

EVALUATION OF HEAVY METALS (ZINC; LEAD  
AND COPPER) TOXICITIES IN FEMALE CLIMBING  
PERCH (*Anabas testudineus*)  
BLOCH (1792)

WASAN ABDULMUNEM TAHA

SULTAN IDRIS EDUCATION UNIVERSITY

2021

EVALUATION OF HEAVY METALS (ZINC; LEAD AND COPPER)  
TOXICITIES IN FEMALE CLIMBING PERCH (*Anabas testudineus*)  
BLOCH (1792)

WASAN ABDULMUNEM TAHA

THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE  
DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND MATHEMATICS  
SULTAN IDRIS EDUCATION UNIVERSITY

2021



----- 3/BO 32  
**Please tick (✓/1)**  
 Project Paper 


  
 Masters by Research 


  
 Masters by Mix Mode 


  
 Ph.D. 

✓

**INSTITUTE OF GRADUATE STUDIES**

## DECLARATION OF ORIGINAL WORK

This declaration is made on the 7 day of 09 / 2021

**i. Student's Declaration:**

I'm Wasan Abdulmunem Taha - P201610012-Faculty of Science and Mathematics  
Hereby declares that the dissertation/thesis for titled Evaluation of Heavy Metals (Zinc;  
Lead and Copper) Toxicities in Female Climbing Perch (*Anabas testudineus*) Bloch  
(1792) is my original work. I have not plagiarized from any other scholar's work and  
any sources that contain copyright had been cited properly for the permitted meanings.  
Any quotations, excerpts, reference or re-publication from or any works that have  
copyright had been clearly and well cited.

well and good.

---

Signature of the student

**ii. Supervisor's Declaration:**

I'm Assoc. Prof. Dr. Syakirah Samsudin hereby certify that the work entitled (Evaluation of Heavy Metals in Female Climbing Perch (*Anabas testudineus*)) was prepared by the above-named student, and was submitted to the Institute of Graduate Studies as a partial / full fulfillment for the conferment of the requirements for Doctor of Philosophy (By Research), and the aforementioned work, to the best of my knowledge, is the said student's work.

Symel.

Signature of the Supervisor

15/9/2021

Date \_\_\_\_\_

**INSTITUT PENGAJIAN SISWAZAH /  
INSTITUTE OF GRADUATE STUDIES**

**BORANG PENGESAHAN PENYERAHAN TESIS/DISERTASI/LAPORAN KERTAS PROJEK  
DECLARATION OF THESIS/DISSERTATION/PROJECT PAPER FORM**

Tajuk / Title: Evaluation of heavy metals (Zinc; Lead and Copper) toxicities in female climbing perch (*Anabas testudineus*) Bloch (1792)

No. Matrik / Matric No.: P20161001002

Saya / I: Wasan Abdulmunem Taha

mengaku membenarkan Tesis/Disertasi/Laporan Kertas Projek (Kedoktoran/Sarjana)\* ini disimpan di Universiti Pendidikan Sultan Idris (Perpustakaan Tuanku Bainun) dengan syarat-syarat kegunaan seperti berikut:-

*acknowledged that Universiti Pendidikan Sultan Idris (Tuanku Bainun Library) reserves the right as follows:-*

1. Tesis/Disertasi/Laporan Kertas Projek ini adalah hak milik UPSI.  
*The thesis is the property of Universiti Pendidikan Sultan Idris*
2. Perpustakaan Tuanku Bainun dibenarkan membuat salinan untuk tujuan rujukan dan penyelidikan.  
*Tuanku Bainun Library has the right to make copies for the purpose of reference and research.*
3. Perpustakaan dibenarkan membuat salinan Tesis/Disertasi ini sebagai bahan pertukaran antara Institusi Pengajian Tinggi.  
*The Library has the right to make copies of the thesis for academic exchange.*
4. Sila tandakan ( ✓ ) bagi pilihan kategori di bawah / Please tick ( ✓ ) from the categories below:-

☐ **SULIT/CONFIDENTIAL**


Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub dalam Akta Rahsia Rasmi 1972. / *Contains confidential information under the Official Secret Act 1972*

☐ **TERHAD/RESTRICTED**

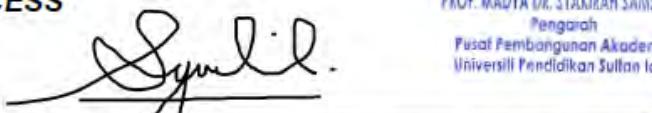
Mengandungi maklumat terhad yang telah ditentukan oleh organisasi/badan di mana penyelidikan ini dijalankan. / *Contains restricted information as specified by the organization where research was done.*

☒ **TIDAK TERHAD / OPEN ACCESS**

PROF. MADYA DR. SYAKIRAH SAMSUDIN  
Pengerah  
Pusat Pembangunan Akademik  
Universiti Pendidikan Sultan Idris

  
(Tandatangan Pelajar/ Signature)

Tarikh: 17 / 06 / 2021

  
(Tandatangan Penyelia / Signature of Supervisor)  
& (Nama & Cop Rasmi / Name & Official Stamp)  
18/06/2021

Catatan: Jika Tesis/Disertasi ini **SULIT @ TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai **SULIT** dan **TERHAD**.

*Notes: If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the related authority/organization mentioning the period of confidentiality and reasons for the said confidentiality or restriction*

## ACKNOWLEDGMENT

*“In the name of Allah, the Most Gracious and the Most Merciful”*

Alhamdulillah, first and foremost, praise be Allah, the Cherisher and Sustainer of the World, and to the Prophet Muhammad (Peace and Blessings of Allah Be Upon Him) who was sent by Allah to be a great teacher to the mankind. I am very grateful to my supervisor Assoc. Prof. Dr. Syakirah Samsudin for guiding me during my work on this research. I would like to express my sincere thanks and gratitude to her for her continuous guidance, support, and patience. I am also very grateful to my family especially my father and mother for their support blessing, patience, love, and encouragement since my childhood. I extend my love and appreciation to my husband Ehab for his continuous support and encouragement. Finally, I would like to thank all my friends who have helped me and encouraged me. This research would not have come to fruition without all the help and supports. Thank them, and Allah blesses them.



## ABSTRACT

The purpose of this study was to evaluate effect of heavy metals on the blood parameters, biochemical indicators, and histological changes in female *Anabas testudineus* Bloch (1792). A total number of 200 female *A. testudineus* fish were exposed to (zinc chloride 2mg/L, lead chloride 2 mg/L, and copper chloride 1.5 mg/L) for 35 days. The numbers of red and white blood cells were counted by using hemocytometer, while blood glucose and liver enzymes were measured by spectrophotometer. The concentrations of heavy metals in the muscles were determined by using Atomic Absorption Spectrophotometer. The tissues of ovary, liver, gut, and gills were collected and processed for histological analyses. The results of this study showed that exposure to these heavy metals led to a significant decreased ( $p < 0.05$ ) in the number of red blood cells, the weight of ovaries, and liver compared to the control group. However, there were a significant increments ( $p < 0.05$ ) observed on the number of white blood cells, blood glucose level, and liver enzymes; Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), and Alkaline Phosphatase (ALP) as compared to the control group. A significant increased ( $p < 0.05$ ) in the concentration of the heavy metals was identified, and the concentration of heavy metals in the muscle tissues was in the following order;  $Zn > Cu > Pb$ . Histology in all tissues examined assessment showed exhibited cellular bulging, swelling, and necrosis. As a conclusion, heavy metals exposure to female *A. testudineus* shows there are negative impacts on the blood parameters, blood glucose and liver enzymes. In addition, histological study on the selected organs ovaries, liver, intestine, and gills also negatively affected. This study implicates that heavy metals contributed negative effect on *A. testudineus* physiology and information from this study can be further used to evaluate and propose solution to manage negative impact of heavy metals on fish.





## PENILAIAN KETOKSIKAN LOGAM BERAT (ZINK, PLUMBUM DAN KUPRUM) TERHADAP IKAN PUYU BETINA (*Anabas testudineus*) BLOCH (1792)

### ABSTRAK

Tujuan kajian ini adalah untuk menilai kesan logam berat pada parameter darah, petunjuk biokimia, dan perubahan histologi pada *Anabas testudineus* Bloch (1792) betina. Sebanyak 200 ekor *A. testudineus* betina didedahkan kepada ( zink klorida 2mg / L, plumbum klorida 2 mg / L, dan kuprum klorida 1.5 mg / L) selama 35 hari. Jumlah sel darah merah dan putih dihitung dengan menggunakan hemasitometer. Manakala glukosa darah dan enzim hati diukur dengan spektrofotometer. Kepekatan logam berat pada otot ditentukan dengan menggunakan Spektrofotometer Penyerapan Atom. Tisu ovari, hati, usus, dan insang dikumpulkan dan diproses untuk analisis histologi. Hasil kajian ini menunjukkan bahawa pendedahan kepada logam-logam berat ini menyebabkan penurunan yang signifikan ( $p < 0.05$ ) pada jumlah sel darah merah, berat ovari, dan hati berbanding dengan kumpulan kawalan. Manakala terdapat peningkatan yang ketara ( $p < 0.05$ ) pada jumlah sel darah putih, tahap glukosa darah, dan enzim hati; Alanin Aminotransferase (ALT), Aspartat Transaminase (AST), Alkali Fosfatase (ALP) berbanding dengan kumpulan kawalan. Peningkatan yang signifikan ( $p < 0.05$ ) pada kepekatan logam berat telah dikenal pasti, dan kepekatan logam berat dalam tisu otot berada dalam urutan berikut;  $Zn > Cu > Pb$ . Penilaian histologi menunjukkan pembedahan sel, bengkak, dan nekrosis pada tisu yang dikaji. Sebagai kesimpulan, pendedahan logam berat kepada *A. testudineus* betina menunjukkan terdapat kesan negatif terhadap parameter darah, glukosa darah dan enzim hati. Di samping itu, kajian histologi pada organ ovari, hati, usus, dan insang yang terpilih juga terkesan secara negatif. Implikasi kajian menunjukkan bahawa logam-logam berat memberi kesan negatif kepada fisiologi *A. testudineus* dan maklumat daripada kajian ini boleh digunakan bagi menilai dan mencadangkan penyelesaian untuk mengurus kesan logam-logam berat terhadap ikan.



## TABLE OF CONTENTS

	<b>Page</b>
<b>DECLARATION OF ORIGINAL WORK</b>	ii
<b>DECLARATION OF THESIS</b>	iii
<b>ACKNOWLEDGEMENT</b>	iv
<b>ABSTRACT</b>	v
<b>ABSTRAK</b>	vi
<b>TABLE OF CONTENTS</b>	vii
<b>LIST OF TABLES</b>	xiv
<b>LIST OF FIGURES</b>	xv
<b>LIST OF ABBREVIATIONS</b>	xviii
<b>CHAPTER 1 INTRODUCTION</b>	
1.1 Background of The Study	1
1.2 Problem Statement	2
1.3 Research Objectives	5
1.4 Research Questions	5
1.5 Research Hypothesis	6
1.6 Significance of the Study	6
1.7 Theoretical Importance the Study	7
1.8 Scope and Limitations	8
<b>CHAPTER 2 LITERATURE REVIEW</b>	
2.1 Inroduction	10
2.2 General Description of <i>Anabas testudineus</i>	12
2.2.1 Taxonomic Hierarchy	13



2.2.2	Identification of Male and Female of <i>A. testudineus</i>	14
2.2.3	Popular Names of <i>A. testudineus</i>	15
2.2.4	Types of <i>Anabas</i>	15
2.2.5	Habitat of <i>A. testudineus</i>	16
2.2.6	Deployment Areas of <i>A. testudineus</i>	17
2.2.7	Feeding Behavior of <i>A. testudineus</i>	18
2.3	Anatomical Composition of <i>A. testudineus</i>	18
2.3.1	Ovary of <i>A. testudineus</i>	19
2.3.1.1	Gonadosomatic Index (GSI)	20
2.3.2	Liver of <i>A. testudineus</i>	22
2.3.3	Intestine of <i>A. testudineus</i>	25
2.3.4	Gills of <i>A. testudineus</i>	26
2.4	Heavy Metals Risks and Aquatic Organisms	29
2.5	Heavy Metals and Physiological Disorders	31
2.6	Effect of Malaysian Water Pollution on Fish	32
2.7	Malaysian Water Environments and Heavy Metals	34
2.8	Malaysian Water Pollutions and Fish	36
2.9	Sources and Chemical Description of Zinc Chloride	37
2.9.1	Chemical Properties of Zinc Chloride	38
2.9.2	Toxicity of Zinc Chloride on Fish	39
2.10	Effect of Zinc on Physiological Activities of Fish	40
2.10.1	Effect of Zinc on Blood of Fish	40
2.10.2	Effect of Zinc on Liver of Fish	41
2.10.3	Effect of Zinc on Reproductive Organs of Fish	41
2.10.4	Toxicity of Zinc on Human	42

2.11	Source and Chemical Description of Lead Chloride	43
2.11.1	Chemical Properties of Lead Chloride	44
2.11.2	Toxicity of Lead Chloride on Fish	44
2.12	Effect of Lead on Fish Physiological Events	45
2.12.1	Effect of Lead on Blood Components of Fish	46
2.12.2	Effects of Lead on Liver of Fish	46
2.12.3	Lead Toxicity on Human	47
2.13	Sources and Chemical Description of Copper Chloride	48
2.13.1	Chemical Properties of Copper Chloride	49
2.13.2	Toxicity of Copper Chloride on Fish	49
2.14	Effect of Copper on Physiological Activities of Fish	51
2.14.1	Effect of Copper on Blood Components of Fish	51
2.14.2	Effect of Copper on Liver of Fish	52
2.14.3	Toxicity of Copper on Human	53

### CHAPTER 3 RESEARCH METHODOLOGY

3.1	General Methodology	54
3.2	Site of the Study	56
3.3	Procurement of <i>A. testudineus</i>	56
3.3.1	Acclimatization of <i>A. testudineus</i>	57
3.3.2	Parameters of Water Quality	58
3.3.3	Handling of <i>A. testudineus</i>	59
3.4	Experimental Design	59
3.4.1	Determination of Acute Lethal Dose	60
3.4.2	Experiment Setting	61
3.4.3	Anesthesia	62

3.4.4	Collection of Blood Samples	63
3.4.4.1	Blood Collection of Caudal Artery	63
3.4.5	Blood Analysis of <i>A. testudineus</i>	64
3.4.5.1	Blood Cell Counts	64
3.4.6	Biochemical Analysis of <i>A. testudineus</i>	65
3.4.6.1	Blood Glucose Analysis of <i>A. testudineus</i>	65
3.4.6.2	Procedure of Blood Glucose Analysis	66
3.4.7	Functions of Liver in <i>A. testudineus</i>	67
3.4.7.1	Measuring Alanine Aminotransferase (ALT) Enzyme	67
3.4.7.2	Procedure of Preparing (ALT) Enzyme Reagent	68
3.4.7.3	Measuring Aspartate Aminotransferase (AST) Enzyme	68
3.4.7.4	Procedure of Preparing (AST) Enzyme Reagent	70
3.4.7.5	Measuring Alkaline phosphatase (ALP) Enzyme	70
3.4.7.6	Procedure of Preparing (ALP) Enzyme Reagent	71
3.5	Determination of Heavy Metal in the Muscles of <i>A. testudineus</i>	71
3.6	Preparation of Microscopic Sections	72
3.6.1	Fixation	73
3.6.2	Dehydration	73
3.6.3	Clearing	73
3.6.4	Embedding	74
3.6.5	Trimming and Sectioning	74

3.6.6	Staining	75
3.6.7	Mounting	75
3.6.8	Microscopic Study and Microscopic Photograph	76
3.7	Statistical Analysis	76

## CHAPTER 4 RESULTS AND DISCUSSION

4.1	Toxicity Study of Acute Lethal Dose Test	77
4.1.1	Determination Acute Lethal Dose of Zinc Chloride	78
4.1.2	Determination Acute Lethal Dose of Lead Chloride	79
4.1.3	Determination Acute Lethal Dose of Copper Chloride	80
4.2	Effect of Heavy Metals on Blood Components of Female <i>A. testudineus</i>	81
4.2.1	Effect of Heavy Metals on Red Blood Cell Counts of Female <i>A. testudineus</i>	82
4.2.2	Effect of Heavy Metals on White Blood Cell Counts of Female <i>A. testudineus</i>	87
4.3	Effect of Heavy Metals on Biochemical Changes in Female <i>A. testudineus</i>	91
4.3.1	Effect of Heavy Metals on the Level of Blood Glucose in Female <i>A. testudineus</i>	92
4.3.2	Effect of Heavy Metals on the Concentration of Alanine Aminotransferase (ALT) Enzyme in Female <i>A. testudineus</i>	97
4.3.3	Effect of Heavy Metals on the Concentration of Aspartate Transaminase (AST) Enzyme in Female <i>A. testudineus</i>	103
4.3.4	Effect of Heavy Metals on the Concentration of Alkaline Phosphate (ALP) Enzyme in Female <i>A. testudineus</i>	108
4.4	Heavy Metals Concentration in the Muscles of Female <i>A. testudineus</i>	114

4.5	Effect of Heavy Metals on the Organs Index of Female <i>A. testudineus</i>	121
4.5.1	Effect of Heavy Metals on the Gonadosomatic Index of Female <i>A. testudineus</i>	121
4.5.2	Effect of Heavy Metals on the Hepatosomatic Index of Female <i>A. testudineus</i>	126
4.6	Histological Studies in Female <i>A. testudineus</i> Exposed to Heavy Metals	131
4.7	Histological Changes in the Ovary of Female <i>A. testudineus</i>	132
4.7.1	Histological Structure of Control Group Ovary in Female <i>A. testudineus</i>	133
4.7.2	Effect of Heavy Metals on Ovary Tissue	134
4.7.2.1	Effect of Zinc Chloride on the Ovary tissue of Female <i>A. testudineus</i>	134
4.7.2.2	Effect of Lead Chloride on the Ovary Tissue of Female <i>A. testudineus</i>	136
4.7.2.3	Effect of Copper Chloride on the Ovary Tissue of Female <i>A. testudineus</i>	140
4.8	Histological Changes in the Liver of Female <i>A. testudineus</i>	145
4.8.1	Histological Structure of Control Group Liver in Female <i>A. testudineus</i>	145
4.8.2	Effect of Heavy Metal on the Liver Tissue of Female <i>A. testudineus</i>	146
4.8.2.1	Effect of Zinc Chloride on the Liver Tissue of Female <i>A. testudineus</i>	147
4.8.2.2	Effect of Lead Chloride on the Liver Tissue of Female <i>A. testudineus</i>	149
4.8.2.3	Effect of Copper Chloride on the Liver Tissue of Female <i>A. testudineus</i>	152
4.9	Histological Change of Intestine in Female <i>A. testudineus</i>	157
4.9.1	Histological Structure of Control Group Intestine in Female <i>A. testudineus</i>	157

4.9.2	Effect of Heavy Metals on the Intestine Tissue in Female <i>A. testudineus</i>	159
4.9.2.1	Effect of Zinc Chloride on the Intestine Tissue Female <i>A. testudineus</i>	159
4.9.2.2	Effect of Lead chloride on the Intestine Tissue of Female <i>A. testudineus</i>	161
4.9.2.3	Effect of Copper Chloride on the Intestine Tissue of Female <i>A. testudineus</i>	163
4.10	Histological Change of Gills in Female <i>A. testudineus</i>	167
4.10.1	Histological Structure of Control Group Gills in Female <i>A. testudineus</i>	168
4.10.2	Effect of Heavy Metal on the Gill Tissue of Female <i>A. testudineus</i>	169
4.10.2.1	Effect of Zinc Chloride on the Gill Tissue of Female <i>A. testudineus</i>	170
4.10.2.2	Effect of Lead Chloride on the Gill Tissue of Female <i>A. testudineus</i>	172
4.10.2.3	Effect of Copper Chloride on the Gill Tissue of Female <i>A. testudineus</i>	174

## CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1	Conclusion	179
5.2	Recommendations	181

<b>REFERENCES</b>	183
-------------------	-----

<b>LIST OF PUBLICATION</b>	246
----------------------------	-----

<b>APPENDICES</b>	247
-------------------	-----



## LIST OF TABLES

No. Table		Page
2.1	Types of Anabas species	16
2.2	Heavy metals distributed (Zn, Pb, and Cu) in Peninsular Malaysia of past 20 years	35
3.1	The number of dead fish in determining acute lethal dose.	61
4.1	Summarizing the changes observed in the histological structure of ovaries due to heavy metals exposure	144
4.2	Summarizing the changes observed in the histological structure of liver due to heavy metals exposure	156
4.3	Summarizing the changes observed in the histological structure of intestine due to heavy metals exposure	166
4.4	Summarizing the changes observed in the histological structure of gills due to heavy metals exposure.	178





## LIST OF FIGURES

No. Figure		Page
2.1	Pathway of the transition of heavy metals to the fish	12
2.2	Male (upper) and female (lower) of <i>A. testudineus</i> .	15
2.3	Internal structure of ovary in <i>A. testudineus</i>	20
2.4	internal structure of liver in <i>A. testudineus</i>	25
2.5	internal structure of intestine in <i>A. testudineus</i>	26
2.6	Targetted regions when exposed to zinc	43
2.7	Targetted regions when exposed to lead	48
3.1	Location of high tech aquaculture	57
3.2	Package of <i>A. testudineus</i> Food	59
3.3	Hematological and biochemical analysis	63
4.1	LC50 value of $ZnCl_2$	78
4.2	LC50 value of $PbCl_2$	79
4.3	LC50 value of $CuCl_2$	80
4.4	Changes in red blood cells count of female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	83
4.5	Changes in white blood cells counts of female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	88
4.6	Changes in serum glucose level (mg/dl) of female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	93
4.7	Changes in the level of Alanine Aminotransferase (ALT) enzyme in female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	99







4.8	Changes in the level of Aspartate Transaminase (AST) enzyme of female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	104
4.9	Changes in the level of Alkaline Phosphatase (ALP) enzyme of female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days	110
4.10	Heavy metals Concentration in the muscles of female <i>A. testudineus</i> following of zinc (2.0 mg/L), lead chloride, and (1.5 mg/L) of copper chloride exposure for 35 days	116
4.11	Effect of heavy metals on gonadosomatic index in female <i>A. testudineus</i> following of zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days.	122
4.12	Effect of heavy metals on hepatosomatic index in female <i>A. testudineus</i> following zinc chloride (2.0 mg/L), lead chloride (2.0 mg/L) and copper chloride (1.5 mg/L) exposure for 35 days in female.	127
4.13	Histological structure of ovary in control group.	133
4.14	Histological structure of ovary in zinc chloride group	135
4.15	Histological structure of ovary in lead chloride group	139
4.16	Histological structure of ovary in copper chloride group	142
4.17	Histological structure of liver in control group	146
4.18	Histological structure of liver in zinc chloride group	148
4.19	Histological structure of liver in lead chloride group	151
4.20	Histological structure of liver in copper chloride group	154
4.21	Histological structure of intestine in control group	158
4.22	Histological structure of intestine in zinc chloride group	160
4.23	Histological structure of intestine in lead chloride group	162
4.24	Histological structure of intestine in copper chloride group	164
4.25	Histological structure of gills in control group	169





4.26	Histological structure of gills in zinc chloride group	170
4.27	Histological structure of gills in lead chloride group	173
4.28	Histological structures of gills in copper chloride group	



## LIST OF ABBREVIATIONS

<i>A.testudineus</i>	<i>Anabas testudineus</i>
AchE	Acetylc cholinesterase
Al	Aluminum
ALAD	Aminolevulinate Dehydratase
ALP	Alkaline phosphate
ALT	Alanine amino transfrase
AST	Aspartate amino transfrase
<i>C.punctatus</i>	<i>Corydoras punctatus</i>
CA	Carbonic Anhydrase
CAT	Catalase
Cd	Cadmium
Co	Cobalt
Co <sub>2</sub>	Carbon dioxide
Cr	Chromium
Cu	Copper
CuCl <sub>2</sub>	Copper chloride
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
Fe	Iron
GSHPx	Glutathione peroxidase
GSH-R	Glutathione-Reductase

GSSG	glutathione disulfide
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
Hb	Hemoglobin
HcT	Hematocrit
Hg	Mercury
LPo	Lipid peroxidation
MAD	Malondialdehyde
MAPK	Mitoge activated protein kinase
McH	Mean corpuscular hemoglobin
McHc	Mean corpuscular hemoglobin concentration
McV	Mean corpuscular volume
MDH	MalateDehydrogenase
Mo	Molybdenum
MPO	Meloperoxidase
MRCs	Mitochondria Rich Cells
NADPH	Nicotinamide adenine dinucleotide phosphate
Ni	Nickel
NKA	Na <sup>+</sup> /K <sup>+</sup> ATPase
<i>O. mossambicus</i>	<i>Oreochromis mossambicus</i>
<i>O. niloticus</i>	<i>Oreochromis niloticus</i>
O.S	Oxidative Stress
O-2	Supper oxide anion
OH	Hydroxyl radical
Pb	Lead



Pb c <sub>l</sub> 2	Lead chloride
RBCs	Red Blood Cells
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
Sn	Tin
SOD	Sopperoxide dismutase
<i>T.leeri</i>	<i>Trichogaster.leeri</i>
Trxs	Thioredoxins
WBCs	White Blood Cells
WHO	World Health Organization
Zn	Zinc
Znc <sub>l</sub> 2	Zinc chloride



## CHAPTER 1

### INTRODUCTION

The current chapter presents a brief background of the study, problem statement, research objectives and questions, the significance and theoretical importance of this study, finally; the scope and limitation.

#### 1.1 Background of the study

The physical and chemical properties of the aquatic environment affect the biological and physiological indicators of fish (Mohammed et al., 2016), aquatic environment pollution by heavy metals pose a serious threat the health of aquatic living organisms (Ali, Khan, & Ilahi, 2019; Bassem et al., 2020; Haseena et al., 2017; Koff et al., 2016; & Rahman et al., 2012). The permissible limits of zinc in fresh water and sea water

are 0.0766 mg/l respectively (Duruibe et al., 2007); while the permissible limits of lead in fresh water and sea water are 0.18–1.00; 0.02–0.05 mg/l, the permissible limits of copper in fresh water and sea water are 0.1 mg/l (Mehana et al., 2020)

Several studies have concluded that they are heavy metals accumulation in large quantities in the coasts and Malaysian fish (Yunus et al. 2011; Bashir et al. 2012). A study on the Port Dickson coast to evaluate the heavy metals impacts on fish confirmed these allegations that fishes in this coast do accumulate heavy metals (Praveena & Lin, 2015). Estuaries and rivers have not been spared the heavy metals pollutants (Vasanthi et al., 2019). A study reported that exposure of *Anabas testudineus* to sub-lethal concentrations of heavy metals caused to hepatocytes degeneration and necrosis, as well as gill tissues hemorrhage (Benjamin & Kutty, 2019; & Fatema et al., 2019).

Heavy metals pollutant had a series of adverse effects on the humans health (Jan et al., 2015); where symptoms that arisen as a result of heavy metals poisoning included intellectual disability in children, dementia in adults, central nervous system disorders, liver , kidney diseases, insomnia, and depression (Flora, Mittal & Mehta, 2008).

## 1.2 Problem Statement

The ecosystem is the main repository of heavy metals pollutants (Hook, Gallagher & Batley, 2016). Fishes can give early warning about the heavy metals effects that may later affect human health (Kroon, Streten & Harries, 2017). Fish consumed all over the world because of nutritional value, its high quality proteins, in addition its content high



omega-3 fatty acid, its content low of saturated fat, as well as high level of vitamin-B (Arulkumar, Paramasivam & Rajaram, 2017; Durmuş, 2019; Özden et al., 2018). Bioaccumulation of heavy metals in the aquatic organisms causes to change their biological activities (Khoshnood, 2017), such as negative influence on reproductive ability, physiological activities, cellular, gene poisoning, and genetic mutations (Mattos et al., 2017).

Fast economic development in Malaysia has led to the heavy metals accumulation in aquatic environments, that's led increased water pollution in its coastal regions (Bashir et al., 2013). The agricultural and industrial activities worsen the scenario by contributed to pollution of seawater by heavy metal and accumulate it later in commercially important marine fishes (Salam et al., 2019; Salleh & Halim 2018; Sarkar et al., 2016). It is known that heavy metal compounds impacts on the hematological and histological indicators of several body organs in different fish species. Therefore, consumption of fish with a high level of heavy metal pollutants (Salamat, Movahedinia & Mohammadi, 2014), causing respiratory dysfunction, hepatic, kidney damage, bone disease, hypertension, influence on reproductive ability and tumors (Satarug, Vesey & Gobe, 2017).

Abdel-Tawwab, 2016; Celik et al., (2013); Javed & Usmani, (2014); and Suganthi et al., (2015) studies have indicated significant changes occurred of hematological, biochemical and histological indicators as a result of accumulation zinc compounds in the fish body. Research on freshwater fish indicated that fish exposed to lead compounds caused significant changes in hematological; biochemical; and histological parameters (Aich et al., 2015; Ribeiro et al., 2013; Verma, Gupta, & Gupta,





2020). Authman et al., (2015); Khabbazi et al., (2014); Moosavi & Shamushaki, (2015); Nunes et al., (2020); and Ostaszewska et al., (2016) they indicated that accumulation of copper in the fish body causes harmful effects on hematological and biochemical indicators. A study conducted on *A. testudineus* observed a significant accumulation of heavy metal in the muscles, in addition, it effected on the blood and enzymatic parameters (Dey et al., 2019; Majed et al., 2019).

Maximum levels in ( $\mu\text{g/g}$  weight) of heavy metals in fish described in literature and range of concentrations found in commercial fishes of zinc; lead; and copper were respectively 40; 0.5; and 30  $\mu\text{g/g}$  as FAO/WHO was recommends (Elhabris et al., 2013); therefore, increased concentration of these minerals above the recommended level leads to the occurred of all the previously mentioned disorders

These previous studies provided sufficient evidence about the effect of several heavy metal compounds on the hematological, and histological indicators of different body organs in various fish species, but no similar studies on the effect of zinc, lead and copper chlorides on blood components, biochemical indicators, and histological changes in ovaries, livers, intestines, and gills of female *A. testudineus*. Since *A. testudineus* is widely consumed by Malaysia, it is worthy to evaluate these heavy metals effect on the *A. testudineus*.



### 1.3 Research Objectives

In general, the research aimed to identify the effects of environmental pollution on hematological, biochemical, and histological changes of female *A. testudineus*. More specifically, the research objectives are as the followings:

1. To study the effect of heavy metals on the blood components (red and white blood cells count).
2. To identify the effect of heavy metals on biochemical changes in female *A. testudineus*.
3. To identify most accumulated heavy metals in the muscles of female *A. testudineus*.
4. To evaluate the effect of heavy metals on the histological structure of ovary, liver, intestine, and gills in female *A. testudineus*.



### 1.4 Research Questions

- 1- How heavy metals do affected the blood components (red and white blood cell count)?
- 2- Do heavy metals cause significant changes in biochemical changes of female *A. testudineus*?
- 3- Which heavy metals accumulated the most in the muscles of female *A. testudineus*?
- 4- How heavy metals do affected the histological structure of female *A. testudineus* ovary, liver, intestine, and gills?



## 1.5 Research Hypothesis

- 1- There is no significant different in red and white blood cells count in the female *A. testideneus* treated by heavy metals (zinc; lead and copper chlorides).
- 2- There is no significant diferent in biochemical changes of female *A. testudineus* treated by heavy metals (zinc; lead and copper chlorides).
- 3- There is no significant diferent in the level of metal accumulated in the muscles of female *A. testideneus* exposed to heavy metals (zinc; lead and copper chlorides).

## 1.6 Significance of the Study

Practically, through a review of previous studies, the effect of different compounds of zinc, lead and copper on many organs of different types of fish was concluded, but the effect of zinc, lead and copper chlorides on female *A. testideneus* was not addressed therefore this study will be conducted to investigate the effect of heavy metals on female *A. testideneus* in order to bridge the gap related to the effect of these chlorides on female *A. testideneus*.

Since *A. testideneus* fish is consumed in large quantities by the consumers of Asian countries the conduct of the current study is very necessary in order to keep *A. testudineus* fish from the impact of different heavy metals. These heavy metals directly affect human health, if a human consumed fishes contaminated by heavy metals. Thus, the current study was able to provide more information about the effects of heavy

metals (zinc, lead, and copper chlorides) on female *A. testudineus*. Therefore present information encourages doing future studies about the effects of different chlorides on the physiological and biological activities of different types of fish or aquatic organisms.

### 1.7 Theoretical Importance the Study

This study contributed through the literature review approach to provide an overview of existing information about the heavy metals impact, and highlighted the research methodology of heavy metals affected on fish biology. This study also contributed to fulfilling the lack of research in this research area. This study can bring several benefits as well, imposes a sort of organization on the publications mass, and provided researchers with important insights into the research field.

The current study is necessary because it can reveal gaps in previous researches, and new mapping of the literature on evaluated of heavy metals effects on physiological and biological indicators of fish or other aquatic organisms. Due to the scarcity of available studies of the heavy metals effects on the hematological changes, biochemical and histological indicators in female climbing perch *A. testudineus* (Bloch, 1792), it is necessary to determine the heavy metals effects especially  $ZnCl_2$ ;  $PbCl_2$ ; and  $CuCl_2$ , on the hematological changes, biochemical and histological indicators in female *A. testudineus*.



## 1.8 Scope and Limitations

The scope of this research is defined by the following considerations:

1. This research focuses on the methodology to evaluate effects of heavy metals (zinc, lead, copper chlorides) on blood components (RBCs and WBCs), and biochemical changes (blood glucose and liver enzymes) of female *A. testudineus* based on a statistical analysis of the data, which obtained from the results of fish blood analysis.
2. This research focuses on the methodology to evaluate effects of heavy metals (zinc, lead, copper chlorides) on the histological structure of female *A. testudineus*, for many tissues such as ovary, liver, intestine, and gills, to proof the validity of the proposed methodology.

