HEAVY METALS IN WATER, SEDIMENT AND WHITELEG SHRIMP *(Litopenaeus vannamei)* FROM AQUACULTURE PONDS IN LIKAS, SABAH

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DECLARATION

I hereby declare that the materials in this thesis are my own except for the quotations, equations and a summary that sources of each work was mentioned in the text.

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ABSTRACT

The issue of heavy metal toxicity has received considerable attention in aquaculture field. Most studies of heavy metal in aquaculture have only focused on fish species and molluscs but not shrimp. Thus, the key research objective of this study is to identify and study selected heavy metals in water, sediment and *Litopenaeus vannamei* (Whiteleg Shrimp) in the study area. In this study, the water samples were filtered and preserved with acid while for the mature shrimps (5 months old) and sediment were collected and dried. Sample of 1g (shrimp and sediment) and 1 ml (water) were digested using H_2SO_4 and HNO_3 for 2 hours and filtered. The heavy metal concentration in water, sediment and shrimp samples was determined using ICP-OES. The water sample multivariate statistical analysis showed that primary contributing heavy metals are Cu, Fe, Mn and Zn, followed by Cd and Pb and least contributing heavy metals is Cr and Ni in water. Heavy metals in sediments shows the abundant heavy metals is Fe, Cr, Mn and Zn while the least abundant is Pb and Cd. The present research shows the ranking profile of heavy metals concentration is Fe> Cu> Zn> Cr> Mn> As> Ni> Pb> Cd. Most of the heavy metals are accumulated in the head of the shrimp whereas the least is in the shell of shrimp. In this research, all BCFp-w values are classified under low potential. It was found the BCFp-s values for Cu for shrimp head (BCFp-s=26.84), shrimp flesh (BCFp-s= 3.36) and shrimp shell (BCFp-s=18.11) are highest. This indicates that the shrimp contained more metals than sediment. The BCFp-s values for Cd, Cr, Fe, Mn, Ni and Pb were lower than 1.0, which means limited ability of these heavy metals to be accumulated by the shrimp. In this research, it is shown that bioaccumulation of heavy metals in shrimp and water are negative relationship while it shows positive relationship between heavy metals concentration in water quality and sediment. Bioaccumulation of heavy metals in shrimp and sediment shows similar trend which is negative relationship.

ABSTRAK

LOGAM BERAT DALAM AIR, SEDIMEN DAN UDANG PUTIH (*Litopenaeus vannamei*) DI KOLAM TERNAKAN AKUAKULTUR, LIKAS, SABAH

Isu ketoksikan logam berat telah banyak mendapat perhatian dalam bidang akuakultur. Kebanyakan kajian logam berat di akuakultur hanya memberi tumpuan kepada spesies ikan dan moluska tetapi bukan udang. Oleh itu, objektif penyelidikan utama kajian ini adalah untuk mengenal pasti dan mengkaji logam berat terpilih dalam air, sedimen dan Litopenaeus vannamei (Udang Putih) di kawasan kajian. Dalam kajian ini, sampel air ditapis dan dipelihara dengan asid manakala untuk udang yang matang (5 bulan) dan sedimen dikumpulkan dan dikeringkan. Sampel 1g (udang dan sedimen) dan 1 ml (air) dicerna menggunakan asid sulfur (H_2SO_4) dan asid nitrik (HNO_3) selama 2 jam dan ditapis. Kepekatan logam berat di dalam sampel air, sedimen dan udang telah ditentukan menggunakan ICP-OES. Analisis statistik multivariat sampel air menunjukkan bahawa penyumbang logam berat yang utama adalah Cu, Fe, Mn dan Zn, diikuti oleh Cd dan Pb dan paling kurang menyumbang logam berat adalah Cr dan Ni dalam air. Kepekatan logam berat di dalam sedimen menunjukkan bahawa Fe, Cr, Mn dan Zn adalah paling tinggi manakala paling rendah adalah Pb dan Cd. Kajian ini menunjukkan profil kepekatan logam berat ialah Fe> Cu> Zn> Cr> Mn> As> Ni> Pb> Cd. Kebanyakan logam berat dikumpul di kepala udang sedangkan yang paling kecil adalah dalam kulit udang. Dalam kajian ini, semua nilai BCFp-w diklasifikasikan dalam potensi rendah. Didapati nilai BCFp untuk Cu untuk kepala udang (BCFp-s = 26.84), daging udang (BCFp-s = 3.36) dan kulit udang (BCFp-s = 18.11) adalah yang tertinggi. Ini menunjukkan bahawa udang mengandungi lebih banyak logam daripada sedimen. Nilai-nilai BCFp bagi Cd, Cr, Fe, Mn, Ni dan Pb adalah lebih rendah daripada 1.0, yang bermaksud kemampuan yang terhad untuk logam-logam berat ini berkumpul di dalam udang. Dalam kajian ini didapati bioakumulasi logam berat dalam udang dan air adalah berhubungan negatif manakala hubungan positif didapati antara kepekatan logam berat dalam air dan sedimen. Bioakumulasi logam berat dalam udang dan sedimen menunjukkan tren yang sama iaitu hubungan negatif.

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LIST OF ABBREVIATIONS / SYMBOL

As	Arsenic
Be	Beryllium
Cd	Cadmium
Cr	Chromium
Cu	Copper
CN	Cyanide
Pb	Lead
Hg	Mercury
Ni	Nickel
Se	Selenium
Ag	Silver
Tl	Thallium
Zn	Zinc
TAN	Total Ammoniacal Nitrogen

µg/kg	microgram per kilogram
mg /kg	milligram per kilogram
mg/L	milligram per liter
µg/g	microgram per gram
°C	Degree Celcius
>	Greater than
<	Smaller than
≥	Greater than or equal to
%	Percentage
±	plus or minus

LIST OF EQUATION

Equation 3.1 $Metal\ concentration, \frac{mg}{L} = A \times \frac{B}{v}$

Equation 3.2 $Dilution\ factor = \frac{Final\ volume, mL}{Initial\ volume, mL}$

Equation 3.3 $$Concentration\ of\ metal, mg/kg = \frac{\left(Concentration\ of\ metal, \frac{mg}{L}\right) x\ volume\ of\ sample\ (ml)}{Sample\ weight, g\ x\ 1000}$$

Equation 3.4 $y_{mn} = z_{m1}x_{1n} + z_{m2}x_{2n} + z_{m3}x_{3n} + \ldots + z_{mi}x_{in}$

Equation 3.5 $f(G_i) = k_i + \sum_{j=i}^{n} w_{ij}P_{ij}$

Equation 3.6 $Z_{no} = \sum Q_{mn}R_{no}$

Equation 3.7 $BCF = C_B = C_{WT}$ or $C_B = C_{WD}$

Equation 4.1 Prawn Conc. = 7.0716+0.0967*Sediment Conc.+ 5.401*Water Conc.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Aquaculture is one of the currently fastest growing food producing sector in the world due to high demands from the consumers. It is an important source of food, nutrition, income and livelihoods for hundreds of millions of people around the world. Aquaculture productions have reached about 11.9 million tonnes in 2014 with continuing positive trend that has resulted in a 37% increase in the last decade (FAO, 2016). The four main contributing groups are tunas, lobsters, shrimps and cephalopods which registered new record catches in 2014. Malaysia is one of the top 15 world aquaculture producers in the 2014 production with 521 thousand tonnes.

Asia is the biggest contributor in the world aquaculture production of food fish (fishes, crustaceans, mollusks, amphibians, reptiles and other aquatic animal for human consumption) since year 2001 (FAO Fisheries and Aquaculture Department, 2013). Aquaculture industry is expanding in many regions of the world including Malaysia. Malaysian brackish water white shrimp aquaculture industry performed a sustainable growth of production from 2011 to 2014 (Annual Fisheries Statistics, 2011-2014). Shrimp is one of the main aquaculture species in international trade in year 2008 with production of 3,450,000 tonnes (Ababouch & Karunasagar, 2013). The *Litopenaeus vannamei (Whiteleg Shrimp)* is predominant in Malaysia market.

According to Sharihan *et al.*, (2018), Malaysia aquaculture sector are facing six major issues and challenges faces specifically issues of fish stock depletion, climate changes, current disease, media influences in the sector, non-compliance feeding practiced and poor interaction between stakeholders. Global depletion of aquaculture livestock is caused by massive exploitation and ineffective fisheries management (Begg & Waldman, 1999). Moreover, the alarming disease commonly found in shrimp are Early Mortality Syndrome (EMS) and White Spot Disease Sharihan et al., (2018). EMS is a shrimp disease with no causative pathogen has caused mass mortalities during the first 30 days culture of *Penaeus monodon* and *Litopenaeus vannamei* whereas White Spot disease caused by White Spot Syndrome Virus (Leaño and Mohan, 2012).

From now until 2030, the Agenda for Sustainable Development and the Sustainable Development Goals (SDGs) have several objectives that are directly relevant to aquaculture development such as SDGs 2 and 14. The SDGs 2 is about Zero Hunger where its objectives are to end hunger, achieve food security and improved nutrition and promote sustainable agriculture (FAO, 2019). In this rapid growing global population with increasing water and land scarcity, soil, land and biodiversity degradation and more frequent and severe weather events, the greatest challenges are to ensure enough quality food to meet their nutritional needs for a healthy life. In other hands, SDGs 14 is Life Below Water which focuses on conserve and sustainably use the oceans, seas and marine resources (FAO, 2019). Aquaculture is the fastest growing food sector and has the potential to produce the fish needed to help meet the demands of a growing population. However, unmanaged aquaculture expansion and practices can cause pollution and rising of carbon dioxide level in the atmosphere which contributing to ocean acidification. Besides, overfishing threatening the livelihoods. The SDGs is one of the ways to ensure the sustainability of aquaculture industry.

FAO has taken into account of sustainable development within the framework of its own Blue Growth Initiative to accelerate its work in support of sustainable management of living aquatic resources, balancing their use and conservation in an economically, socially and environmentally responsible manner. Wilfart *et al.*, (2013) foresee that aquaculture industry will face four inevitable challenges which are increase cultivable areas without decreasing biodiversity or increase water demand, improving food quality, producing ecosystem services and adapting to climate changes. Hence, it is necessary to design aquaculture systems which use fewer chemical products, more renewable resources and make the best use of ecosystem services without impairing their regeneration.

Aquaculture activities resulted an impact on the concentration of heavy metals in water and sediment. Shrimp culture could modify the concentration of heavy metals in the river water (Ling *et al.*, 2010). Shrimp farms are located in the estuaries where it supplies the brackish water needed and it also received the discharge of the pond effluent during harvesting (Ling *et al*, 2012). There are study findings of environmental degradation due to loading of pollutants from shrimp farming. Estuaries and coastal area exhibit a wide array of human impacts that can compromise their ecological integrity, because of rapid population growth and uncontrolled development in many coastal regions worldwide (Kulikova *et al.*, 1985). Hence, aquaculture which uses water from the river, estuary and coastal area is prone to external pollution and the aquaculture products (fish, shrimps) can be a health risk if consumed. This is potentially serious as aquaculture grows rapidly especially for tiger shrimp, high value of seafood community whose consumer demands far exceeds the supply (Caeiro, 2005; Forstner & Wittmann, 1983; Kulilova *et al.*, 1985; Sharif *et al.*, 2008).

Estuaries and coastal area receive significant inputs from both point and non-point upstream sources and from metropolitan areas, tourism and industries along the estuarine edge (Caeiro, 2005). According to Dural (2007), heavy metal pollution in estuaries and coastal area has been recognized as a serious environmental concern. In general, studies on heavy metals can be important in two main aspects which are public health and the aquatic environment. In the public health point of view, the attention has been drawn to the necessity of measuring the accumulation of heavy metals especially those particularly pose an imminent health hazard to humans and included in "black list" of metals (Cd, Pb, Hg and etc). In the aquatic environment point of view, the main issue has been to prevent biological deterioration and to identify the sources which could threaten ecological equilibrium. In this regard, the more abundant metals such as copper, zinc and manganese may sometimes represent greater hazard than lead, mercury and cadmium (Kinne, 1984).

1.2 Research questions

In this research, there are a few research questions that have been taken into account which are:

a) What is the concentration level of selected heavy metals in *Litopenaeus vannamei (Whiteleg Shrimp)* different body parts?

b) What is the concentration level of selected heavy metals in water of aquaculture pond?

c) What is the concentration level of selected heavy metals in sediment of aquaculture pond?

d) Does heavy metals concentration in *Litopenaeus vannamei (Whiteleg Shrimp)* affected by heavy metals concentration of water and sediment?

e) Does heavy metals concentration in water affected by heavy metals concentration of sediment?

f) What is the bioconcentration of heavy metal in *Litopenaeus vannamei (Whiteleg Shrimp)*?

1.3 Objectives of study

This research aims to analyse the heavy metals in *Litopenaeus vannamei (Whiteleg Shrimp)*, water and sediment in selected aquaculture ponds. The objectives of this study are:

a) To identify the level of selected heavy metals in *Litopenaeus vannamei (Whiteleg Shrimp)* and its surrounding water and sediment in study area.

b) To study the relationship between heavy metals in water, sediment and *Litopenaeus vannamei (Whiteleg Shrimp)*.

c) To determine the bioconcentration of heavy metal in *Litopenaeus vannamei (Whiteleg Shrimp)*.

1.4 Scope and significance of study

The scope of study focused on one aquaculture site as this research was carried out on-site of operating aquaculture ponds. This study made comparison between two aquaculture ponds in the same period of time and location to provide clearer picture and as control. The sediments were taken after the shrimps have been harvested and water discharged from the pond. The sediment sample was limited to only one pond due to constraint of business owner in access to the pond at specific period of time. The heavy metal concentration of different parts of shrimp, water and sediment were determined. The relationship between shrimp, water and sediment were also done and analyzed using Principal Component Analysis.

This research focused on three parts. The first part was to identify the level of selected heavy metals in *Litopenaeus vannamei (Whiteleg Shrimp)* and its surrounding water and sediment in study area. The shrimp sample was taken from Kingfisher Inanam, Kota Kinabalu, Sabah. In Malaysia particularly Sabah, there is no background studies on the *Litopenaeus vannamei (Whiteleg Shrimp)* in aquaculture pond. Similar studies had been carried out on different species of shrimp which is *Penaeus monodon*. Hence, the identification of heavy metals in shrimp and its surrounding water and sediment is essential in providing background information for this study. Besides that, this background information is useful in determining the pollutant level of the river and estuaries of the aquaculture activities. This research will also enable stakeholders to plan sustainable aquaculture activities in river or estuaries which can gain maximum profits without degrading our environment quality.

The second part of this study focused on determination of the relationships between heavy metals in water, sediment and *Litopenaeus vannamei (Whiteleg Shrimp)*. At present, numerous studies on the relationship between heavy metals in water and sediment were carried out, however there are a lack of studies on the relationship between the three elements which are water, sediment and *Litopenaeus vannamei (Whiteleg Shrimp)*. This relationship study enables us to better understand the impact of pollutant level of one aspect to another. The knowledge gained will provide important insight in reducing pollutant level in all aspect and considering on surrounding environment of aquaculture where all aspects are interconnected.

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