METAL CONTAMINANTS AND CHLORAMPHENICOL IN *Litopenaeus vannamei* AND *Penaeus silasi* AT VARIOUS SUPPLY CHAIN STAGES IN JOHOR BAHRU, AND THEIR HEALTH RISK ASSESSMENT

NURAZIRA BINTI ANUAR

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

OCTOBER 2022

ACKNOWLEDGEMENT

First and foremost, I would like to praise Allah the Almighty, the Most Gracious, and the Most Merciful for His blessing given to me during my study and in completing this thesis. May Allah's blessing goes to His final Prophet Muhammad (peace be up on him), his family and his companions.

In preparing this thesis, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. In particular, I would like to express my gratitude and sincere appreciation to my main thesis supervisor, Ts. Dr. Naji Arafat Mahat, for encouragement, guidance, and valuable advices. I am also very thankful to my co-supervisor Dr. Aemi Syazwani Abdul Keyon and Dr. Mohamad Afiq Mohamed Huri for their guidance and motivation. Without their continued support and interest, this thesis would not have been the same as presented here.

I wish to thank Prof. Ida Idayu Muhammad and Dr. Nor Hayati Sapie from Faculty of Engineering, Universiti Teknologi Malaysia, Dr. Mohd Fazrul Hisam bin Abd Aziz from Faculty of Fishery and Food Science, Universiti Malaysia Terengganu, all Centre of research laboratory (PPA) staffs at Universiti Teknologi Malaysia especially Encik Fuad Omar, for their assistance throughout this research. I am also indebted to the Ministry of Higher Education for funding my research study under the research grant of Malaysian Research University Network.

My sincere appreciation also extends to all my postgraduate friends, Wajihan, Idayu, Ereena, Gandu, Aida, and others who have provided assistance at various occasions. Their views and tips are useful indeed. Unfortunately, it is not possible to list all of them in this limited space. I am grateful and blessed with them through this journey.

Finally, I must express my very profound gratitude to my parents and family for providing me with unfailing support (moral and financial) and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

ABSTRACT

Contamination by toxic metals and antibiotics (like chloramphenicol) in white shrimps can be detrimental to human health. Hence, this present research evaluated the concentrations of As, Pb, Cd and total Hg and chloramphenicol as prescribed by the Malaysian Food Act in *Penaeus silasi* and *Litopenaeus vannamei* shrimps sampled from the different supply chain stages. The sampling was made at landing sites, major markets and grocers in Johor Bahru, Johor, Malaysia during the six sampling intervals. A Perkin-Elmer Analyst PinAAcle 900T atomic absorption spectrometer equipped with graphite furnace (GFAAS) and autosampler was used for quantitating the concentrations of As, Pb, and Cd. A flow injection mercury/hydride system (FI-MHS) was utilized for analysing the total Hg. The results revealed the median As and Cd concentrations that ranged between below detection limit (bdl) - 167.5 mg/kg and bdl - 0.46 mg/kg, respectively. As for total Hg, the concentrations ranged between 0.02 - 1.13 mg/kg. Although Pb and chloramphenicol were not detected, As and total Hg concentrations varied significantly across the six sampling intervals ($P < 0.05$), exceeded the maximum permitted proportions prescribed by the Malaysian Food Act. Identified as *P. silasi*, shrimps from landing sites Mersing and Pontian had significantly higher As and total Hg concentrations than those of *L. vannamei* from major markets and their surrounding grocers ($P < 0.05$). Alarmingly, Hazard Index for *P. silasi* from landing sites Mersing and Pontian as well as *L. vannamei* from major markets and grocers exceeded 1.00. Hence, formulating suitable intervention programs and devising efficient removal technologies are paramount for benefiting the public at large.

ABSTRAK

Pencemaran logam toksik dan antibiotic (seperti kloramfenikol) dalam udang putih boleh menjejaskan kesihatan manusia. Justeru, kajian ini yang menilai kepekatan bahan kontaminasi logam (As, Pb, Cd dan Hg terkumpul) dan kloramfenikol seperti yang ditetapkan oleh Akta Makanan Malaysia dalam udang *Penaeus silasi* dan *Litopenaeus vannamei* yang disampel pada pelbagai peringkat rantaian bekalan. Pensampelan dilakukan pada tapak pendaratan, pasar utama dan kedai runcit di Johor Bahru, Johor, Malaysia semasa enam selang pensampelan. Spektrometer serapan atom PinAAcle 900T Penganalisis Perkin-Elmer yang dilengkapi dengan relau grafit (GFAAS) dan penyampel automatik digunakan dalam menentukan kepekatan As, Pb dan Cd. Sistem suntikan merkuri/hidrida (FI-MHS) digunakan untuk menganalisis Hg terkumpul. Keputusan menunjukkan median kepekatan As dan Cd yang masing-masing berjulat antara di bawah had pengesanan (bdl) - 167.5 mg/kg dan bdl - 0.46 mg/kg. Bagi Hg terkumpul, kepekatannya berjulat antara 0.02 - 1.13 mg/kg.Walaupun Pb dan kloramfenikol tidak dikesan, kepekatan As dan Hg terkumpul berbeza secara signifikan merentas enam selang pensampelan ($P < 0.05$), melebihi perkadaran maksimum dibenarkan yang ditetapkan oleh Akta Makanan Malaysia. Dikenal pasti sebagai *P. silasi*, udang dari tapak pendaratan Mersing dan Pontian mempunyai kepekatan As dan Hg terkumpul yang lebih tinggi secara signifikan berbanding *L. vannamei* dari pasar utama dan kedai runcit di sekitarnya ($P < 0.05$). Apa yang membimbangkan, Indeks Bahaya untuk *P. silasi* dari tapak pendaratan Mersing dan Pontian serta *L. vannamei* dari pasar utama dan kedai runcit adalah melebihi 1.00. Oleh yang demikian, perumusan program intervensi yang sesuai dan menghasilkan teknologi penyingkiran yang cekap adalah utama dalam memanfaatkan khalayak ramai.

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LIST OF ABBREVIATIONS

AAS	-	Atomic absorption spectroscopy
AC	-	Average consumer
ADI	-	Allowable daily intake
ANZFSC	-	Australia New Zealand Food Standard Code
AOAC	-	Association of Official Analytical Collaboration
As	-	Arsenic
Bdl	-	Below detection limit
BW	-	Body Weight
CAP	-	Chloramphenicol
Cd	-	Cadmium
CE	-	Capillary electrophoresis
Co	-	Cobalt
Cr	-	Chromium
Cu	-	Copper
DAD	-	Diode array detector
DI	-	Deionized
DNA	-	Deoxyribonucleic acid
DOF	-	Department of Fishery
Dw	-	Dry weight
EC	-	European Commission
EDI	-	Estimation daily intake
ELISA	-	Enzyme-linked immunosorbent assay
EU	-	European Union
EWI	-	Estimated weekly intake
FAAS	-	Flame atomic absorption spectroscopy
FAO	-	Food and Agriculture Organization
Fe	-	Iron
FI-MHS	-	Flow injection mercury/hydride system

FSSAI	-	Food Safety and Standards Authority of India
GFAAS	-	Graphite furnace atomic absorption spectroscopy
GPS	-	Global positioning system
H_2O_2	-	Hydrogen peroxide
HC	-	High consumer
HCL	-	Hydrochloric acid
Hg	-	Mercury
HI	-	Hazard index
HNO_3	-	Nitric acid
HPLC	-	High performance liquid chromatography
HQ	-	Hazard quotient
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
IUPAC	-	International Union of Pure and Applied Chemistry
JECFA	-	Joint FAO/WHO Expert Committee on Food Additives
KMNO4	-	Potassium manganate (VII)
LC-MS	-	Liquid chromatography-mass spectrometry
LLOQ	-	Lower limit of quantification
LOD	-	Limit of detection
LOQ	-	Limit of quantification
MeHg	-	Methyl Mercury
Mn	-	Manganese
Mo	-	Molybdenum
MOFL	-	Ministry of Fisheries and Livestock
MPPs	-	Maximum permitted proportions
MRLs	-	Maximum residue limit
MRPL	-	Minimum required performance limit
MYR	-	Malaysia ringgit
Ni	-	Nickel
P	-	Probability
Pb	-	Lead
PTDI	-	Provisional tolerable daily intake
PTWI	-	Provisional tolerable weekly intake
Rb	-	Rubidium

RfD	-	Oral reference dosage
RM	-	Ringgit Malaysia
RSD	-	Relative standard deviation
Se	-	Selenium
SEAFDEC	-	Southeast Asian Fisheries Development Centre
SnCl2	-	Tin (II) chloride
Sp.	-	Species
THQ	-	Target hazard quotient
UPLC-MS/MS	-	Ultra high performance liquid chromatography-tandem mass spectrometry
USA	-	United stated of America
USA EPA	-	Environmental Protection Agency
USFDA	-	United States of America Food and Drug Administration
V	-	Vanadium
Viz.	-	Namely
WHO	-	World health organization
WHO/FAO	-	World Health Organization/ Food Agriculture Organization
ww	-	Wet weight
WWTPs	-	Waste water treatment plant
Zn	-	Zinc

LIST OF SYMBOLS

ITEMS	**DESCRIPTIONS**
g/day	Gram per day
ºC	Celsius
mg/kg	Milligram per kilogram
%	Percent
µg/L	Microgram per litre
v/v	Volume per volume
w/w	Weight per weight
µg/g	Microgram per gram
ng/g	Nanogram per gram
µg/kg	Microgram per kilogram
mg/kg	Milligram per kilogram
kg	Kilogram
mg	Milligram
µL	Microliter
min	Minute
mL	Millilitre
nm	Nanometre
mg/L	Milligram per litre
mL/min	Millilitre per minute
≈	Approximate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

White shrimp (*Litopenaeus vannamei)* and false white shrimp (*Penaeus silasi*) are popular aquaculture products in many countries including Malaysia (Okpala et al., 2014; Hidayati et al., 2020). According to the Malaysia Department of Fisheries (DOF), Malaysia has been producing 50,000 metric tons of shrimps a year, approximately 13% of aquaculture total production volume. This production has contributed to approximately MYR 1.3 billion worth of fishery exports (DOF, 2020). As a matter of fact in Peninsular Malaysia, *L. vannamei* has been exclusively farmed in captivity (Abidin, M.N., Personal communication, Department of Fishery Johor, August 25, 2020), while others species of shrimps (including *P. silasi*) can be harvested from the ocean (Anandkumar et al., 2017). Exclusively for Johor (the southernmost state of peninsular Malaysia), about 4457.94 metric tons of shrimps have been traded in the market for the year of 2019 alone, accountable for approximately MYR 175 million in economic values (Abidin, M.N., Personal communication, Department of Fishery Johor, August 25, 2020). The escalating demand for shrimps has been attributable to its comparatively affordable price and favorable nutritional values (Pires et al., 2018), as well as the rapidly growing population (Ghee-Thean et al., 2016). Interestingly, despite being cheaper in prices than that of black tiger shrimps (*Penaeus monodon*), *L.vannamei* has been reported to have a higher protein content (18.8 %) than that of the former (17.1%) (Sriket et al., 2007). Despite slightly lower protein content in *P. silasi* (13.6 %) (European Commission, 2021) than those of *L. vannamei* and *P. monodon,* the false white shrimp can still be considered as an important source of protein. Such findings would translate as *L. vannamei* and *P. silasi* being good sources of amino acids, especially those of essential ones for human consumption (Gunalan et al., 2013).

Similar to shellfishes and crabs, shrimps scavenge on a wide range of materials including debris and other bottom-dwelling animals (Food and Agriculture Organization, FAO, 2008), as well as having the tendency to accumulate toxicants, especially metal contaminants prevailing in the aquatic environment (Batvari et al., 2016). In this context, studies revealed that the sensitivity of crustaceans (including shrimps) to accumulate metal contaminants is largely influenced by their stages of development, reproductive cycle, molting stage and nutritional condition (Zhang et al., 2017). It has been indicated that pollution by metal contaminants in the aquatic ecosystem is one of the most alarming, decade-long environmental issues due to their inherent toxicity, non-degradability and persistency in water (Wang et al., 2013). Such a situation eventually leads to toxic accumulations in aquatic organisms (Olmedo et al., 2013). Industrial, agricultural and mining activities appear to be among the major sources of anthropogenic contamination of the aquatic ecosystem for metal contaminants (Raissy, 2016). In addition, contamination of the aquatic ecosystem with pharmaceutical drugs would further exert an unwanted environmental stressor on aquatic organisms (McCallum et al., 2019). Being highly stable, pharmaceutical drugs can be considered as “pseudo persistent” pollutants, and these drugs are able to target specific metabolic and molecular pathways in humans and animals because they are biologically active (Trombini et al., 2019). Hence, the fact that humans can be exposed to contamination by these metal contaminants and pharmaceutical drugs through the food chain with consumption of seafood (Ullah et al., 2017b) cannot be neglected.

In this context, researchers (Rabiul Islam et al., 2017;Mahat et al., 2018) indicated the possible toxicities of metal contaminants via ingestion of seafood following acute and chronic exposures. The adverse health implications included brain damage, paralysis, anemia and disruption of vital body systems (Rabiul Islam et al., 2017; Mahat et al., 2018). The World Health Organization (WHO, 2016) also reported about the increasing incidences of fetus malformations and deaths, as well as preterm and/or low birth weight babies among mothers exposed to toxic metals. Therefore, to safeguard human health, many countries (including Malaysia) have prescribed the Maximum Permitted Proportions (MPPs) for a number of metal contaminants, as well as other contaminants such as pharmaceutical compounds in the various types of seafood including shrimps (Griboff et al., 2020; Rajan and Ishak, 2017; Vandermeersch et al., 2015). In Malaysia, the metal contaminants outlined in the law are arsenic (As), cadmium (Cd), lead (Pb), and total mercury (total Hg). The law also bans any presence of chloramphenicol, nitrofuran and beta agonist (excluding Ractopamine) in any types of foods (Malaysia Food Act 1983 (Act 281) and Regulations (2021)), accentuating their severe toxicities for human.

Being a broad spectrum antibiotic, chloramphenicol has been illegally used in aquaculture for promoting growth and production, as well as treating bacterial diseases of aquatic products (like white shrimps) (Suseno et al., 2016). Because chloramphenicol can be carcinogenic and possibly suppress the human bone marrow, as well as affecting various vital systems in the human body (including that of the reproductive system), its utilization in food producing animals for human consumption has been banned by many developed countries (Chen et al., 2020). Importantly, it has also been indicated that bacterial antibiotic resistance such as chloramphenicol can be augmented by heavy metals (e.g. species of arsenic) even at sub-toxic levels which subsequently may cause higher health risk towards human and the environment (Chen et al., 2015). It is pertinent to mention here that shrimps exported from Malaysia has been banned and placed into import alert by the United States of America Food and Drug Administration, (USFDA), presumably attributable to the presence of traces of illegal antibiotics of chloramphenicol (USFDA, 2020). Responding to such a ban, the DOF has issued a press release denying such an allegation, attributing the element of transshipment from other countries as the reason for such contaminated shrimps (DOF, 2020).

Considering that continuous monitoring of metal contaminants is imperative, various analytical methods developed using different instruments have been reported. The methods ranged from flame atomic absorption spectroscopy (FAAS) (Mahat et al., 2018), and graphite furnace atomic absorption spectroscopy (GFAAS) (Elgammal et al., 2019), inductively coupled plasma optical emission spectroscopy (ICP-OES) (Giri and Singh, 2014), and capillary electrophoresis (CE) (LI et al., 2016). The use of GFAAS has been acquiring popularity due to its low detection limits while requiring minimum sample manipulation as well as its relative simplicity and the short duration to obtain a result (Zhong et al., 2016). As for pharmaceutical compounds, the use of High-Performance Liquid Chromatography (HPLC) as well as Gas chromatography (GC) to analyze seafood products has been prevalently reported (Cháfer-Pericás et al., 2010; Mottaleb et al., 2016). This chromatographic techniques provide sensitive and selective method for the simultaneous quantification of different pharmaceutical drugs (Siddiqui et al., 2017). However, these approaches are time-consuming and necessitating the use of highly specialized experts as well as pricey instruments. Chloramphenicol residues can now be detected by microbiological, enzymatic, and immunological assays (Liu et al., 2019). Enzyme-linked immunosorbent assay (ELISA) technique for quantitative detection of chloramphenicol has been highly recommended nowadays because of several advantages. The method is highly accurate and precise, as well as quick and easy to handle; even at the limit of detection, the analysis of large volumes of samples can be done in short time (Sarwer et al., 2017). In this context, this current study used the ELISA technique to determine the level of chloramphenicol residues in the tissues of 'white shrimps'. As such, developing validated and robust analytical methods for detecting and quantitating the different toxicants such as metal contaminants and chloramphenicol in important aquaculture commodities like white shrimps appears relevant.

Despite the pertinence of these ecotoxicology and health issues, review of literature reveals only limited number of scientific articles endeavoring on the detection and quantitation of selected metal contaminants in shrimps from Malaysia as well as its relevant health risk assessment. Specific study on pharmaceutical contaminants like chloramphenicol in shrimps remains unreported so far. While investigating the concentrations of Cu, Cd, Cr, Co, Ni, Pb, Mn, Zn and Rb in several marine species of shrimps from the Miri coast of Sarawak, Anandkumar et al. (2017) reported that they were lower than that of the permissible limits of Malaysian and international guidelines for seafood. The authors further reported low estimated daily intake (EDI) and hazard indices, concluding that the shrimps may not cause acute toxicities and therefore, safe for human consumption. However, because the work by Anandkumar et al. (2017) did not include the full list of metal contaminants as prescribed by the Malaysian Food Act 1983 (Act 281) and Regulations (2021) while estimating its hazard indices (As and Hg were excluded), the real status of safety for consuming the shrimps may not be well represented. The fact that the description on the sampling period as well as the complete analytical validation data are also not provided, the robustness of the results reported by the authors can be at dispute.

In another study, Rajan and Ishak (2017) reported that ‘there is no calculated significant risk from metal toxicity by the consumption of shrimps’ from several local wet markets in Selangor. However, the fact that (a) the sampling was done only for 3 months in 2015, (b) with small sample size from the unreported sources, (c) the analysis did not include As and Hg (d) with no analytical validation data reported, the interpretation drawn by the authors may prove outdated and erroneous. Similar issues as indicated for the work by Rajan and Ishak (2017) also prevailed for the study on shrimps conducted in Kingfisher Inanam, Kota Kinabalu, Sabah reported by Lee et al. (2017). In a more recent study, Annual et al. (2018) reported the concentrations of total Hg and methylmercury (MeHg) alone in several species of crustaceans, cephalopods and fishes from West Peninsular Malaysia (Perak and Selangor). While concluding that the concentrations observed for these two compounds as below the Malaysian Food Regulation for fish and fishery products, suitable inference on the safe consumption of such seafood products for human cannot be explicitly corroborated. This is because their work did not include all the metal contaminants prescribed by the Malaysian Food Act 1983 (Act 281) and Regulations (2021). Interestingly, it has been advocated that the shrimps of *Penaeus merguiensis* from Serdang (Selangor) (Yap et al., 2019a) and *Acetes* sp. from Pantai Klebang (Malacca) (Yap et al., 2019b) as safe for human consumption. Conversely, in-depth evaluations of these two articles revealed that they were both undertaken (a) in a single instance during year 2007 (b) focusing on only one or two relevant metal contaminants prescribed by the law. In addition, the two articles also (c) did not provide suitable description on the sampling methods as well as (d) the relevant analytical validation data. The lack of such important pieces of information may hinder appropriate and empirically justifiable public health conclusion to be made. Thus, suitable studies addressing these limitations are necessary.

1.2 Problem Statement

The global and domestic changes in the preference of consumers from consuming red meat (ruminant products) to white meat (e.g. shrimp) (Ghee-Thean et al., 2016) as a source of protein (Okpala et al., 2014) have been reported. Since contamination by metal contaminants (Mahat et al., 2018) and pharmaceutical compounds (Griboff et al., 2020) like chloramphenicol in aquatic organisms has been indicated, continuous monitoring of such contaminants in important aquaculture commodities like *L. vannamei and P. silasi* sold to the consumers appears imperative. Being an important metropolitan city connected to Singapore *via* a causeway across the Straits of Johor as well as other states within Peninsular Malaysia, Johor Bahru is strategically important for international trading.

Despite the categorical placement of Malaysian shrimp exporters on the US FDA red list due to the detection of chloramphenicol since 2018, such tainted shrimps have been reportedly sold in Malaysia for domestic consumption (Malay Mail, 2020, January 4). In the immediate response by the DOF (2020), a press statement associating the presence of chloramphenicol to the element of transshipment, by illegally importing shrimps and re-exporting the commodity to the USA, was made on 4th January 2020. However, no tangible evidence has been offered to support such a claim. Such a negative connotation has not only tarnished the reputation of Malaysia as one of the aquaculture products exporting countries in Asia, but also detrimentally affects the well-being of the population, causing public health and economic issues that merit scientific clarification.

Specifically for Malaysia, its Food Act 1983 (Act 281) and Regulations (2021) outlines the Maximum Permitted Proportions (MPPs) (wet weight) for As, Pb, and Cd as 1.0 mg/kg, with slightly lower MPP of 0.5 mg/kg for total Hg) in crustaceans/shrimps. The same act also clearly bans any presence of chloramphenicol in any type of foods, accentuating its severe toxicity for human consumption. While review of literature does not reveal any study that evaluated chloramphenicol in shrimps from Malaysia, studies on the amount of metal contaminants in the same matrix appear to suffer a great deal of limitations. The limitations include (a) analysis that excluded As and Hg, leading to inappropriate conclusion on its risk assessment (e.g. Yap et al., 2019), (b) inadequate description of sampling procedure and locations (e.g. Rajan and Ishak, 2017), (c) outdated sampling time (e.g. Anual et al., 2018) as well as (d) incomplete validation data, (e.g. Rajan and Ishak, 2017). Moreover, there is no medium to long-term study that investigated all the four metal contaminants (As, Pb, Cd and total Hg) and chloramphenicol in *L. vannamei* and *P. silasi* sold at various supply chain stages (seafood landing sites, major markets and retail grocers) in Malaysia.

While it is important to ensure that the concentrations of metal contaminants (*viz.* As, Pb, Cd and total Hg) in *L. vannamei* and *P. silasi* comply fully with the prescribed MPPs, it is also paramount to perform its health risk assessment (target hazard quotient (THQ) and hazard index (HI)). Such evaluations are important because the long-term consumption of metal contaminants (even at low concentrations) can cause detrimental effects on human (Rabiul Islam et al., 2017). The effects include disruption of numerous physiological and biochemical processes, resulting in various health problems including thalassemia, dermatitis, brain and kidney damages or worse, cancer (WHO, 2021). According to WHO (2022), eating food contaminated with germs, viruses, parasites, or chemical compounds such as heavy metals causes over 200 different diseases. These diseases considerably contribute to the worldwide disease and mortality burden. Every year, about one in every ten people worldwide become ill as a result of tainted food, resulting in over 420 000 fatalities. Hence, this study was aimed at determining the amounts of metal contaminants as prescribed by the Malaysian law (i.e. As, Pb, Cd and total Hg) and chloramphenicol in *L. vannamei* and *P. silasi* obtained from various supply chain stages (*viz.* seafood landing sites, major markets and retail grocers) in Johor Bahru, Malaysia. Additionally, this research too was aimed at investigating its health risk assessment which may prove beneficial for the relevant health authorities in developing and implementing appropriate intervention strategies for the community as a whole. Figure 1.1 represents the conceptual framework of this research study.

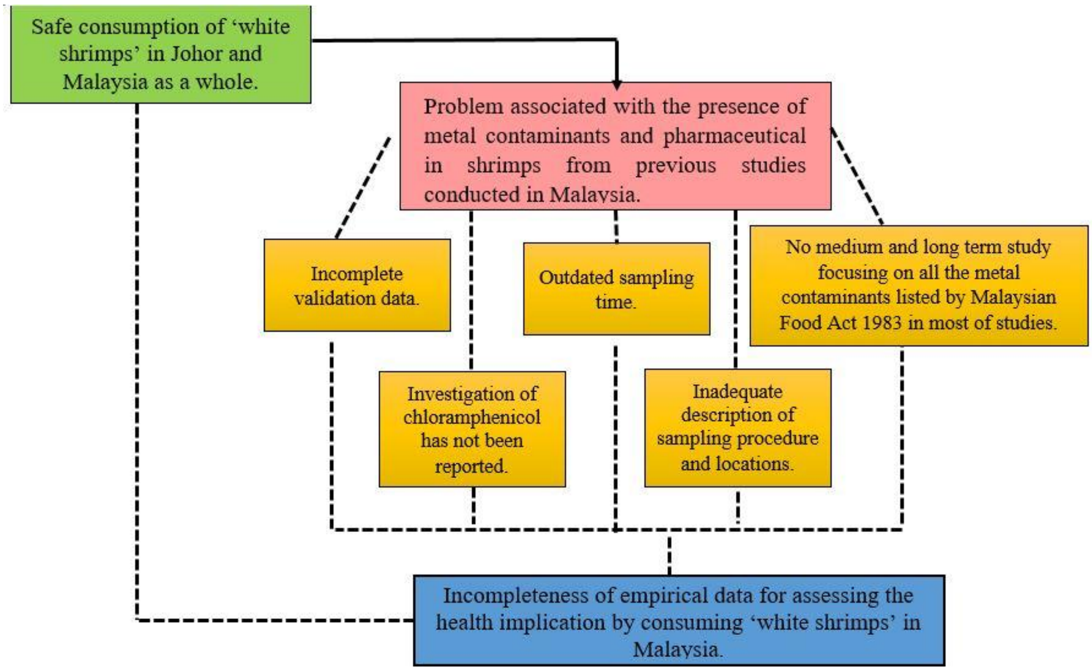

Figure 1.1 Conceptual framework of this research study.

1.3 Objectives and Hypothesis

The objectives of this present study were:

1. To compare the concentrations of As, Pb, Cd, total Hg and chloramphenicol in 'white shrimps' sampled from seafood landing sites with those of major markets and their surrounding retail grocers in Johor Bahru.
2. To evaluate the health risk assessment associated with the long-term consumption of contaminated 'white shrimps' in Johor Bahru.

It was hypothesized that there were significant differences in the concentrations of metal contaminants and chloramphenicol in 'white shrimps' sampled from seafood landing sites with those of major markets and their surrounding retail grocers in Johor Bahru.

1.4 Scope of Study

The study involved stratified random sampling of ‘white shrimps’ sampled from three different stages of supply chain in Johor Bahru *viz.* the Pontian and Mersing seafood landing sites, major markets A and B, as well as surrounding retail grocers that were situated within the 2 km radius of major markets (A and B) over six sampling intervals. Using the validated GFAAS and flow injection mercury/hydride system (FI-MHS) as well as ELISA, quantitative analysis of As, Pb and Cd, as well as total Hg and chloramphenicol in ‘white shrimps’ samples were attempted. The concentrations of metal contaminants in ‘white shrimps’ samples at the different stages of supply chain were compared with the MPPs prescribed by the Food Act 1983 (Act 281) and Regulations (2021). The EDI, THQ, and HI were used to evaluate the health risk assessment for consuming this shrimp for human.

1.5 Significance of study

The findings reported here revealed the extent of contaminations of As, Cd, Pb, total Hg and chloramphenicol in ‘white shrimps’ sold at various stages of supply chain (*viz*. seafood landing sites, major markets and retail grocers) in Johor Bahru, in view of identifying the possible points of contamination. The fact that this study was the first ever attempt to evaluate the health risk assessment for consuming contaminated ‘white shrimps’ in Johor Bahru, the data may prove useful for the relevant health authority to construct suitable intervention remedies for benefiting the community. This traceability study for shrimps at various stages of supply chain in Johor is deemed useful for strengthening the overall safety and security of our seafood industry. Therefore, formulating suitable intervention programs and devising efficient removal technologies are paramount for benefiting the public at large.

REFERENCES

Abuquerque, F. E. A., Minervino, A. H. H., Miranda, M., Herrero-Latorre, C., Barrêto Júnior, R. A., Oliveira, F. L. C., Dias, S. R., Ortolani, E. L., and López-Alonso, M. (2020). 'Toxic and essential trace element concentrations in the freshwater shrimp *Macrobrachium amazonicum* in the Lower Amazon, Brazil'. *Journal of Food Composition and Analysis*, 86(June 2019), 103361. https://doi.org/10.1016/j.jfca.2019.103361

Ali, T. G., Abdul Keyon, A. S., and Mahat, N. A. (2021). 'Occurrence of heavy metals and their removal in *Perna viridis* mussels using chemical methods: a review'. *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-021-17343-3

Amlund, H., Sele, V., and Sloth, J. J. (2019). 'Arsenic exposure from seafood consumption'. In *Encyclopedia of Environmental Health* (2nd ed., Issue October 2017). Elsevier Inc. https://doi.org/10.1016/B978-0-12-409548-9.10929-7

Anandkumar, A., Nagarajan, R., Prabakaran, K., and Rajaram, R. (2017). 'Trace metal dynamics and risk assessment in the commercially important marine shrimp species collected from the Miri coast, Sarawak, East Malaysia'. *Regional Studies in Marine Science*, 16, 79–88. https://doi.org/10.1016/j.rsma.2017.08.007

Anual, Z. F., Maher, W., Krikowa, F., Hakim, L., Ahmad, N. I., and Foster, S. (2018). 'Mercury and risk assessment from consumption of crustaceans, cephalopods and fish from West Peninsular Malaysia'. *Microchemical Journal*, 140(April), 214–221. https://doi.org/10.1016/j.microc.2018.04.024

AOAC (2005). *Official method of Analysis*. 18th Edition. Washington DC: Association of Officiating Analytical Chemists.

Ashfaq, M., Nawaz Khan, K., Saif Ur Rehman, M., Mustafa, G., Faizan Nazar, M., Sun, Q., Iqbal, J., Mulla, S. I., and Yu, C. P. (2017). 'Ecological risk assessment of pharmaceuticals in the receiving environment of pharmaceutical wastewater in Pakistan'. *Ecotoxicology and Environmental Safety*, 136(October 2016), 31–39. https://doi.org/10.1016/j.ecoenv.2016.10.029

Aytekin, T., Kargın, D., Çoğun, H. Y., Temiz, Ö., Varkal, H. S., and Kargın, F. (2019). 'Accumulation and health risk assessment of heavy metals in tissues of the shrimp and fish species from the Yumurtalik coast of Iskenderun Gulf, Turkey'. *Heliyon*, 5(8). https://doi.org/10.1016/j.heliyon.2019.e02131

Azril Annuar. (2020). 'US food regulator bans Malaysian shrimp exporters over illegal antibiotics use'. *Malay Mail*, 1–5. https://malaysia.news.yahoo.com/us-food-regulator-bans-malaysian-014207116.html

Bahmani, K., Shahbazi, Y., and Nikousefat, Z. (2020). 'Monitoring and risk assessment of tetracycline residues in foods of animal origin'. *Food Science and Biotechnology*, 29(3), 441–448. https://doi.org/10.1007/s10068-019-00665-x

Baki, M. A., Hossain, M. M., Akter, J., Quraishi, S. B., Haque Shojib, M. F., Atique Ullah, A. K. M., and Khan, M. F. (2018). 'Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh'. *Ecotoxicology and Environmental Safety*, *159*(April), 153–163. https://doi.org/10.1016/j.ecoenv.2018.04.035

Batvari, B. P. D., Sivakumar, S., Shanthi, K., Lee, K. J., Oh, B. T., Krishnamoorthy, R., and Kamala-Kannan, S. (2016). 'Heavy metals accumulation in crab and shrimps from Pulicat lake, north Chennai coastal region, southeast coast of India'. *Toxicology and Industrial Health*, 32(1), 1–6. https://doi.org/10.1177/0748233713475500

Briffa, J., Sinagra, E., and Blundell, R. (2020). 'Heavy metal pollution in the environment and their toxicological effects on humans'. *Heliyon*, 6(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Briggs, Mathew, Funge-Smith, S., Subasnighe, R. P., and Phillips, M. (2005). *Shrimp trade , marketing and economics Market Value and Market Competition of Asia and The Pacific with Latin*. FAO. https:// www.fao.org/3/a0086e/A0086E09.htm

Briggs, Matthew, Funge-Smith, S., Subasinghe, R., and Phillips, M. (2004). 'Advantages and disadvantages of surgical approaches to petroclival lesions'. *Food and Agriculture Organization of The United Nations Regional Office For Asia and The Pacific*. https://doi.org/10.1016/j.wneu.2010.12.045

Britannica. (2021). *Shrimp*. Encyclopedia Britannica. https://www.britannica.com/animal/shrimp-crustacean

Candra, Y. A., Syaifullah, M., Irawan, B., Putranto, T. W. C., Hidayati, D., and Soegianto, A. (2019). 'Concentrations of metals in mantis shrimp *Harpiosquilla harpax*(de Haan, 1844) collected from the eastern region of Java Sea Indonesia, and potential risks to human health'. *Regional Studies in Marine Science*, 26, 100507. https://doi.org/10.1016/j.rsma.2019.100507

Cháfer-Pericás, C., Maquieira, Á., Puchades, R., Company, B., Miralles, J., and Moreno, A. (2010). 'Multiresidue determination of antibiotics in aquaculture fish samples by HPLC-MS/MS'. *Aquaculture Research*, *41*(9), 217–225. https://doi.org/10.1111/j.1365-2109.2010.02504.x

Chahid, A., Hilali, M., Benlhachimi, A., and Bouzid, T. (2014). 'Contents of cadmium, mercury and lead in fish from the Atlantic sea (Morocco) determined by atomic absorption spectrometry'. *Food Chemistry*, 147, 357–360. https://doi.org/10.1016/j.foodchem.2013.10.008

Chan, T. Y. (1998). 'Shrimps and Prawns'. In *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 2. Cephalopods, crustaceans, holothurians and sharks.* (pp. 687–1396).

Chen, D., Delmas, J. M., Hurtaud-Pessel, D., and Verdon, E. (2020). 'Development of a multi-class method to determine nitroimidazoles, nitrofurans, pharmacologically active dyes and chloramphenicol in aquaculture products by liquid chromatography-tandem mass spectrometry'. *Food Chemistry*, 311(November 2019), 125924. https://doi.org/10.1016/j.foodchem.2019.125924

Chen, H., Liu, S., Xu, X. R., Liu, S. S., Zhou, G. J., Sun, K. F., Zhao, J. L., and Ying, G. G. (2015). 'Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure'. *Marine Pollution Bulletin*, 90(1–2), 181–187. https://doi.org/10.1016/j.marpolbul.2014.10.053

Chen, S., Li, X., Sun, G., Zhang, Y., Su, J., and Ye, J. (2015). Heavy metal induced antibiotic resistance in bacterium LSJC7. International Journal of Molecular Sciences, 16(10), 23390–23404. https://doi.org/10.3390/ijms161023390

Dee, K. H., Abdullah, F., Md Nasir, S. N. A., Appalasamy, S., Mohd Ghazi, R., and Eh Rak, A. (2019). Health Risk Assessment of Heavy Metals from Smoked *Corbicula fluminea* Collected on Roadside Vendors at Kelantan, Malaysia. *BioMed Research International*, 2019. https://doi.org/10.1155/2019/9596810

Dehghani, M., Sharifian, S., Taherizadeh, M. R., and Nabavi, M. (2021). 'Tracing the heavy metals zinc, lead and nickel in banana shrimp (*Penaeus merguiensis*) from the Persian Gulf and human health risk assessment'. *Environmental Science and Pollution Research*, 28(29), 38817–38828. https://doi.org/10.1007/s11356-021-13063-w

Dinh, Q. T., Munoz, G., Vo Duy, S., Tien Do, D., Bayen, S., and Sauvé, S. (2020). 'Analysis of sulfonamides, fluoroquinolones, tetracyclines, triphenylmethane dyes and other veterinary drug residues in cultured and wild seafood sold in Montreal, Canada'. *Journal of Food Composition and Analysis*, 94(August). https://doi.org/10.1016/j.jfca.2020.103630

Diop, M., Howsam, M., Diop, C., Goossens, J. F., Diouf, A., and Amara, R. (2016). 'Assessment of trace element contamination and bioaccumulation in algae (*Ulva lactuca*), mussels (*Perna perna*), shrimp (*Penaeus kerathurus*), and fish (*Mugil cephalus*, Saratherondon melanotheron) along the Senegalese coast'. *Marine Pollution Bulletin*, 103(1–2), 339–343. https://doi.org/10.1016/j.marpolbul.2015.12.038

DOF. (2020). *Udang Ternakan Di Malaysia Selamat Dimakan*. Department of Fisheries Malaysia. https://www.dof.gov.my/index.php/pages/view/3779

Done, H. Y., and Halden, R. U. (2015). 'Reconnaissance of 47 antibiotics and associated microbial risks in seafood sold in the United States'. *Journal of Hazardous Materials*, 282, 10–17. https://doi.org/10.1016/j.jhazmat.2014.08.075

Elgammal, S. M., Khorshed, M. A., and Ismail, E. H. (2019). 'Determination of heavy metal content in whey protein samples from markets in Giza, Egypt, using inductively coupled plasma optical emission spectrometry and graphite furnace atomic absorption spectrometry: A probabilistic risk assessment study'. *Journal of Food Composition and Analysis*, 84(August). https://doi.org/10.1016/j.jfca.2019.103300

Emerenciano, M. G. C., Rombenso, A. N., Vieira, F. D. N., Martins, M. A., Coman, G. J., Truong, H. H., Noble, T. H., and Simon, C. J. (2022). 'Intensification of Penaeid Shrimp Culture: An Applied Review of Advances in Production Systems, Nutrition and Breeding'. *Animals*, 12(3). https://doi.org/10.3390/ani12030236

European Commission. (2006). COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs. In *Official Journal of the European Union* (Issue 364).

European Commission. (2021). *Penaeus silasi*. European Commission. https://fish-commercial-names.ec.europa.eu/fish-names/species_en?sn=27771

Ezemonye, L. I., Adebayo, P. O., Enuneku, A. A., Tongo, I., and Ogbomida, E. (2019). Potential health risk consequences of heavy metal concentrations in surface water, shrimp (*Macrobrachium macrobrachion*) and fish (*Brycinus longipinnis*) from Benin River, Nigeria. *Toxicology Reports*, 6(November 2018), 1–9. https://doi.org/10.1016/j.toxrep.2018.11.010

Seafood Health Facts (2016). *Making Smart choices Balancing the Benefits and Risks of Seafood Consumption*. National Integrated Food Safety Initiative of National Institute of Food and Agriculture. https://www.seafoodhealthfacts.org/seafood-choices/description-top-commercial-seafood-items/shrimp

FAO (2004). *Introductions and movement of Penaeus vannamei and Penaeus stylirostris in Asia and the Pacific*. Food and Agriculture Organization. http://www.fao.org/3/ad505e/ad505e04.htm

FAO (2020). *Shrimp Market Reports*. Food and Agriculture Organization. https://www.fao.org/in-action/globefish/market-reports/shrimp/en/

FAO (2022). *Penaeus vannamei*. Food and Agriculture Organization. https://www.fao.org/fishery/en/culturedspecies/Penaeus_vannamei/en

Fatema, K., Naher, K., Tr, C., Ma, I., Tamim, U., Sm, H., Sma, I., and Ali, M. P. (2015). *Determination of Toxic Metal Accumulation in Shrimps by Atomic*. 2(3). https://doi.org/10.4172/2380-2391.

Ghee-Thean, L., Islam, G. M. N., and Ismail, M. M. (2016). 'Malaysian white shrimp (*P. vannamei*) aquaculture: An application of stochastic frontier analysis on technical efficiency'. *International Food Research Journal*, 23(2), 638–645.

Ghimire, S., Flury, M., Scheenstra, E. J., and Miles, C. A. (2019). 'Toxicity of the Non-Steroidal Anti-inflammatory Drugs (NSAIDs) acetylsalicylic acid, paracetamol, diclofenac, ibuprofen and naproxen towards freshwater invertebrates: a review'. *Science of the Total Environment*, 135577. https://doi.org/10.1016/j.scitotenv.2019.135577

Gillett, R. D., and Food and Agriculture Organization of the United Nations. (2008). *Global study of shrimp fisheries*. Rome: Food and Agriculture Organization of the United Nations.

Giri, S., and Singh, A. K. (2014). 'Assessment of human health risk for heavy metals in fish and shrimp collected from Subarnarekha river, India'. *International Journal of Environmental Health Research*, 24(5), 429–449. https://doi.org/10.1080/09603123.2013.857391

Griboff, J., Carrizo, J. C., Bonansea, R. I., Valdés, M. E., Wunderlin, D. A., and Amé, M. V. (2020). 'Multiantibiotic residues in commercial fish from Argentina. The presence of mixtures of antibiotics in edible fish, a challenge to health risk assessment'. *Food Chemistry*, 332(June), 127380.

https://doi.org/10.1016/j.foodchem.2020.127380

Gunalan B, Nina Tabitha S., S. P. and T. A. (2013). 'Nutritive value of cultured white leg shrimp *Litopenaeus vannamei*'. *International Journal of Fisheries and Aquaculture*, 5(7), 166–171. https://doi.org/10.5897/IJFA2013.0333

Habte, G., Choi, J. Y., Nho, E. Y., Oh, S. Y., Khan, N., Choi, H., Park, K. S., and Kim, K. S. (2015). 'Determination of toxic heavy metal levels in commonly consumed species of shrimp and shellfish using ICP-MS/OES'. *Food Science and Biotechnology*, 24(1), 373–378. https://doi.org/10.1007/s10068-015-0049-4

Hidayati, N. V., Prudent, P., Asia, L., Vassalo, L., Torre, F., Widowati, I., Sabdono, A., Syakti, A. D., and Doumenq, P. (2020). 'Assessment of the ecological and human health risks from metals in shrimp aquaculture environments in Central Java, Indonesia'. *Environmental Science and Pollution Research*, 27(33), 41668–41687. https://doi.org/10.1007/s11356-020-09967-8

Ismail, Z., Nabil, M., and Shabani, R. (2016). 'Detection of Furazolidone Metabolites in Cultured Shrimps in Penang Market'. *Australian Journal of Basic and Applied Sciences*, November, 1–6.

Joint FAO/WHO Expert Committee on Food Additives, JECFA (1999) Reports of the 53rd meeting of the joint FAO/WHO expert committee on food additives (JECFA). In: JECFA/53/TRS. Rome, Italy

Joint FAO/WHO Expert Committee on Food Additives, JECFA (2003): Summary and conclusions of the 61st meeting of the joint FAO/ WHO expert committee on food additives (JECFA). JECFA/61/ SC;

Jeyaletchumi, P., Hekambaram, M., Azhar, A., Param, J. S. P. S., Ghani, A. A. S., Harliani, R., and Rafidah, I. (2019). 'Safety of frozen shrimp imported through entry points in Selangor, Malaysia'. *International Journal of Innovative Technology and Exploring Engineering*, 8(11 Special issue 2), 246–248. https://doi.org/10.35940/ijitee.K1038.09811S219

Jiang, D., Du, X., Liu, Q., Hao, N., and Wang, K. (2019). 'MoS 2 /nitrogen doped graphene hydrogels p-n heterojunction: Efficient charge transfer property for highly sensitive and selective photoelectrochemical analysis of chloramphenicol'. *Biosensors and Bioelectronics*, 126(November 2018), 463–469. https://doi.org/10.1016/j.bios.2018.11.018

Jović, M., and Stanković, S. (2014). 'Human exposure to trace metals and possible public health risks via consumption of mussels Mytilus galloprovincialis from the Adriatic coastal area'. In FAO (Ed.), *Food and Chemical Toxicology*, 70(2014). FAO. https://doi.org/10.1016/j.fct.2014.05.012

Kamal, T., Tanoli, M., Mumtaz, M., Ali, N., and Ayub, S. (2015). 'Bioconcentration Potential Studies of Heavy Metals in *Fenneropenaeus penicillatus* (Jaira or Red Tail Shrimp) along the Littoral States of Karachi City'. *Journal of Basic & Applied Sciences*, 11, 611–618. https://doi.org/10.6000/1927-5129.2015.11.82

Karageorgou, E., Christoforidou, S., Ioannidou, M., Psomas, E., and Samouris, G. (2018). 'Detection of β-lactams and chloramphenicol residues in raw milk — Development and application of an HPLC-DAD method in comparison with microbial inhibition assays'. *Foods*, 7(6), 1–12.

https://doi.org/10.3390/foods7060082

Kaya, G., and Turkoglu, S. (2017). 'Bioaccumulation of Heavy Metals in Various Tissues of Some Fish Species and Green Tiger Shrimp (*Penaeus semisulcatus*) from İskenderun Bay, Turkey, and Risk Assessment for Human Health'. *Biological Trace Element Research*, 180(2), 314–326. https://doi.org/10.1007/s12011-017-0996-0

Knapp, C.W., McCluskey, S.M., Singh, B.K., Campbell, C.D., Hudson, G., and Graham, D.W (2011). Antibiotic resistance gene abundances correlate with metal and geochemical conditions in archived Scottish soils. *PLoS ONE*, 6, e27300.

Lee, W. P., Payus, C., Ali, S. A. M., and Vun, L. W. (2017). 'Selected Heavy Metals in *Penaeus vannamei* (White Prawn) in Aquaculture Pond near Likas Lagoon, Sabah, Malaysia'. *International Journal of Environmental Science and Development*, 8(7), 530–533. https://doi.org/10.18178/ijesd.2017.8.7.1010

Li, H., Yang, L., He, L., Ma, Y., Yan, X., Wu, L., and Zhang, Z. (2020). 'Kinetics and mechanisms of chloramphenicol degradation in aqueous solutions using heat-assisted nZVI activation of persulfate'. *Journal of Molecular Liquids*, 313, 113511. https://doi.org/10.1016/j.molliq.2020.113511

Li, J. X., Sun, C. J., Zheng, L., Jiang, F. H., Yin, X. F., Chen, J. H., and Wang, X. R. (2016). 'Determination of Lead Species in Algae by Capillary Electrophoresis-Inductively Coupled Plasma-Mass Spectrometry'. *Chinese Journal of Analytical Chemistry*, 44(11), 1659–1664. https://doi.org/10.1016/S1872-2040(16)60970-2

Liao, I. C., and Chien, Y. The Pacific White Shrimp, *Litopenaeus vannamei*, in Asia: The World’s Most Widely Cultured Alien Crustacean. In: Bella S. Galil, Paul F. Clark, James T. Carlton. *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts*. Springer Dordrecht. 489-513; (2011)

Liu, C., Deng, D., Xu, D., Wu, K., Yang, H., Zhao, K., Li, J., and Deng, A. (2019). 'Development of a monoclonal antibody based-ELISA for the detection of chloramphenicol in shrimp, feed and milk samples and validation by LC-MS/MS coupled with immunoaffinity clean-up'. *Analytical Methods*, 11(4), 507–516. https://doi.org/10.1039/c8ay02284d

Liu, Q., Xu, X., Zeng, J., Shi, X., Liao, Y., Du, P., Tang, Y., Huang, W., Chen, Q., and Shou, L. (2019). 'Heavy metal concentrations in commercial marine organisms from Xiangshan Bay, China, and the potential health risks'. *Marine Pollution Bulletin*, 141(36), 215–226. https://doi.org/10.1016/j.marpolbul.2019.02.058

Liu, X., Cao, Z., Yuan, Z., Zhang, J., Guo, X., Yang, Y., He, F., Zhao, Y., and Xu, J. (2018). 'Insight into the kinetics and mechanism of removal of aqueous chlorinated nitroaromatic antibiotic chloramphenicol by nanoscale zero-valent iron'. *Chemical Engineering Journal*, 334(June 2017), 508–518. https://doi.org/10.1016/j.cej.2017.10.060

Mahat, N. A., Muktar, N. K., Ismail, R., Abdul Razak, F. I., Abdul Wahab, R., and Abdul Keyon, A. S. (2018). 'Toxic metals in *Perna viridis* mussel and surface seawater in Pasir Gudang coastal area, Malaysia, and its health implications'. *Environmental Science and Pollution Research*, 25(30), 30224–30235. https://doi.org/10.1007/s11356-018-3033-8

Man, C. N., Gam, L. H., Ismail, S., Lajis, R., and Awang, R. (2006). 'Simple, rapid and sensitive assay method for simultaneous quantification of urinary nicotine and cotinine using gas chromatography-mass spectrometry'. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 844(2), 322–327. https://doi.org/10.1016/j.jchromb.2006.07.029

Malaysia Food act 1983 (Act 281) and regulations (2021). Petaling Jaya: International law book services.

McCallum, E. S., Sundelin, A., Fick, J., Alanärä, A., Klaminder, J., Hellström, G., and Brodin, T. (2019). 'Investigating tissue bioconcentration and the behavioural effects of two pharmaceutical pollutants on sea trout (*Salmo trutta*) in the laboratory and field'. *Aquatic Toxicology*, 207(August 2018), 170–178. https://doi.org/10.1016/j.aquatox.2018.11.028

Md Yunus, S., Hamzah, Z., Ariffin, N. A. N., and Muslim, M. B. (2014). 'Cadmium, chromium, copper, lead, ferum and zinc levels in the cockles (*Anadara granosa*) from Kuala Selangor, Malaysia'. *Malaysian Journal of Analytical Sciences*, 18(3), 514–521.

Mottaleb, M. A., Stowe, C., Johnson, D. R., Meziani, M. J., and Mottaleb, M. A. (2016). 'Pharmaceuticals in grocery market fish fillets by gas chromatography-mass spectrometry'. *Food Chemistry*, 190, 529–536. https://doi.org/10.1016/j.foodchem.2015.06.003

Muthu, M. ., and Motoh, H. (1979). *A new species of penaeus from North Borneo (4).*

Ngah, C. W. Z. C. W., and Yahya, M. A. (2012). 'Optimisation of digestion method for determination of arsenic in shrimp paste sample using atomic absorption spectrometry'. *Food Chemistry*, *134*(4), 2406–2410. https://doi.org/10.1016/j.foodchem.2012.04.032

Okpala, C. O. R., Choo, W. S., and Dykes, G. A. (2014). 'Quality and shelf life assessment of Pacific white shrimp (*Litopenaeus vannamei*) freshly harvested and stored on ice'. *LWT - Food Science and Technology*, 55(1), 110–116. https://doi.org/10.1016/j.lwt.2013.07.020

Olmedo, P., Pla, A., Hernández, A. F., Barbier, F., Ayouni, L., and Gil, F. (2013). 'Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers'. *Environment International*, 59, 63–72. https://doi.org/10.1016/j.envint.2013.05.005

Omobepade, B. P., Akinsorotan, A. M., Ajibare, A. O., Ogunbusola, E. M., Ariyomo, T. O., Jimoh, J. O., Odeyemi, K. M., Okeke, O. S., Falabake, M. A., Adeniji, S. M., and Adedapo, A. M. (2020). 'Heavy Metal Concentration in White Shrimp *Nematopalaemon hastatus* and their Associated Ecological and Health Risk in the Nigerian Continental Shelf Bayode'. *Journal of Membrane Science and Research*, 24(2), 301–316. http://www.msrjournal.com/article_36914.html

Pilehvar, S., Dardenne, F., Blust, R., and De Wael, K. (2012). 'Electrochemical sensing of phenicol antibiotics at gold'. *International Journal of Electrochemical Science*, 7(6), 5000–5011.

Pires, D. R., de Morais, A. C. N., Coelho, C. C. S., Marinho, A. F., Góes, L. C. D. S. A., Augusta, I. M., Ferreira, F. S., and Saldanha, T. (2018). 'Nutritional composition, fatty acids and cholesterol levels in Atlantic white shrimp (*Litopenaeus schimitti*)'. *International Food Research Journal*, 25(1), 151–157.

Plichta, S. B., and Kelvin, E. (2013). *Munro's Statistical Methods for Health Care Research*. In Angewandte Chemie International Edition, 6(11), 951–952. (Vol. 13, Issue April).

Rabiul Islam, G. M., Habib, M. R., Waid, J. L., Rahman, M. S., Kabir, J., Akter, S., and Jolly, Y. N. (2017). 'Heavy metal contamination of freshwater prawn (*Macrobrachium rosenbergii)* and prawn feed in Bangladesh: A market-based study to highlight probable health risks'. *Chemosphere*, 170, 282–289. https://doi.org/10.1016/j.chemosphere.2016.11.163

Raissy, M. (2016). 'Assessment of health risk from heavy metal contamination of shellfish from the Persian Gulf'. *Environmental Monitoring and Assessment*, 188(1), 1–7. https://doi.org/10.1007/s10661-015-5071-8

Rajan, S., and Ishak, N. S. (2017). 'Estimation of target hazard quotients and potential health risks for metals by consumption of shrimp *(Litopenaeus vannamei*) in Selangor, Malaysia'. *Sains Malaysiana*, 46(10), 1825–1830. https://doi.org/10.17576/jsm-2017-4610-20

Rodrigues, J., Albino, S., Silva, S., Cravo, A., Cardoso, V. V., Benoliel, M. J., and Almeida, C. M. M. (2019). 'Development of a Multiresidue Method for the Determination of 24 Pharmaceuticals in Clams by QuEChERS and Liquid Chromatography-Triple Quadrupole Tandem Mass Spectrometry'. *Food Analytical Methods*.

Sarwer, M. G., Rony, M. M. H., Sharmin, Ms. S., Chowdhury, A. K. J., and Bhowmik, S. (2017). 'ELISA validation and determination of cut-off level for chloramphenicol (CAP) residues in shrimp and fish'. *Our Nature*, 15(1–2), 13–18. https://doi.org/10.3126/on.v15i1-2.18789

SEAFDEC. (2005). Regional Technical Consultation on the Aquaculture of *Penaeus vannamei* and Other Exotic Shrimps in Southeast Asia. (Issue March). https://repository.seafdec.org.ph/bitstream/handle/10862/1717/RTC-P.vannamei.pdf?sequence=1&isAllowed=y

Siddiqui, M. R., AlOthman, Z. A., and Rahman, N. (2017). 'Analytical techniques in pharmaceutical analysis: A review'. *Arabian Journal of Chemistry*, *10*, S1409–S1421. https://doi.org/10.1016/j.arabjc.2013.04.016

Singh, A., Narayan, S., Datta, and Ansal, M. D. (2017). 'Biometric characteristics of pacific white shrimp, *Litopenaeus vannamei* (boone, 1931) cultured in the salt affected area of district Fazilka (Punjab), India'. *Indian Journal of Ecology*, *44*(3), 628–631.

Soltani, N., Moore, F., Keshavarzi, B., Sorooshian, A., and Javid, R. (2019). 'Potentially toxic elements (PTEs) and polycyclic aromatic hydrocarbons (PAHs) in fish and prawn in the Persian Gulf, Iran'. *Ecotoxicology and Environmental Safety*, 173(January), 251–265. https://doi.org/10.1016/j.ecoenv.2019.02.005

Song, B., Lei, M., Chen, T., Zheng, Y., Xie, Y., Li, X., and Gao, D. (2009). 'Assessing the health risk of heavy metals in vegetables to the general population in Beijing, China'. *Journal of Environmental Sciences*, 21(12), 1702–1709.

https://doi.org/10.1016/S1001-0742(08)62476-6

Sriket, P., Benjakul, S., Visessanguan, W., and Kijroongrojana, K. (2007). 'Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (Penaeus vannamei) meats'. *Food Chemistry*, 103(4), 1199–1207. https://doi.org/10.1016/j.foodchem.2006.10.039

Susakate, S., Poapolathep, S., Chokejaroenrat, C., Tanhan, P., Hajslova, J., Giorgi, M., Saimek, K., Zhang, Z., and Poapolathep, A. (2019). 'Multiclass analysis of antimicrobial drugs in shrimp muscle by ultra high performance liquid chromatography-tandem mass spectrometry'. *Journal of Food and Drug Analysis*, 27(1), 118–134. https://doi.org/10.1016/j.jfda.2018.06.003

Suseno, H., Hudiyono, S., and Muslim, M. (2016). 'Elimination of Chloramphenicol by Tiger Shrimp (*Penaeus monodon*) and White Shrimp (*Litopenaeus vannamei*)'. *HAYATI Journal of Biosciences*, *23*(3), 117–120. https://doi.org/10.1016/j.hjb.2016.07.001

Swapna, K. M., Rajesh, R., and Lakshmanan, P. T. (2012). 'Incidence of antibiotic residues in farmed shrimps from the southern states of India'. *Indian Journal of Marine Sciences*, *41*(4), 344–347.

Tajik, H., Malekinejad, H., Razavi-Rouhani, S. M., Pajouhi, M. R., Mahmoudi, R., and Haghnazari, A. (2010). 'Chloramphenicol residues in chicken liver, kidney and muscle: A comparison among the antibacterial residues monitoring methods of Four Plate Test, ELISA and HPLC'. *Food and Chemical Toxicology*, 48(8–9), 2464–2468. https://doi.org/10.1016/j.fct.2010.06.014

Timchenko, Y. V. (2021). 'Advantages and Disadvantages of High-Performance Liquid Chromatography (HPCL)'. *Journal of Environmental Analytical Chemistry*, *8*, 2391.

Tiwari, G., and Tiwari, R. (2010). 'Bioanalytical method validation: An updated review'. *Pharmaceutical Methods*, 1(1), 25. https://doi.org/10.4103/2229-4708.72226

Trombini, C., Hampel, M., and Blasco, J. (2019). 'Assessing the effect of human pharmaceuticals (carbamazepine, diclofenac and ibuprofen) on the marine clam Ruditapes philippinarum: An integrative and multibiomarker approach'. *Aquatic Toxicology*, 208(December 2018), 146–156. https://doi.org/10.1016/j.aquatox.2019.01.004

Tsai, M. Y., Lin, C. F., Yang, W. C., Lin, C. T., Hung, K. H., and Chang, G. R. (2019). 'Health risk assessment of banned veterinary drugs and quinolone residues in shrimp through liquid chromatography-tandem mass spectrometry'. *Applied Sciences (Switzerland)*, 9(12), 1–11. https://doi.org/10.3390/app9122463

Uchida, K., Konishi, Y., Harada, K., Okihashi, M., Yamaguchi, T., Do, M. H. N., Thi Bui, L., Duc Nguyen, T., Do Nguyen, P., Thi Khong, D., Thi Tran, H., Nam Nguyen, T., Viet Le, H., Van Chau, V., Thi Van Dao, K., Thi Ngoc Nguyen, H., Kajimura, K., Kumeda, Y., Tran Pham, K., … Yamamoto, Y. (2016). 'Monitoring of Antibiotic Residues in Aquatic Products in Urban and Rural Areas of Vietnam'. *Journal of Agricultural and Food Chemistry*, 64(31), 6133–6138.

https://doi.org/10.1021/acs.jafc.6b00091

Ullah, A. K. M. A., Maksud, M. A., Khan, S. R., Lutfa, L. N., and Quraishi, S. B. (2017a). 'Development and validation of a GF-AAS method and its application for the trace level determination of Pb, Cd, and Cr in fish feed samples commonly used in the hatcheries of Bangladesh'. *Journal of Analytical Science and Technology*, 8(1). https://doi.org/10.1186/s40543-017-0124-y

Ullah, A. K. M. A., Maksud, M. A., Khan, S. R., Lutfa, L. N., and Quraishi, S. B. (2017). 'Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh'. *Toxicology Reports*, *4(O*ctober), 574–579. https://doi.org/10.1016/j.toxrep.2017.10.002

Urtado, P. I. P. E., Obbins, L. A. L. D., Rooks, B. R. W. B., and Hambliss, C. K. E. C. (2009). 'Pharmaceuticals and Personal Care Products in the Environment: Occurrence of Pharmaceuticals and Personal Care Products in Fish: Results of A National Pilot Study in The United States'. *Environmental Toxicology and Chemistry*, 28(12), 2587–2597.

US EPA (2010). *Calibration Curves : Program Use / Needs.* US Environmental Protection Agency. https://19january2017snapshot.epa.gov/sites/production/files/2014-05/documents/calibration-guide-ref-final-oct2010.pdf

US EPA (2020). *Regional Screening Levels (RSLs) - User ' s Guide Regional Screening Levels (RSLs)*. US Environmental Protection Agency. https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide#special

US EPA (2011) *Risk-based concentration table*. US Environmental Protection Agency, Washington

US EPA (2004). Integrated Risk Information System: Lead. *United States Environmental Protection Agency (EPA)*, 1–15.

US FDA (2018). *Bioanalytical method validation Guidance for Industry* (Issue May).

Vandermeersch, G., Lourenço, H. M., Alvarez-Muñoz, D., Cunha, S., Diogène, J., Cano-Sancho, G., Sloth, J. J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J. O., Marques, A., and Robbens, J. (2015). 'Environmental contaminants of emerging concern in seafood - European database on contaminant levels'. *Environmental Research*, 143, 29–45. https://doi.org/10.1016/j.envres.2015.06.011

Wang, S. L., Xu, X. R., Sun, Y. X., Liu, J. L., and Li, H. Bin. (2013). 'Heavy metal pollution in coastal areas of South China: A review'. *Marine Pollution Bulletin*, 76(1–2), 7–15. https://doi.org/10.1016/j.marpolbul.2013.08.025

Wang, X. G., Su, F. Z., Zhang, J. J., Cheng, F., Hu, W. Q., and Ding, Z. (2019). 'Construction land sprawl and reclamation in the Johor River Estuary of Malaysia since 1973'. *Ocean and Coastal Management*, 171(August 2018), 87–95. https://doi.org/10.1016/j.ocecoaman.2019.01.006

Won, S. Y., Lee, C. H., Chang, H. S., Kim, S. O., Lee, S. H., and Kim, D. S. (2011).'Monitoring of 14 sulfonamide antibiotic residues in marine products using HPLC-PDA and LC-MS/MS'. *Food Control*, 22(7), 1101–1107. https://doi.org/10.1016/j.foodcont.2011.01.005

WHO (2021). *Lead poisoning*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

WHO (2022). *Foodborne Diseases*. World Health Organization. https://www.who.int/health-topics/foodborne-diseases#tab=tab_1

Xie, H., Hao, H., Xu, N., Liang, X., Gao, D., Xu, Y., Gao, Y., Tao, H., and Wong, M. (2019). 'Pharmaceuticals and personal care products in water, sediments, aquatic organisms, and fish feeds in the Pearl River Delta: Occurrence, distribution, potential sources, and health risk assessment'. *Science of the Total Environment*, 659, 230–239. https://doi.org/10.1016/j.scitotenv.2018.12.222

Yan, J., Su, F., and Wang, M. (2017). 'The development processes and regional differentiation of both banks of the Strait of Malacca during 1980–2010'. *Ocean and Coastal Management*, 139, 141–152. https://doi.org/10.1016/j.ocecoaman.2017.02.012

Yan, M., Wang, W., Huang, X., Wang, X., and Wang, Y. (2020). 'Interactive effects of dietary cholesterol and phospholipids on the growth performance, expression of immune-related genes and resistance against Vibrio alginolyticus in white shrimp (*Litopenaeus vannamei*)'. *Fish and Shellfish Immunology*, 97(November 2019), 100–107. https://doi.org/10.1016/j.fsi.2019.11.048

Yang, J., Ji, G., Gao, Y., Fu, W., Irfan, M., Mu, L., Zhang, Y., and Li, A. (2020). 'High-yield and high-performance porous biochar produced from pyrolysis of peanut shell with low-dose ammonium polyphosphate for chloramphenicol adsorption'. *Journal of Cleaner Production*, 264, 121516. https://doi.org/10.1016/j.jclepro.2020.121516

Yap, C. kong, Cheng, W. H., Ali, M. H., Nulit, R., Peng, S. H. T., Ismail, M. S., and Leow, C. S. (2019a). 'Health Risk Assessment of Heavy Metals in Prawn *Penaeus merguiensis* Collected in 2007 from Sri Serdang Market, Peninsular Malaysia'. *Acta Scientifci Nutritional Health*, 3(8), 109–113. https://doi.org/10.31080/asnh.2019.03.0376

Yap, C. kong, Cheng, W. H., Ali, M. H., Nulit, R., Peng, S. H. T., Ismail, M. S., and Leow, C. S. (2019b). 'Heath Risk Assessment of Heavy Metals in Shrimp *Acetes* sp. and Cincalok Collected in 2007 from Pantai Klebang, Peninsular Malaysia'. *Journal of Toxicology and Risk Assessment*, 5(2), 29–31. https://doi.org/10.23937/2572-4061.1510028

Yu, B., Wang, X., Dong, K. F., Xiao, G., and Ma, D. (2020). 'Heavy metal concentrations in aquatic organisms (fishes, shrimp and crabs) and health risk assessment in China'. *Marine Pollution Bulletin*, 159(December 2019). https://doi.org/10.1016/j.marpolbul.2020.111505

Zhang, C., Yu, K., Li, F., and Xiang, J. (2017). 'Acute toxic effects of zinc and mercury on survival, standard metabolism, and metal accumulation in juvenile ridgetail white prawn, Exopalaemon carinicauda'. *Ecotoxicology and Environmental Safety*, 145(August), 549–556. https://doi.org/10.1016/j.ecoenv.2017.07.075

Zhang, J., Zhao, R., Cao, L., Lei, Y., Liu, J., Feng, J., Fu, W., Li, X., and Li, B. (2020). 'High-efficiency biodegradation of chloramphenicol by enriched bacterial consortia: Kinetics study and bacterial community characterization'. *Journal of Hazardous Materials*, 384(March 2019), 121344. https://doi.org/10.1016/j.jhazmat.2019.121344

Zhang, R., Pei, J., Zhang, R., Wang, S., Zeng, W., Huang, D., Wang, Y., Zhang, Y., Wang, Y., and Yu, K. (2018). 'Occurrence and distribution of antibiotics in mariculture farms, estuaries and the coast of the Beibu Gulf, China: Bioconcentration and diet safety of seafood'. *Ecotoxicology and Environmental Safety*, 154(November 2017), 27–35. https://doi.org/10.1016/j.ecoenv.2018.02.006

Zhong, W. S., Ren, T., and Zhao, L. J. (2016).'Determination of Pb (Lead), Cd (Cadmium), Cr (Chromium), Cu (Copper), and Ni (Nickel) in Chinese tea with high-resolution continuum source graphite furnace atomic absorption spectrometry'. *Journal of Food and Drug Analysis*, 24(1), 46–55. https://doi.org/10.1016/j.jfda.2015.04.010

Zhu, M., Long, X., and Wu, S. (2018). 'Effects of dietary trehalose on the growth performance and nonspecific immunity of white shrimps (*Litopenaeus vannamei*)'. *Fish and Shellfish Immunology*, 78(February), 127–130. https://doi.org/10.1016/j.fsi.2018.04.035

LIST OF PUBLICATION

Conference Proceeding

1. Anuar, N., Mahat, NA., and Muhammad, II. (2022). Metal Contaminants in Litopenaeus Vannamei and Penaeus Silasi at Various Supply Chain Stages in Johor Bahru, Malaysia and Their Health Risk Assessment. *Proceedings Science and Mathematics* 6, 1–5.

ISI Journal Article (Under review)

1. Anuar, N., Mahat, NA., Muhammad, II., Keyon, ASA., Huri, MAM., Yunus, NA., Zaidel, DNA., Mutalib, NAA., Radu, S., and Azman, AR. Metal contaminants and chloramphenicol in Litopenaeus vannamei and Penaeus silasi at various supply chain stages in Johor Bahru, Malaysia and their health risk assessment. Submitted to *Journal of King Saud University-Science* (WOS: Q2).

Other publications

1. Ting, C. W., Mahat, N. A., Azman, A. R., Muda, N. W., and Anuar, N. (2021). Performance of the Nanobio-Based Reagent for Visualising Wet Fingerprints Exposed to Different Levels of Water Salinity. *Journal of Clinical and Health Sciences*, 6(1(Special)), 32. https://doi.org/10.24191/jchs.v6i1(special).13169