UNIVERSITI PUTRA MALAYSIA

BIOACCUMULATION OF HEAVY METALS AND METALLOTHIONEIN EFFECTS ON REPRODUCTIVE BIOLOGY OF ASIAN SWAMP EEL (MONOPTERUS ALBUS ZUIEW, 1793) IN PADDY FIELDS IN TUMPAT, KELANTAN, MALAYSIA

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By

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

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BIOACCUMULATION OF HEAVY METALS AND METALLOTHIONEIN EFFECTS ON REPRODUCTIVE BIOLOGY OF ASIAN SWAMP EEL (*Monopterus albus* ZUIEW, 1793) IN PADDY FIELDS IN TUMPAT, KELANTAN, MALAYSIA

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The current application of excessive agrochemical fertilizers during the four paddy seasons could potentially elevate the concentration levels of Zn, Cu, Cd, Ni and Pb in paddy soils. Hence, concentrations of Zn, Cu, Cd, Ni and Pb in paddy soils and Asian swamp eel, *Monopterus albus*, were evaluated to determine the availability, distribution, overall classification and risk associated with application of agrochemical fertilizers and pesticides introduced by rice farmers. A four step sequential extraction technique (SET) was conducted to establish the speciation, mobility, availability and origin of heavy metals such as zinc, copper, cadmium, nickel and lead. These four fractions are termed as exchangeable (EFLE), acid-reducible, oxidisable-organic and resistant fractions in the paddy soil. Nine tissues of Asian swamp eel were selected, namely, gills, liver, kidneys, digestive tract, stomach contents, gonads, bone, skin and muscle; which were digested using the acid digestion method. In comparison to the values of average shale of the studied metals, total Pb and Cd were higher than the shale values, whereas the total Zn, Cu and Ni concentrations were found to be below the values. Generally, most of the metals studied were significantly associated with the resistant fraction, followed by oxidisable-organic and acid-reduction fractions. This indicates that the paddy soils of Tumpat are naturally rich with the metals studied. Meanwhile, the least average partitioning patterns in EFLE in paddy seasons indicate low Zn, Cu, Cd, Ni and Pb mobility. Among the metals studied, availability of average Cd and Pb levels was found to be abundant in most seasons based on enrichment factors and contamination factors. However, none of the studied metal concentrations in the paddy soils were as high as the toxic effect threshold (TET) value. Since Asian swamp eel is a long-lived organism, the longer the exposure to those pollutants, the higher the concentrations of metals were

bio-accumulated into it. Levels of Zn, Cu, Cd, Ni and Pb in the stomach contents were found to be higher than other tissues studied. Muscle and skin were shown to have the least heavy metal concentrations. To evaluate the health risks to the Malaysian population through consumption of Asian swamp eel, daily intakes and target hazard quotient of heavy metals were estimated on the basis of the concentrations of heavy metals in muscle and skin of Asian swamp eel. Based on the estimated daily intake and THQ of Cd and Pb in skin and muscle in 2011 and 2012, the results suggest that Cd and Pb in skin and muscle of Asian swamp eel was polluted by Cd and Pb, which have higher risk to be consumed. Metallothionein (MT) concentrations in gills, liver and muscle tissues of *M. albus* were determined to assess the biological response of Asian swamp eel to the level of some metals. The concentrations of Zn, Cu, Cd, Ni and Pb (µg/g wet weight) in the tissues studied were correlated with MT contents. MT concentrations were found to be high in the liver, gills and muscles during plowing, growing, and harvesting seasons. However, a significant correlation ($p < 0.01$) was found between MTs concentrations in liver and Zn metals. No correlations were found between MTs contents in gills and muscle with the studied metals. In this study, the gonadal histological procedure was made as a basis for sex determination in Asian swamp eel, *M. albus*. Thirty-two females, three males and two intersexes of Asian swamp eel were considered. The presence of a high number of matured oocyte 3 and 4 during plowing and seedling seasons have shown that these seasons could be considered as breeding seasons for Asian swamp eels. The availability of food and nutrients (agrochemical fertilizers) were attributed to the ovarian development of Asian swamp eel. In conclusion, the concentrations of Zn, Cu, Cd, Ni and Pb in paddy soils varied with paddy cycles in 2011 and 2012. The presence of Zn, Cu, Cd, Ni and Pb in Asian swamp eels' tissues indicates that these eels have the ability to accumulate and partially remove the heavy metals which required future research study.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Doktor Falsafah

BIOAKUMULASI LOGAM BERAT DAN KESAN METALLOTHIONEIN KE ATAS PEMBIAKAN BIOLOGI PADA BELUT SAWAH (*Monopterus albus* ZUIEW, 1793) DI SAWAH PADI DI TUMPAT, KELANTAN, MALAYSIA

Oleh

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Penggunaan baja agrokimia berlebihan semasa empat musim padi berpotensi meningkatkan tahap kepekatan Zn, Cu, Cd, Ni dan Pb di sawah padi pada masa kini. Oleh itu, kepekatan Zn, Cu, Cd, Ni dan Pb di dalam tanah padi dan belut akan dinilai untuk menentukan taburan, ketersediaan, klasifikasi keseluruhan dan risiko yang berhubung kait dengan penggunaan baja dan racun perosak yang diamalkan oleh para petani. Empat langkah pengekstrakan berurutan (SET) telah dijalankan untuk menentukan spesies, pergerakan, ketersediaan dan keaslian Zn, Cu, Cd, Ni dan Pb. Keempat-empat pecahan tersebut dinamakan sebagai pecahan ditukar, pecahan asid-dikurangkan, pecahan organic dioksidakan dan pecahan sisa dalam tanah sawah. Untuk belut sawah padi, sembilan tisu belut telah dipilih iaitu insang, hati, buah pinggang, saluran penghadaman, kandungan perut, telur, tulang, kulit dan otot. Kesemua tisu ini telah dianalisis dengan menggunakan kaedah penghadaman asid. Sebagai perbandingan dengan nilai purata syal, jumlah Pb dan Cd menunjukkan lebih tinggi daripada nilai syal sementara jumlah kepekatan Zn, Cu dan Ni menunjukkan dibawah nilai syal. Umumnya, kesemua logam berat yang dikaji menunjukkan tahap hubungan signifikasi dengan pecahan sisa, diikuti dengan pecahan organik dioksidakan dan pecahan asik-dikurangkan dalam tanah sawah padi. Ini menunjukkan tanah sawah padi kaya dengan kehadiran keaslian mineral Zn, Cu, Cd, Ni dan Pb. Sementara itu, pecahan ditukar dikategorikan sebagai pecahan yang mempunyai kepekatan paling sedikit, dimana menunjukkan pergerakan logam berat tahap rendah. Hasil kajian menunjukkan faktor pengayaan dan pencemaran bagi logam Cd dan Pb adalah tertinggi di kalangan logam yang dikaji untuk musim-musim padi. Bagaimanapun, kepekatan logam berat yang dikaji tidak menunjukkan nilai yang lebih tinggi daripada nilai kesan toksik ambang. Memandangkan belut sawah merupakan sejenis organisma yang dapat hidup lebih lama, semakin lama pendedahan kepada bahan

pencemar, semakin tinggi kepekatan logam yang terkumpul di dalam belut sawah. Kepekatan logam berat yang dikaji (Zn, Cu, Cd, Ni dan Pb) dalam kandungan perut didapati tinggi daripada tisu-tisu belut lain yang dikaji. Disamping itu, otot dan kulit menunjukkan kepekatan logam berat yang sedikit. Risiko kesihatan melalui pemakanan belut sawah oleh penduduk Malaysia dinilai menggunakan pengambilan seharian dan sasaran hasil bahagi bahaya ke atas pengambilan otot dan kulit belut sawah. Berdasarkan kepada anggaran pengambilan harian dan THQ Cd dan Pb dalam kulit dan otot pada 2011 dan 2012, hasil menunjukkan Cd dan Pb dalam kulit dan otot belut sawah padi dicemari oleh ketoksikan logam Cd dan Pb. Kepekatan metallothionein (MT) dalam tisu seperti insang, hati dan otot *M.albus* ditentukan untuk menilai tindak balas biologi belut sawah ke sesuatu tahap logam. Oleh yang demikian, kepekatan Zn, Cu, Cd, Ni dan Pb (µg/g berat basah) di dalam tisu yang dikaji telah dihubung kaitkan dengan kandungan MT. Kepekatan MT didapati telah meningkat di dalam hati, insang, dan otot semasa musim membajak, musim pertumbuhan dan musim menuai padi. Walau bagaimanapun, hubungan signifikan ($p < 0.01$) didapati antara kepekatan MT dalam hati dengan logam Zn. Tiada korelasi didapati diantara kandungan MT di dalam insang dan otot dengan logan dikaji. Prosedur histologi telur belut telah dilakukan sebagai asas untuk penentuan seks belut sawah, *M. albus*. Dari pemerhatian slaid, terdapat tiga puluh dua ekor betina, tiga ekor jantan dan dua ekor interseks belut sawah. Bilangan yang tinggi oosit 3 dan oosit di musim membajak dan menabur menunjukkan kedua-dua musim tersebut berpotensi diklasifikan sebagai musim membiak untuk belut sawah. Kehadiran makanan dan nutrisi (baja agrokimia) menjadi faktor kematangan ovari belut sawah. Sebagai kesimpulan, kehadiran logam Zn, Cu, Cd, Ni dan Pb di dalm belut sawah padi menunjukkan belut tersebut mempunyai keupayaan mengumpul dan menyahkan logam berat dimana memerlukan kajian penyelidikan pada masa akan datang.

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I certify that a Thesis Examination Committee has met on 26 October 2016 to conduct the final examination of Sow Ai Yin on her thesis entitled "Bioaccumulation of Heavy Metals and Metallothionein Effects on Reproductive Biology of Asian Swamp Eel (*Monopterus albus* Zuiew, 1793) in Paddy Fields in Tumpat, Kelantan, Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
ºC	Degree Celcius
µg/g	Microgram per gram
µm	Micrometer
µ/kg/day	Micro per kilogram per day
µl	Microlitre
AAS	Atomic Absorption Spectrophotometer AAS
BAF	Bioaccumulation Factor
CRM	Certified Reference Material
Ca	Calcium
CF	Contamination Factor
cm	Centimetre
CO_2	Carbon Dioxide
DW	Distilled Water
DTNB	5,5-dithiobis-2-nitrobenzoic acid
d.w	Dry weight
EDTA	Ethylenediaminetetraacetic Acid
EF	Enrichment Factor
EFLE	Easily, freely, Leachable and Exchangeable
FW	Fresh water
g	Gram
g/day	gram/day

H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
HNO_3	Nitric acid
I_{geo}	Geoaccumulation index
kg/m^3	Kilogram per cubic metre
kDA	Kilodalton
K	Calium
mg/day	Milligram per day
ml	Millilitre
mm	Millimetre
nm	Nanometre
M	Molarity
mM	Millimolar
mol/l	Mole per litre
MΩ	Mega ohms
Mg	Magnesium
$NH_2OH.HCl$	Hydroxylammonium chloride
NH_4CH_3COO	Ammonium acetate
N_2	Nitrogen
NaH_2PO_4	Na-phosphate
P	Phosphorus
ppm	Part per million
PLI	Pollution Load Index
PMSF	Phenylmethylsuphonylfuride

rpm	Revolution per minute
SET	Sequential extraction technique
S.D	Standard Deviation
SQG	Sediment Quality Guidelines
TEL	Threshold Effect Level
TET	Toxic Effect Threshold
w.w	wet weight
w/v	weight per volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

Pollution is of great concern in our society, despite Malaysia showing a relatively positive environmental record (WWF, 2005). Malaysia is not spared from global environmental issues such as global warming, climate change, and acid rain. This has been extensively discussed since the 1970's. Most pollution studies have focused on the effect of hazardous chemicals such as organic and inorganic pollutants. One of the most critical forms of pollution is heavy metals pollution. The content of inorganic pollutants, particularly heavy metals in soils and food, has been well studied but the focus has mainly been in residential, recreational, coastal, industrial and land fill areas. The existence of hazardous chemicals such as pesticides, heavy metals, hydrocarbons, dioxins, and endocrine chemicals in the environment cause a great concern. These chemicals are known to cause acute or chronic effects on living organisms (fishes, wildlife) by disrupting the reproductive systems and internal organs (WWF, 2005).

Studies of heavy metals pollution in Malaysia have focused mostly on marine (Yap et al., 2002a; 2002b; Yap et al., 2003a, 2003b; Ong et al., 2009) and freshwater (Ahmad and Shuhaimi-Othman, 2010) ecosystems. In addition, as agriculture is an important sector to the growth of Malaysia's economy, various studies of heavy metals pollution in agricultural lands have been conducted for example a study of heavy metals in agricultural soils from Cameron Highlands, Pahang, and Cheras, Kuala Lumpur (Khairiah et al., 2006) and Kedah and Penang (Jamil et al., 2011). Heavy metals have the potential to accumulate, which may affect the end users.

Rice is a staple food that is grown in many parts of the world. It is part of a good diet and is central to the dietary habits of people in the Southeast Asian region. After Kedah and Selangor, Kelantan is one of the major rice-growing areas in Malaysia. In Kelantan, the majority of paddy fields are under the controll of the Kemubu Agricultural Development Authority (Kemubu). Several paddy fields which are located in Kelantan are actively used for paddy planting and some of them are no longer suitable for rice production. They also serve as habitats for many living aquatic organism, particularly small fish and invertebrate species such as Asian swamp eels. In paddy field systems, paddies are planted using several processes of paddy practice management which involve the tillage of flooded soil (puddling), followed by rice transplantation and cultivation in a submerged environment in time for

the harvest (ZI-TONG, 1983; Sahrawat, 2005). Generally, there the four season of rice cultivation in Kelantan are ploughing, planting of seedlings, growing and harvesting.

The introduction of heavy metals into the paddy plantation ecosystem is the result of various agricultural practices which likely involve the aggressive application of agrochemical fertilizers, herbicides, pesticides, and inlets of contaminated water. The introduction of agrochemical fertilizers and pesticides by farmers onto paddy fields has caused the build-up of pollutants over the years. This repeated process of paddy management practice over many years could have increased the availability of pollutants in the paddy soil. For example, ploughing could result in the resurfacing of pollutants from the previous paddy cycles. In addition, the usage of fertilizer during the seeding and growing seasons could enhance the availability of pollutants in paddy soils. Once pollutants enter the environment, some of the metals may be transformed into other forms which usually occur from loosely bound fractions (such as exchangeable fractions) to strongly bound fractions (such as Fe-Mn oxide and organic matter bound fractions) (Lu et al., 2005). They have also shown a high tendency to accumulate and biomagnify in water, sediment, soil and aquatic food chains, causing harmful effects on plant, living aquatic organisms, and human life depending upon the concentration (Forstner and Wittman, 1981). Since humans are the final consumers in food chain system, there is a high tendency for these pollutants to accumulate in human organs, which in turn may cause severe to chronic toxicity when the level of pollutants exceeds the level that is required for normal body metabolism. Thus, the main objective of this research is to investigate the level of Zn, Cu, Cd, Ni and Pb concentration and distribution in existing paddy field areas as well as identify the associated risks.

A selection of Asian swamp eels was chosen as the bio-indicator in order to assess the environmental health of paddy fields in this area. Several bio-indicators agents have been used previously in coastal and marine environments in Malaysia such as *Perna viridis* (Ismail, 1990), *Oryzias javanicus* (Khodadoust et al., 2013), *Telescopium telescopium* (Ismail and Safahieh, 2005), *Nerita lineate* (Ismail and Ramli, 1997), *Uca annulipes* (Ismail et al., 1991), *Dotilla myctiroides* (Zulkifli et al., 2012a), *Thais* spp. (Mohamat-Yusuff et al., 2010; 2011), *Anguilla bicolour bicolour* (Arai et al., 2012) and *Periophthlmodon schlosseri* (Ikram et al., 2010; Zulkifli et al., 2012b). Ismail (1994) reported the use of freshwater molluscs, snails and bivalves as bio-indicators in assessing the presence of toxic chemicals in rice fields of Kuala Klawang in. However, the Asian swamp eel has not been used as a bio-indicator in paddy field areas.

The choice of Asian swamp eels is based on a few criteria. They are easy to collect, abundant and long-living. They are also able to tolerate different concentrations of salinity. Due to its unique characteristics, especially its ability of burrowing to 1.5 m depth and living in moist land without food for up

to 3 months, the Asian swamp eel is the only fish species that exists in rice cultivation areas. Therefore, Asian swamp eels could easily be exposed to pollutants. Since the Asian swamp eel is a long-living organism, the chances of exposure and accumulation of heavy metals in particular is high in paddy field areas of Kelantan. They could be exposed to heavy metals throughout the different paddy planting seasons, allowing for a broad health risk assessment to consumers of Asian swamp eel to be conducted.

1.2 Objective of this study

The specific objectives of the study are:

i. to determine the heavy metal (Zn, Cu, Cd, Ni and Pb) levels in paddy soils collected based on four paddy seasons (plowing, seedling, growing, and harvesting) from paddy fields in Tumpat, Kelantan. The soil samples are analysed using the sequential extraction technique (SET);
ii. to examine the bioaccumulation and uptake of Zn, Cu, Cd, Ni and Pb in different tissues of Asian swamp eel, *M. albus* in the four paddy seasons, and their relation to the bioavailability of heavy metal concentration in paddy soils;
iii. to evaluate the relationship of metallothionein (MT) with Zn, Cu, Cd, Ni and Pb concentrations in selected tissues (gills, liver and muscle) of Asian swamp eels; and
iv. to investigate the reproductive biology of Asian swamp eels by using the basic histological process and gonadal somatic index

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LIST OF PUBLICATIONS

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