associated type of fish and a pretest assessment on history should be conducted. Besides that, diagnostic tests can also be used for further evaluation in the occurrence of an allergy.

Nowadays, various methods are used to diagnose the allergies of other shellfish and crab allergies such as the measurements of specific IgE (sIgE) and skin prick testing (SPT). These methods are being used extensively in clinical settings due to efficient implementation (Roberts et al., 2016; Caubet et al., 2012). When a patient has a reaction such as a life-menacing reaction or blindness, a titrated oral test should be offered (Caubet et al., 2012). Basically, a way to prevent the occurrence of crab allergy is to avoid consuming foods with crab-based ingredients (Sicherer & Sampson, 2010). However, prevention is not always practical owing to the cross-reactivity between the allergens and accidental or unintended infection or the inclusion of crab-based ingredients in certain sauces and spices (Sicherer & Sampson, 2010). Pharmacological treatment is one of the useful ways to decrease the symptoms of an allergy with a particular avoidance diet (Caubet & Sampson, 2012). In the case of anaphylaxis, it is best to use the anticholinergic agents, corticosteroids or epinephrine and antihistamines together when the symptoms appear (Matricardi et al., 2016; Sicherer & Sampson, 2010).

Crabs are among the ‘big eight’ food groups that can cause food allergy. Specifically, mud crab contributes to food allergens in adults and comprises various species that may have similar and different allergens. It was well-documented that tropomyosin was the most important of shellfish major allergens including crabs. Tropomyosin is highly stable to heat and is IgE reactive. Tropomyosin-specific IgE is commonly used to clinically estimate the outcomes of shrimp allergy, delivering a predictive accuracy value of 0.72. Evidently, around 60% of the patients had clinically displayed an allergic reaction to the crustaceans from specific IgE binding to tropomyosin. The reaction between the IgE reactivity and tropomyosin is said to be a good predictor to detect the crab allergy rather than conducting the skin prick test (SPT) or extracting the crabs. Beside tropomyosin, other major allergens in the mud crab *Scylla serrata* are arginine kinase, sarcoplasmic calcium-binding proteins, troponin, α-actin and smooth endoplasmic reticulum Ca^{2+}ATPase (Shen et al., 2011).

Consumers should be aware that the different amounts of seafood consumption for each individual may differ from one to the other. It is discovered that various substances can be discovered abundantly in seafood than any other sources of food. Furthermore, it can also cause various clinical symptoms. Based on a common clinical history, an individual who is encountered with a food allergy will be referred to the experts or a non-atopic etiology will be suggested such as contamination with parasites, bacteria, and viruses. Due to similar clinical reactions to the affected individual, it is thus important to differentiate the contrasting reactions from the actual shellfish allergy and to understand the nature of the molecule within the offending allergens in order to enhance the component-resolved diagnosis (Lopata, Kleine-Tebbe & Kamath, 2017).

Different processing methods that involve cooking might affect the allergenic characteristics of food allergens including crabs (Yu et al., 2010). While shellfish are universally suitable to be eaten in both forms raw and cooked, many studies have affirmed that they are allergenic. Basically, cooked crabs are normally controlled to some form of thermal processes mostly boiling to assure microbiological security or to improve flavors and texture. Continuously, the thermal treatment may alter the structure of the protein by encouraging the denaturation through the loss in the secondary and or tertiary structure levels, the production of new molecular chains, aggregation, as well as other conformational alterations that may eventually lead to variations in allergen reactivity (Mondoulet et al., 2005). Moreover, the decreases in the allergen reactivity can be due to heating, as have been mentioned in some previous studies (Chen & Phillips, 2005; Mondoulet et al., 2005; Beyer et al., 2001). In different cases, the heat contributes to the rise of allergen strength by exposing the new IgE-binding positions (Leszczynska et al., 2003). Hence, the treatment shows the allergenicity of the crab and tropomyosin is designated to be a heat-stable protein to the major shellfish allergen (Yu & Chen, 2010). However, the allergen of crab is sensitive to heat (Xue et al., 2016; Abramovitch et al., 2013).

1.2 Problems Statement

All organisms are essentially made up of water. Numerous pollutants enter water bodies in large amounts thus causing disturbances in the ecosystem and on all-natural and anthropogenic activities including urban overflow, wastewater, industries and agronomy (Sharif et al., 2015; Mohamed, 2012; Bai et al., 2009; Chen et al., 2003).

Each year, large amounts of contaminants pose negative short- and long-term effects on marine life and the environment (Massoud et al., 2006). Biological-based and heavy metal contaminants can suspend in water or accrue at various levels, in the residue and body of the organisms, which subsequently can be transferred from the food-chain and could affect the general health of human beings (Cheung & Wang, 2008). Many research on the bioaccumulation of heavy metals in fishery products, particularly mud crabs has been conducted and the studies show that the bioaccumulation of these metals occurs in the tissue of the shellfish (Verbeke et al., 2005). However, at present, little is known about the habitat quality and bioaccumulation of heavy metals and biological contaminants in the *S. olivacea* in Malaysia.

Heavy metal buildup along with biological contaminants in the body of aquatic organisms has been found to trigger allergic reactions in the bodies of their consumers. This is due to the ingestion of contaminated seafood by hydrocarbons and other contaminants, which attracts much attention from scientists to study the impact of human-made perturbations on the ecosystems (Corder et al., 2016). Frequently, the allergy is the result of the ingestion of contaminated seafood which is found in coastal zones where the high processing and consumption of shellfish are well-known (James et al., 1997). A study has reported the presence of heavy metals such as nickel (Ni) and zinc (Zn) in the mud crabs, which increases the chances of allergenic sensitivity to its consumers (Kandah & Meunier, 2007). The bioaccumulation of arsenic (As) in the crab can lead to many human diseases and increases the risks of skin problems including allergy (Gok et al., 2016; Järup, 2003). Currently, studies on the correlation of habitat quality, accumulation of heavy metals, biological contaminants and allergic reactions to *S. olivacea* among consumers are rarely reported.

Globally, seafood is considered to have important nutritional value, driven further by the spike in international trade of numerous seafood products (Belton & Thilsted, 2014; Jeebhay et al., 2010; Lopata et al., 2007). However, consuming crab including the *S. olivacea* can generate an allergic reaction that is mediated by IgE antibodies, which can cause a fatal effect. In fact, the crab has been ranked as the top seven causes of food allergic reactions in the world (Pawankar et al., 2011). Among portunid crabs, mud crabs are the most widely distributed and cultured group.

IgE-driven allergies in individuals suffering from rhinoconjunctivitis, asthma, urticaria, anaphylaxis, atopic eczema, food and drug allergy can be reliably diagnosed using the Skin Prick Test (SPT) (Heinzerling et al., 2013). SPT is usually performed using crab extracts that are prepared from crab meats. However, to date, the crab extracts commercially available are prepared from crab species other than *S. olivacea,* which might have different protein contents and allergenicity. The sources of crab parts that are used for extraction are not well defined. Thus, to produce more accurate diagnostic tests for the detection of specific IgE against *S. olivacea* allergens, an allergen extract that is produced from several body parts of the local crab *S. olivacea* is essential in order to obtain accurate diagnostic results. In this work, the *in-house* allergen extracts were prepared from *S. olivacea* to be used in skin prick tests and immunoblotting tests.

The edible flesh of the crab is in the claws, legs, and abdomen (body) (Madsen, Forster, Grefenstette, Harrison & Stern, 2017; Stewart & Reichelt, 1993). However, no reports are available on allergen characterizations of different crab parts particularly the claws, legs, and abdomens, as well as between crab genders. The comparison of allergenic proteins between the gender and the parts of the crabs are considered as an important aspect to be evaluated in allergy studies. The variations of allergenic properties in body parts and crab gender might play an important role in crab allergenicity. A study reported that the highest protein content was found in the meat of the body and lowest in the claw's meat of *S. tranquebarica* (Sreelakshmi & Saraf, 2016). Meanwhile, the meat from female *S. serrata* and *S. tranquebarica* crabs had significantly higher protein content than the males irrespective of species and body parts variation (Sreelakshmi et al., 2016; Zafar et al., 2004; Khan, 1992). Thus, the variations of protein content in the different body parts and gender of crabs might be useful for the clinician and patients to indicate the possibility of variation of allergenicity of crabs concerning their gender and different body parts.

It is highly crucial to identify allergens to gain insight into the exact immune response mediated by IgE. Such identification can also contribute to better diagnostic tests and patient management. At present, there are numerous reports on the identification of major crab allergens such as tropomyosin, arginine kinase and actin from several species of crabs (Han et al., 2018; Liu et al., 2018; Rosmilah et al., 2015; Abramovitch et al., 2013; Rosmilah et al., 2012; Huang et al., 2010; Liang et al., 2008). Currently, no studies have been documented on the classification of major and minor *S. olivacea* allergens.

Prior to being consumed, crabs normally undergo some form of thermal treatments such as boiling and non-thermal treatments such as pickling by acids. As the allergens mainly comprise protein, the structure of the protein may be altered through various processing methods which can cause the changes of the allergens (Chen & Phillips, 2005). Food processing may induce allergen alterations due to epitope destruction or modifications, potentially resulting in either decreasing, enhancing or having no effect on food allergenicity (Rosmilah, Shahnaz, Zailatul & Noormalin, 2012; Sathe, Teuber & Roux, 2005). Impacts of heat treatments on the reactivity of the patients' IgE antibody to numerous shellfish allergens have been widely reported (Zailatul et al., 2015; Zailatul et al., 2012; Yu et al., 2011; Carnes et al., 2007; Martin-Garcia et al., 2007). Nevertheless, little is known about the effects of non-thermal treatments on crab allergenicity. Hence, there is a need to investigate the impact of the various methods for processing crab allergens, and to find appropriate methods for reducing or removing allergen stability and reactivity.

Currently, reports on the correlation between the protein profiles and allergenicity of *S. olivacea* with metals accumulation, biological contaminants and its habitat quality are not available in the literature. Therefore, this current inquiry intends to examine the quality of *S. olivacea* habitats, accumulation of heavy metals and biological contaminants, and subsequently evaluate the correlation between protein profiles of *S. olivacea* from Merobok River, Kedah with metals accumulation, biological contaminants and its habitat quality.

1.3 Research Objectives

Outlined below are the objectives of this research:

i. To evaluate the habitat quality of the mud crab *Scylla olivacea* in Merbok River, Kedah.

ii. To estimate the accumulation of heavy metals and biological contaminants in *S. olivacea* from Merbok River, Kedah

iii. To characterize the protein profiles and allergenic proteins of *S. olivacea.*

iv. To determine the effect of boiling and acid treatments on the stability of protein profiles and allergenic proteins of *S. olivacea.*

v. To evaluate the correlation between protein profiles of *S. olivacea* with metals accumulation, biological contaminants and its habitat quality.

1.4 Research Questions

i. What is the habitat quality of the mud crab *Scylla olivacea* in Merbok River, Kedah?

ii. What is the level of accumulation of heavy metals and biological contaminants in the *S. olivacea* from Merbok River, Kedah?

iii. What are the characteristics of protein profiles and allergenic proteins of *S. olivacea*?

iv. What are the effects of boiling and acid treatments on the stability of protein profiles and allergenic proteins of *S. olivacea.*?

v. Is there any correlation between protein profiles of *S. olivacea* with metals accumulation, biological contaminants and its habitat quality*?*

1.5 The Significance of the Research

The mud crab *Scylla* spp. is an important crab species in the Indo-Pacific regions. This study will provide new data on the status of the habitat quality of the *S. olivacea* in Merbok River, Sungai Petani, Kedah, Malaysia, by conducting water quality assessment using different parameters in the site and the laboratory. In addition, the evaluation of the concentrations of heavy metal levels and biological contaminants in this river will be useful in determining the level of contamination in crabs inhibiting along the Sungai Merbok waters.

Meanwhile, this study will provide data for allergen extract production by selecting the crab with the lowest contaminants as the sources for allergen extracts. These will be the major findings of this study which will expand the current knowledge on the local crab allergy and provide a platform for the advancements in the diagnosis, setting standard allergenic test products as a tool in molecular allergology and enhance the management of allergic patients by establishing immunotherapy. This will benefit clinicians as well as patients.

Mud crab consumption is increasing in different parts of Malaysia and so are the allergies that are related to mud crab. Hence, consumption of crab meat can cause allergic reactions in human beings. This research will also benefit all *S. olivacea* consumers, especially the crab allergic patients, to avoid any adverse crab reactions due to high toxicity by choosing the crab from high-quality habitat.

This study provided new information on protein profiles of protein extracts from the different body parts and genders of the *S. olivacea*, which will be useful for researchers in selecting crab parts and genders with the highest protein bands as the crab source for allergen extracts. The study will also benefit in the management of patients with crab allergy by avoiding crab parts that have more allergenic bands in their diet. The data of allergenicity from the different crab parts and genders is also useful for the food industries to produce crab products with the least allergenicity by removing the most allergenic crab parts in processed foods.

Crab allergies are diagnosed using crude allergen extracts as test allergens, which contain a mixture of major and minor allergens. Thus, identification of crab allergens is the first step towards generating crab allergen components, leading to the establishment of an allergen panel specific for the diagnosis of local crab allergy. It is crucial to identify the main allergens that cause such a reaction as it will lead to a greater understanding of the specific IgE mediated response so that reliable diagnostic examination can be conducted on the patients. In addition, allergen identification can also facilitate allergic reaction prediction.

Humans are easily exposed to crab allergens through the process of the consumption of crab, inhalation, or direct skin contact while processing, cooking, or working with crabs (Izzah et al., 2015). The characterization of thermal and non-thermal stability might be useful in the management of crab allergenic patients such as to reduce the allergic responses including angioedema, urticaria, asthma, diarrhea, eczema, rhinitis, and even life-threatening anaphylaxis (Lopata & Lehrer, 2009). By identifying the stability of crab allergens, a better insight into crab allergenicity can be established. In addition, various processing treatments may be viewed as important methods of preventing allergenicity in susceptible individuals, thereby reducing treatment costs.

This study indicates the relationship between habitat quality and allergenicity of *S. olivacea*. This finding could facilitate the improvement of crab allergic patients management, as high contaminations from the surrounding environment of the *S. olivacea* might result in health risk to the consumers, which lead to improving patients' health and quality of life.

1.6 Scope and Limitation of Study

This study focuses only on the habitat quality and allergenicity of one species of mud crabs, i.e., the orange mud crab *Scylla olivacea.* The crab and samples for habitat quality studies were collected from only three sites in Merbok River at Sungai Petani, Kedah, Malaysia. The sample collection was carried out once every three months over the duration of a year. The evaluation on the habitat quality for the *S. olivacea* was determined based on several parameters including biological oxygen demand (BOD), chemical oxygen demand (COD), pH, temperature, dissolved oxygen (DO), salinity, turbidity, cation for lithium, sodium, ammonium, potassium, magnesium, and calcium as well as anions like chloride, nitrate, bromide, nitrate, sulfate, fluoride and phosphate.

There are many elements responsible for the toxicity of mud crabs. However, due to financial and time constraint, the heavy metals concentrations would only focus on arsenic (As), nickel (Ni), lead (Pb), chromium (Cr), manganese (Mn), iron (Fe), zinc (Zn) and cadmium (Cd). For the biological contaminants, this study focuses only on determining the levels of Coliform and *E. coli*. The presence of the elements and biological contaminants were tested in both the bodies of the mud crabs and their habitat.

The allergen extracts of the crab were prepared individually from both male and female crabs, from three crab parts only, which were the abdomen (body), claws and legs. The protein profile of the mixed crab part extracts were determined by both SDS-PAGE and 2-DE electrophoresis, while the protein profiles for the individual crab parts were analysed solely by SDS-PAGE.

Immunoblotting and 2-DE immunoblotting tests were carried out to determine the major and minor crab allergens using only 30 sera, which were collected from one location, the Allergy Clinic, Hospital Kuala Lumpur. As for the molecular identification of crab, only the major allergenic spot of the major allergens was identified by the mass-spectrometry analysis, which was conducted by Apical Scientific Sdn. Bhd.

The thermal stability of the crab allergens was evaluated by boiling methods only at 6 different durations 10, 20, 30, 40, 50 and 60 minutes, while the non-thermal stability of the crab allergens were determined by only acid treatments using acetic acids at different pH of 2.5, 2.9, 3.2, 3.9, 4.4 and 5.3.

Initially, this study also aimed to indicate the relationship between habitat quality and allergenicity of *S. olivacea*. However, due to insufficient data and history of allergic patients used, this study only focused on correlation study between profile profiles (total protein contents and number of protein bands) and habitat water as well as the heavy metals accumulation in crab bodies.