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

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SULTAN IDRIS EDUCATION UNIVERSITY

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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 chloride1.5 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. testiduneus* 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 pembenjolan 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.

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LIST OF ABBREVIATIONS

A.testudineus	*Anabas testudineus*
AchE	Acetyle cholinesterase
Al	Aluminum
ALAD	Aminolevulinate Dehydratase
ALP	Alkaline phosphate
ALT	Alanine amino transfrase
AST	Aspartate amino transfrase
C.punctatus	*Corydoras punctatus*
CA	Carbonic Anhydrase
CAT	Catalase
Cd	Cadmium
Co	Cobalt
Co2	Carbon dioxide
Cr	Chromium
Cu	Copper
Cu cl2	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
H2O2	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+ /K+ ATPase
O. mossambicus	*Oreochromis mossambicus*
O.niloticus	*Oreochromis niloticus*
O.S	Oxidative Stress
O-2	Supper oxide anion
OH	Hydroxyl radical
Pb	Lead

Pb cl2	Lead chloride
RBCs	Red Blood Cells
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
Sn	Tin
SOD	Sopperoxide dismutase
T.leeri	*Trichogaster.leeri*
Trxs	Thioredoxins
WBCs	White Blood Cells
WHO	World Health Organization
Zn	Zinc
Zncl2	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 wate

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 (µg/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 µg/g as FAO/WHO was recommends (Elnabris 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 comnsumed 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 evalaute 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. testideneus*. 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 to 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.