UNIVERSITI PUTRA MALAYSIA

ECOTOXICOLOGY AND HUMAN HEALTH RISK ASSESSMENT OF HEAVY METAL IN TINY SCALE BARB [Thynnichthys thynnoides (Bleeker, 1852)] FROM UPSTREAM PERAK RIVER, MALAYSIA

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FPAS 2021 6

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

December 2020

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

ECOTOXICOLOGY AND HUMAN HEALTH RISK ASSESSMENT OF HEAVY METAL IN TINY SCALE BARB [*Thynnichthys thynnoides* (Bleeker, 1852)] FROM UPSTREAM PERAK RIVER, MALAYSIA

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December 2020

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The upstream of Perak River has continuously received pollution from anthropogenic activities. These activities are responsible for the release of heavy metals into the environment. Thus, this study aimed to profile the concentrations of heavy metals in water, sediment, and tiny scale barb *Thynnichthys thynnoides* of the upstream of Perak River by integrating analytical and statistical approaches. This study also assessed the potential ecological and human health risks to heavy metals contamination. Furthermore, this study investigated the effect of heavy metal contamination on genetic variation of the tiny scale barb populations of this river using microsatellite loci from cross-species amplification. The concentrations of heavy metals [i.e. aluminum (Al), arsenic (As), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), and nickel (Ni)] were analysed by the inductively coupled plasma mass spectrometry (ICP-MS). The results revealed that the water and sediment of this river were primarily polluted by As and potentially by Cu and Pb. The mean concentrations of As in both water (0.17 mg/L) and sediment (878.6 mg/kg) were observed to exceed the standard quality guidelines recommended for water (0.01 mg/L, Ministry of Health Malaysia) and sediment (70 mg/kg, Hong Kong Environmental Protection Department), respectively. The principal component analysis (PCA) then identified mixed anthropogenic sources related to the pollution mainly from mining, followed by logging and plantation activities. The high bioaccumulations of As in the tiny scale barb tissues were also correlated to the high concentrations of As in the river. The contamination factor (CF) and potential ecological risk index (RI) showed that this Perak River is severely contaminated by As and could pose a serious ecological risk to aquatic organisms. The concentration of As in the muscle tissues of tiny scale barb (1.00 mg/kg) has reached the maximum permissible limit by the Malaysian Food Regulations (1.00 mg/kg) and target hazard quotient (THQ) suggested that daily consumption of this fish would likely to cause adverse health effects to the consumer. Despite the high levels of metals in the tiny scale barb tissues, it shows no correlation with genetic variation in the tiny scale barb populations. However, this study observed reduced genetic diversity in tiny scale barb populations above the Kenering dam compared to the populations below dam. This is

an indication of genetic divergence and inbreeding due to the physical river barrier. Recent population bottlenecks were also observed in the populations above the dam possibly due to the effects of pollution and overfishing. In conclusion, this study has successfully provided data on heavy metals in river ecosystems for the purpose of monitoring, environmental protection and human safety. This study has also provided valuable genetic information for the fisheries sector and native species conservation efforts.

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

EKOTOKSIKOLOGI DAN PENILAIAN RISIKO KESIHATAN MANUSIA TERHADAP LOGAM BERAT DALAM IKAN LOMA [*Thynnichthys thynnoides* (Bleeker, 1852)] DARI HULU SUNGAI PERAK, MALAYSIA

Oleh

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Hulu Sungai Perak masih menerima pencemaran daripada aktiviti-aktiviti antropogenik. Kegiatan ini dilihat bertanggungjawab dalam menyebabkan terlepasnya logam berat ke persekitaran. Oleh itu, kajian ini dilakukan dengan tujuan untuk memaparkan kepekatan logam berat di dalam air, tanah, dan ikan Loma *Thynnichthys thynnoides* di hulu Sungai Perak, melalui pendekatan analitik dan statistik. Kajian ini juga menilai potensi risiko pencemaran logam berat terhadap kesihatan manusia dan ekologi. Selain itu, kajian ini turut meneliti kesan pencemaran logam berat terhadap variasi genetik populasi ikan Loma di sungai ini dengan menggunakan lokus mikrosatelit melalui penggandaan silang spesies. Kepekatan logam berat [iaitu aluminium (Al), arsenik (As), kuprum (Cu), besi (Fe), mangan (Mn), plumbum (Pb), and nikel (Ni)] dianalisis menggunakan Spektrometer Jisim Gandingan Aruhan Plasma (ICP-MS). Hasil kajian menunjukkan bahawa air dan tanah di sungai ini telah dicemari terutamanya dengan As, dan berpotensi dicemari oleh Cu dan Pb. Kepekatan As di dalam air (0.17 mg/L) dan tanah (878.6 mg/kg) dilihat telah melebihi garis panduan kualiti standard yang telah disyorkan untuk air (0.01 mg/L, Kementerian Kesihatan Malaysia) dan tanah (70 mg/kg, Jabatan Perlindungan Alam Sekitar Hong Kong). Seterusnya, analisis komponen utama (PCA) telah mengenal pasti sumber-sumber antropogenik yang menyumbang kepada pencemaran adalah dari aktiviti perlombongan, pembalakan, dan perladangan. Biopengumpulan As yang tinggi di dalam tisu ikan Loma dilihat selari dengan kepekatan As yang tinggi di dalam sungai. Faktor pencemaran (CF) dan potensi indeks risiko ekologi (RI) menunjukkan bahawa hulu Sungai Perak ini sangat teruk dicemari dengan As dan boleh menimbulkan risiko ekologi yang serius kepada organisma akuatik. Kepekatan As di dalam tisu otot ikan Loma (1.00 mg/kg) dilihat telah mencapai had maksimum yang dibenarkan oleh Peraturan Makanan Malaysia (1.00 mg/kg) dan sasaran darjah bahaya (THQ) menunjukkan bahawa pengambilan ikan ini dalam diet harian berkemungkinan mendatangkan kesan buruk kepada kepada kesihatan pengguna. Meskipun tahap logam berat di dalam tisu ikan Loma tinggi, ia tidak menunjukkan sabarang korelasi dengan variasi genetik populasi ikan Loma ini. Walaupun begitu, kajian ini menunjukkan

penurunan dalam kepelbagaian genetik pada populasi ikan Loma di sebelah atas empangan Kenering berbanding dengan populasi di sebelah bawah empangan. Ini membuktikan bahawa berlakunya pembiakbakaan dalam dan evolusi atau perubahan pada genetik disebabkan oleh halangan pada fizikal sungai. Populasi ikan Loma di sebelah atas empangan juga didapati mengalami kesesakan mungkin disebabkan oleh kesan dari pencemaran dan penangkapan ikan yang berlebihan. Kesimpulannya, kajian ini telah berjaya memberikan data mengenai logam berat dalam ekosistem sungai bagi tujuan pemantauan, perlindungan alam sekitar dan keselamatan manusia. Kajian ini juga telah memberikan info genetik yang berharga untuk sektor perikanan serta usaha pemuliharaan spesies asli.

ACKNOWLEDGEMENTS

First and foremost, praises and gratitude to Allah S.W.T. for allowing me to live, learn, and experience a lot of things throughout this PhD journey. Thank you Allah S.W.T. for giving me the strength and courage to complete this.

My grateful appreciation goes to my main supervisor, Dr. Mohd Yusoff Ishak for his great support, patience, and encouragement throughout the studies. I am also thankful to my co-supervisors, Assoc. Prof. Dr. Yuzine Esa, Assoc. Prof. Dr. Mohammad Noor Amal Azmai, and Dr. Syaizwan Zahmir Zulkifli for their willingness to be part of the committee members and assisting whenever needed.

I wish to thank all the staffs and post-graduate students in the Department of Environment (Forestry and Environment) and Department of Aquaculture (Agriculture), UPM for their assistance and cooperation during the studies, especially to Mr. Mohd Sulkifli Ibrahim, Miss Dalina Jaafar, Mr. Abdul Rahman Sokran, Mr. Zairi Ismail, Dr. Safaa A Kadhum, Mohamad Radhi Amonodin, and Nurul Atikah Wahid. My heartiest appreciation also goes to my friends, especially Zarith Sufiani Baharom, Nurhazwani Juzaimi, Nur Rahmah Majiran, Dr. Syahidah Ahmad, Nabilah Fatin Abd Razak, Dr. Nur Fatihah Abd Halid, and Dr. Mohamad Faizul Mat Isa for help and understanding.

I dedicate this thesis to my family, especially my mom, Radziah Othman, and my dad, Ahmad Affandi Kamaruddin, for their support, love, and patience that has given me the motivation to keep on going until the end.

Not to forget, I thank the Ministry of Higher Education and Universiti Putra Malaysia for the funding under Fundamental Research Grant Scheme, Geran Putra IPS, and Graduate Research Fellowship.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:
- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
bp	Base pair
Bq	Becquerel
BHC	Benzene hexachloride
CCE	Carbon chloroform extract
cm	Centimeter
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
FAM	Fluorescein amidites
gDNA	Genomic DNA
g	Gram
h	Hour
H_2O	Water
hpf	Hour post-fertilization
Kg.	Kampung
kb	Kilobase
kg	Kilogram
km	Kilometer
L	Liter
m	Meter
MBAS	Methylene blue active substances
µg	Microgram
µl	Microliter
µm	Micrometer

µS	Micro Siemen
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
ms	Millisecond
ng	Nanogram
nm	Nanometer
NTU	Nephelometric turbidity unit
O&G	Oil and grease
PBS	Phosphate-buffered saline
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
ppm	Part per million
ppt	Part per thousand
rpm	Round per minute
SAR	Sodium adsorption ratio
SD	Standard deviation
sec	Second
sp. or spp.	Species (for singular or plural term)
SPSS	Statistical Package for Social Science
TCU	True color unit
UPM	Universiti Putra Malaysia
UPGMA	Unweighted pair group method with arithmetic mean
UV	Ultraviolet
V	Volt

v/v	Volume per volume
W	Watt
w/v	Weight per volume

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

Rapid industrialisation and population growth in Malaysia have caused serious problems to the water quality and fish population in many rivers in the country (Yap et al., 2003). Beyond that, water pollution has fundamentally emerged as a major problem worldwide and the presence of harmful contaminants in the environment is raising much concern. One of the main causes of water pollution particularly is heavy metals, whereby pollution due to these materials are known to cause adverse health effects towards aquatic organisms and humans alike (Reis, 2013; Okpala et al., 2018).

Heavy metals are introduced into the aquatic environment by both natural and anthropogenic activities through runoff, land-based point discharge, and weathering processes. Heavy metals are considered toxic pollutants in the aquatic environment due to their persistence, toxicity, and ability to be accumulated into the food chain (Bastami et al., 2014). Unlike other pollutants (mainly organic), metals are not degraded or eliminated from the ecosystem (Rajkowska and Protasowicki, 2013). However, they are incorporated among the various aquatic environmental compartments such as water, sediments, suspended solids, and organisms (plants, fish, invertebrates, and microbes) (Azizur Rahman et al., 2012). These metals may occur in the aquatic environment in dissolved, particulate, or complex forms. These metals will accumulate in the food chain and cause various adverse effects or even death to the aquatic organisms.

Fish are exposed to metals both through the water and food chain. In the water, metals remain as free ions of organic and inorganic anions. Hence, these free metal ions are accumulated in fish via direct uptake across the gills (Hodson, 1988). Other than that, these free metal ions can accumulate in algae, plankton, and bacteria, which then are transferred to a higher trophic level of the food chain such as the forage fish and small invertebrates (Azizur Rahman et al., 2012). Finally, these small fishes and invertebrates will be eaten by predator fish thus accumulating a high concentration of metals in the fish organs and tissues (Azizur Rahman et al., 2012).

The tiny scale barb *Thynnichthys thynnoides* (Bleeker, 1852) is a freshwater fish widely distributed in Southeast Asia which inhabits the streams and lakes in Malaysia particularly in Tasik Chini, Pahang (Kutty et al., 2009) as well in Perak River, Perak (Ali and Kadir, 1996). Perak River is the second longest river in Peninsular Malaysia after the Pahang River. Perak River has become an attraction among locals to harvest the tiny scale barbs during their mass migration starting in October every year (Ismail et al., 2015). Even though the IUCN Red List Status has categorised the tiny scale barb as one of the Least Concern species, these species are facing a few threats such as impact from water pollution, overfishing, and habitat degradation due to deforestation (Chong et al., 2010). This apparently will decrease the fish numbers and population which may lead to species absence in certain areas, as absence reported in the southern parts of Peninsular Malaysia (Ambak et al., 2010).

Genetic disturbance or change in fish as a response to pollutant exposure has been discussed for at least three decades (De Flora et al., 1993; Belfiore and Anderson, 2001; Moon et al., 2020). Bickham et al. (2000) and Hamilton et al. (2016) observed the potential reduction of species diversity and population, as well as the predicted loss of genetic variation due to pollutant exposure. Frankham (1995) also stated that the population's ability to adapt to changing environments is dependent on its genetic diversity, and that small populations with less genetic diversity may have reduced fitness and be more vunarable to extinction. Therefore, the genetic approach in evaluating the current status of the fish population and understanding the history of population changes have offered powerful tools in predicting future population directions (Belfiore and Anderson, 2001).

1.2 Problem Statement

Water pollution is a major issue that has a negative impact on the aquatic ecosystem. A high level of heavy metals in the water could have disastrous effects for the ecological balance, changing the diversity of organisms in the water. Heavy metal toxicity's harmful effects on aquatic organisms may eventually have an influence on human lives as well. The metal accumulation in aquatic organisms could enter the human body system when consumed, which then leads to various health effects.

The upper Perak River plays an important role as the water resource for Kenering Reservoir. Rui River as its main tributary is familiarly associated with the provision of various sources of food, while also acting as a breeding ground for aquatic organisms, especially the tiny scale barb (Azmai et al., 2015). Due to the massive amount of tiny scale barb migrating through the river during the spawning season, fishing for the species has become a sought-after activity among locals and tourists alike. However, the low water quality reported in Rui River over the last few years has caused a decline in its catch (Bernama, 2015; Abd Manap, 2016). Despite such reports, the river continuously receives a huge amount of untreated discharge due to anthropogenic activities, such as mining, logging, and plantation. Surface runoff from the upstream mining and logging during the rainy season has caused intense sedimentation along the river and might contribute to the large amount of metal deposition in the river ecosystem. The presence of toxic metals such as arsenic, cadmium, lead, and mercury in waters associated with mining and logging activities, have been found accumulated in fish in levels that exceed the maximum levels for human consumption (Tarras-Wahlberg et al., 2001; Ashraf et al., 2012; Poon et al., 2016; Gusso-Choueri et al., 2018; Alizada et al., 2020). These metals are known as human carcinogens which are able to induce multiple organ damage and failure, even at lower concentrations of exposure (Tchounwou et al., 2012). Because of their high degree of toxicity, these metals are of great public health concern.

Previous studies showed that heavy metal bioaccumulation levels in aquatic organisms accurately reflect the pollution levels in their immediate environment, and this heavy metal stress is related to a loss of genetic diversity at the population level (Fratini et al., 2008; Ungherese et al., 2010). High levels of several metals from mining activities have been found to cause genotoxic effects in fish at molecular and chromosomal levels (Gusso-Choueri et al., 2016). Meanwhile, high metal levels in the thermal power plant effluent were found to cause DNA damage and oxidative stress in fish (Javed et al., 2016). Specifically, these toxic metals may reduce the diversity and abundance of aquatic biota as well as the total aquatic biomass composition. They are particularly harmful to the embryos and larvae of fishes, and deformities at these early stages of life will affect their subsequent survival and growth rate, devastating the fish populations (Lourenço et al., 2017; Ramos et al., 2015).

Since the tiny scale barbs are still being harvested in large amounts annually, the fish need to be used sustainably before extinction occurs in nature. Moreover, little is known about the genetic structure of the tiny scale barb population, especially at the micro-geographical scale such as in this Perak River where cycles of colonisation or extinction may be frequent. Therefore, it is important to measure and monitor the quality of this river ecosystem to prevent the loss of biodiversity and serious human health hazards. Understanding the effects of pollutants on genetic variability is necessary for conserving the evolutionary potential of natural populations. It is fundamental to have management efforts either by conservation or domestication.

1.3 Hypothesis

This research tested the following hypotheses:

1) H_0: The upper Perak River is not polluted by heavy metal contamination from the upstream mining activity.
   H_1: The upper Perak River is mainly polluted by heavy metal contamination from the upstream mining activity.
2) H_0: There is no correlation between bioaccumulation of heavy metals in tiny scale barb tissues and heavy metal concentrations in water and sediment of the upper Perak River.
   H_1: Bioaccumulation of heavy metals in tiny scale barb tissues is correlated with heavy metal concentrations in water and sediment of the upper Perak River.
3) H_0: Heavy metal pollution in the upper Perak River has no effect on genetic variability of tiny scale barb population.
   H_1: Heavy metal pollution in the upper Perak River has a negative effect on genetic variability of tiny scale barb population.
4) H_0: Consumption of tiny scale barb from the upper Perak River will not cause any toxic effect on human health.
   H_1: Consumption of tiny scale barb from the upper Perak River will cause a toxic effect on human health.

1.4 Objectives

This research was carried out to investigate the ecotoxicological effects and human health risk assessment of heavy metal in the tiny scale barbs of the upstream of Perak River. Figure 1.1 shows the framework of this research. The detailed objectives of this research are as follows:

1) To assess the status of heavy metal occurrence and distribution (Al, As, Cu, Fe, Mn, Ni, and Pb) in the water and sediment of the upstream of Perak River.
2) To determine and compare the bioaccumulation level of heavy metals in different parts of tissues (gills, liver, and muscle) of tiny scale barb collected from the upstream of Perak River.
3) To evaluate the effects of heavy metal pollution and bioaccumulation on genetic variability of the tiny scale barb collected from the upstream of Perak River.
4) To determine the human health risk of heavy metals in the tiny scale barb collected from the upstream of Perak River.

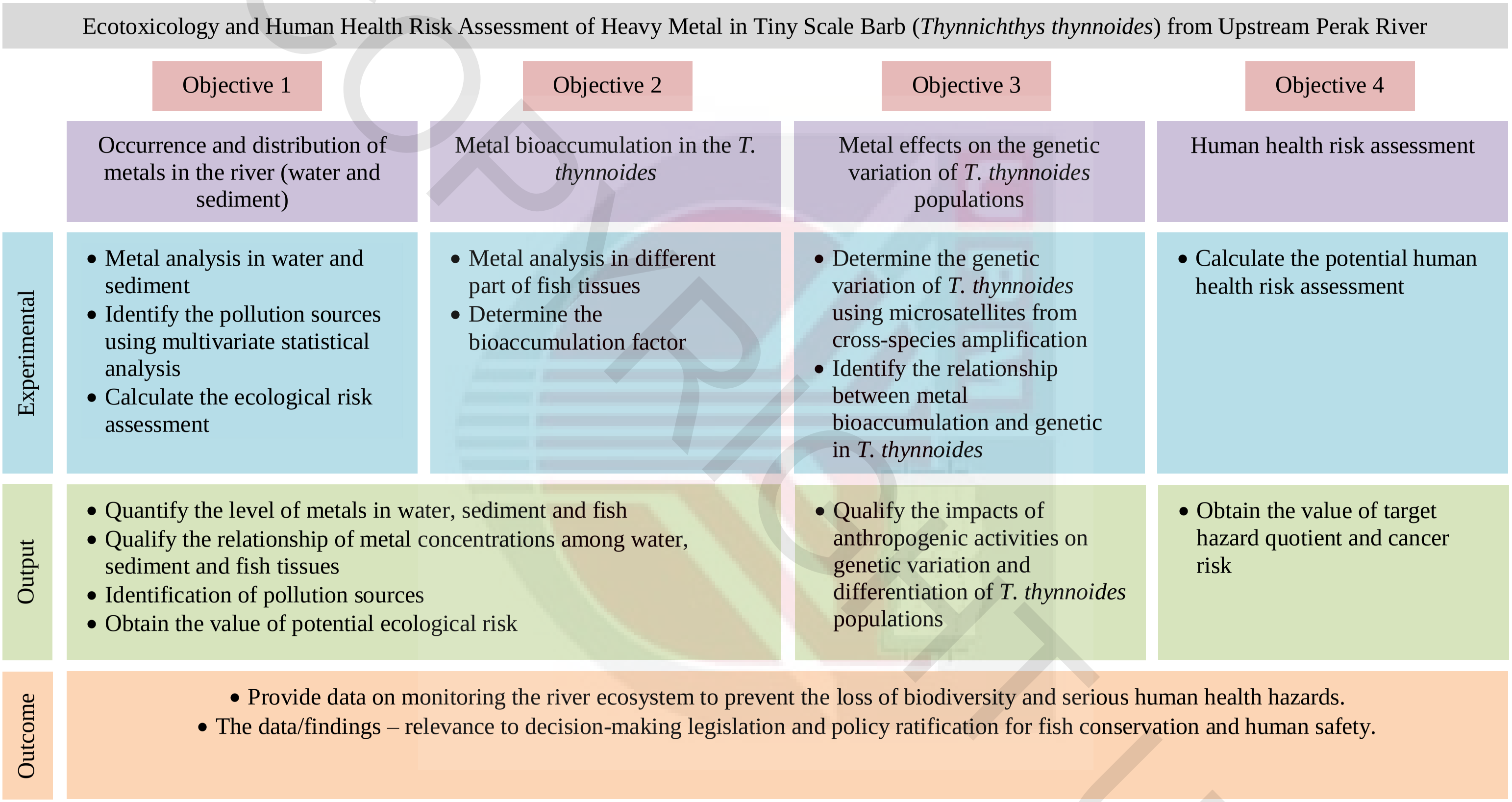

Figure 1.1: The research framework of this study

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