Assesment of Heavy Metals in Nile Tilapia (*Oreochromis niloticus*) Collected from Malacca River, Malaysia

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Abstract: Present study aimed to determine selected heavy metals (Cu, Zn, Pb) concentration in Nile tilapia (*Oreochromis niloticus*) collected from Malacca River, Malaysia. Samples of *O. niloticus* were collected from several locations along the Malacca River, transported back to the laboratory, treated with standard digestion procedures and analyzed with an atomic absorption spectrophotometer (AAS). Recovery heavy metal concentration of the reference material was satisfactorily between 98 to 102 percent. Results demonstrate that Cu in *O. niloticus* from Malacca River was significantly higher than other studied metal. Concentration of heavy metals may vary depending on locally anthropogenic activities. Concentration of heavy metals may not be reduced after short time acclimatization. Liver was found to contain significant amount of heavy metals. Detail studies of various aspects involving *O. nilotiucs* should be conducted as it shows a potential candidate of biomonitoring agent to determine the water pollution in order to manage the aquatic ecosystem.

1 INTRODUCTION

Since several decades ago, Malaysia has emerged as one of the Asian countries that have rapid boost in economy, industry, agriculture and urbanization. However, unsustainable and improper planning for growth and developments by authorizes and communities could severely affect the environment. According to [1], the accumulation of heavy metals such as As, Cu, Pb, Zn, and Fe in the environments are due to the anthropogenic activities or men made. Industrial activities can lead to the severe environmental condition from the untreated sewage discharged. Among the characteristics of the heavy metals are non-biodegradable, very persistence and remain still in the environments (Zulkifli *et al*, 2010). The monitoring of the water sample, sediments and the biota can be used to determine the amount of the heavy metals in the aquatic ecosystem such as river (Naji and Ismail, 2012).

Some heavy metals are easily enriched into fishes including those species that are commercial and having significantly high economic value in markets (Ashraf *et al*, 2012). For example, the Nile tilapia (*Oreochromis niloticus*) is an important fish in many countries including Malaysia and increasingly reared in aquaculture (Cogun *et al*, 2003). Fish is suitable for biomonitoring water quality and easily be interpreted by the consumers in public. Some environmental changes can be detected by the fish and it can be used as monitor and indicator to the pollution (Ashraf *et al*, 2012). Fish is very suitable model to be used in the study of accumulation of heavy metals in different tissues of fish (Cogun *et al*, 2003). According to (Batvari *et al*, 2007), the present of fish in large scale, potentially metal accumulation, long lifespan and optimum range of size and easy to be sampled are the reasons why fish can be used as a good bioindicator and biomonitor. Among the advantages of using tilapia fish are because of their tolerance to poor water quality and the fact that they have wide range of natural food organism such as plankton, detritus, aquatic invertebrates and decomposing organic matter (Popma and Masser, 1999). In addition, tilapia is the most tolerant fish as compared to other freshwater fish in the high salinity, high water temperature and low dissolved oxygen.

Heavy metals can accumulate into biological tissues through the absorption from the water body

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and exposed to the human via consumption from the food web (Zulkifli *et al*, 2014). Bioaccumulation of heavy metals in human body can lead to harmful effect in human health from contamination of heavy metals food chain. The concentration of the heavy metals is increasing as the pass the tropic level from lower to higher tropic level and this phenomenon is known as biomagnifications (Zulkifli *at al*, 2016). The effect on human health later can be acute and chronic effect depends on the level exposure to the heavy metals from the consumption of fish that contain heavy metals such as Zn, Cu, Pb, and Fe. Besides that, some heavy metals can cause neurological and behavior changes in children especially and some of them also carcinogenic, mutagenic, teratogenic and endocrine disruptors (Iavicoli *et al*, 2009). Therefore, present study aimed to determine heavy metals (Zn, Cu, Pb) concentration in *O. niloticus* collected from Malacca River.

2 MATERIALS AND METHOD

2.1 Sample Collection

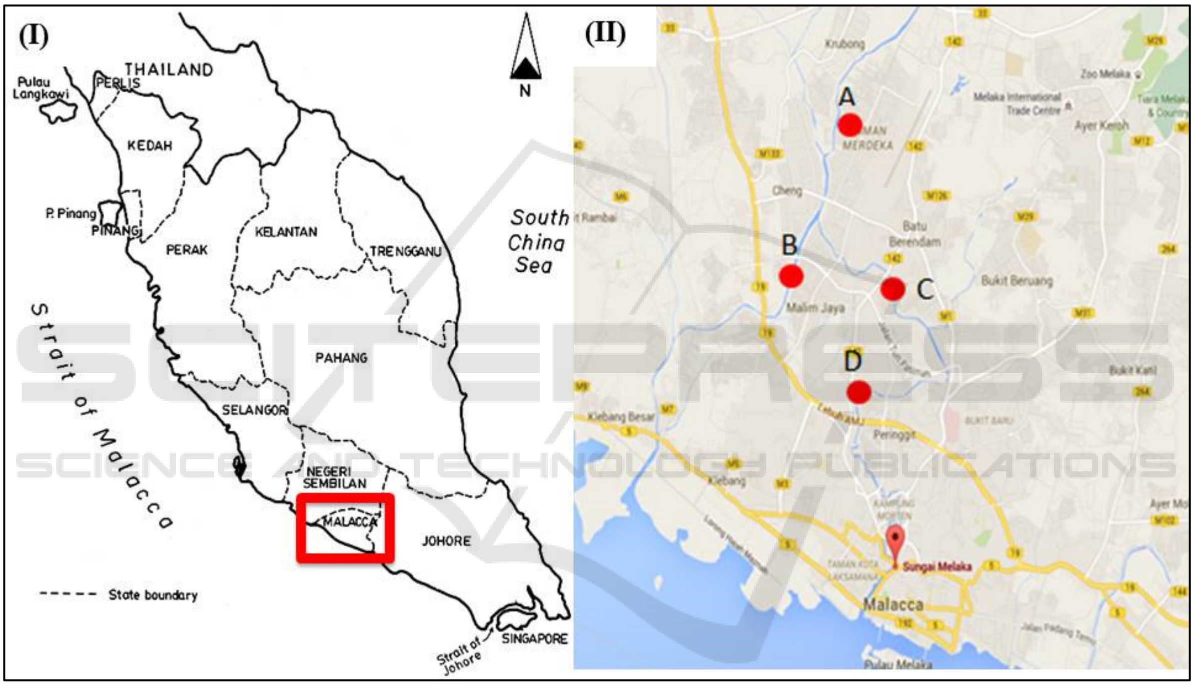

Figure 1: (I) State of Malacca (red box): (II) sampling points along Malacca River (A) Taman Merdeka; (B) Malim; (C) BatuBerendam; (D) Bachang.

A total of samples 206 individuals of tilapia fish (*O. niloticus*) were collected by using a casting net from four different locations, namely Taman Merdeka, Malim, BatuBerendam and Bachang (Figure 1). The standard length (SL) range of the collected fishwas between 18 cm to 23 cm. The fish were divided into two condition of fresh and acclimatized environment. The fresh samples are the samples were immediately stored after collected from the river, while the acclimatized samples were kept alive in a container containing aerated tap water for 24 hours. All samples were transported back to the lab

2.2 Analytical Procedures

For fish samples, all samples from each station will be pooled together. The livers and muscles were dissected separately and the scales were removed. Livers organ is selected due to its function as a detoxify site of toxic chemical in the body. Only tissues along with skin without bone were selected for analysis. Tissues with skin were selected as assuming that people consuming the fish together with skin. Those samples was dried using air-circulating oven at 60°C for 72 hours until the water content loss and the constant weight was obtained.

To digest biological tissues with acid, we followed method established by [11]. In brief, about 10 ml of concentrated nitric acid (HNO_3) was used to digest 1.0g samples in a digestion tubes. The tubes were heated in a digestion block for four hours. The heating process was first preheating for an hour and followed by increased gradually to 140°C for another three hours to completely digesting the biological samples. The temperature was increased gradually to avoid the sample from boil vigorously and may splatter across the tube. This may affect the total of sample digest and alter the precision of the result. After the digestion was completed, all the digested samples were allowed to cool at room temperature for 30 minutes. Then, the samples were diluted with 30 ml of distilled water to a fixed volume (40 ml). Next, Whatman filter paper was used to filter the digested samples and those filtrates was stored in refrigerator until metal determination. The concentration of Zn, Cu, Pb and Fe were analyzed by using Flame-Atomic Absorption Spectrophotometer (FAAS). All the data obtained was converted into µg/g basis. The SRM 2976 - Mussel Tissue (Trace Elements & Methylmercury) was used as the reference material. The recovery of heavy metals was between 98 to 102 percent.

2.3 Statistical Analysis

The data obtained from the AAS instrument were converted into micrograms per gram (µg/g) or parts per million (ppm) to obtain the actual data. All the data obtained were analyzed with multivariate analysis of variance (MANOVA) from IBM SPSS Statistics 20.0 software. Mean data were converted into tables and graph by using Microsoft Excel. All the comparisons obtained were made at least at the 95% level of significance ($p<0.05$).

3 RESULTS AND DISCUSSION

3.1 Accumulation of Heavy Metals in Tilapia Fish (*Oreochromis niloticus*)

Figure 2 shows the distribution of metal (mean ± standard error) Zn, Cu, and Pb in *O. niloticus* fish (muscle and liver) at different sampling sites. Based on the graph, the metal Cu concentration is highest in Malim with mean of 1554.36 µg/g, followed by Taman Merdeka (923.70 µg/g), Bachang (836.84 µg/g), and BatuBerendam (739.47 µg/g). While, metal Zn concentration is highest in Malim with mean of 124.51µg/g is far lower than metal Cu concentration. Metal Pb concentration is highest in Taman Merdeka (87.15µg/g) and lowest in BatuBerendam (13.214µg/g). Multivariate test of MANOVA shows that there are significantly different ($p<0.05$) between distributions of metals at different sampling sites.

Fishes are sometimes positioned at the top level of a food chain. Their condition can be closely related with the surrounding environments, including pollution. The heavy metals concentration in aquatic environments can be estimated from the water, sediments and an organism. *O. niloticus* fish can be one of the biomonitoring agents that can reflex the real environment. Fish also has high potential to bioaccumulate metals through absorption from polluted water, food (biomagnification) and sediment and transfer to human via food chain (Zulkifli *et al*, 2016;Ahmad and Suhaimi, 2010) . The accumulation of heavy metals can be influenced by factors such as gender, location, environment, season and size.

In this study, the concentration of Cu in *O. niloticus* fish including the muscle and liver is the highest in all sampling sites as the concentration of Cu in sediments which higher at all sampling site. However, metal Cu concentration is lower in the river water may due to the dilution factor in rainy season. Metals in the river water are from environmental availability of metal in sediment leaching out and also from direct sources of metal influx and sediments runoff into the river. The main source of Cu is from the industrial area and urban development construction along the Malacca River. Metal Cu is the common metal found in the water and sediment and can bioaccumulate and biomagnified in the fish from its diet (Ahmad and Suhaimi, 2010). The metals enter the fish body through the respiration from the gills, adsorption through skin and detoxification in the liver (Shouta *et al*, 2010).

Zn and Pb have significantly lower concentration in the *O. niloticus* fish which can be related to the predicted lower concentration of Zn and Cu in the sediment and water samples. Other study by (Ahmad and Suhaimi, 2010) in Lake Chini, Pahang shows that the concentration of Zn is the highest in fish however in this study the concentration of Zn and Pb in fish is higher than the fish in Chini Lake due to the a lot of human activities along Malacca River.

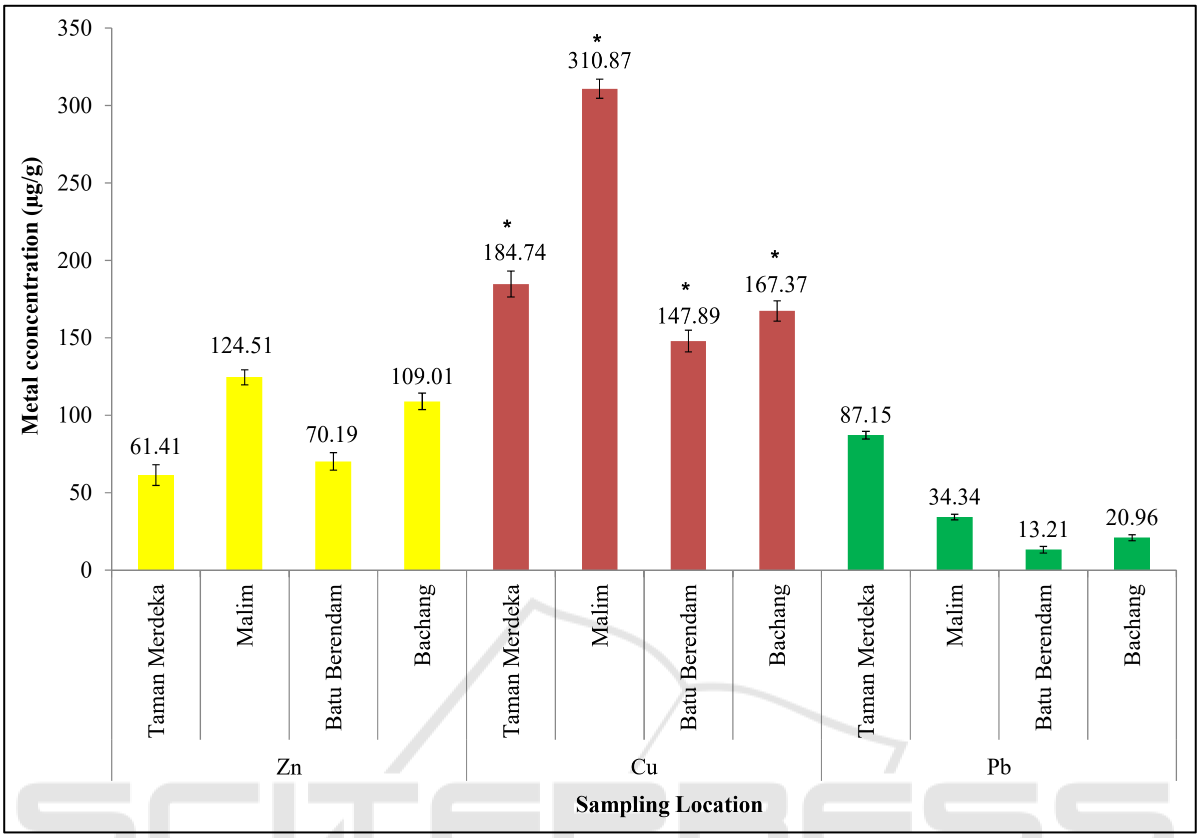

Figure 2: Distribution of the heavy metals concentration (mean ± standard error; µg/g dry weight) of Zn, Cu and Pb in *O. niloticus* fish at four different sampling sites.(Remark: * is the actual values need to be multiply with 5).

3.2 Distribution of Heavy Metals between Conditions

Figure 3 shows the comparison of metal concentration (mean ± standard error) in *O. niloticus* fish with different conditions of fresh and acclimatized sample. Based on the graph, the acclimatized sample has higher metal concentration as compared to fresh sample with metal Cu has highest concentration with mean of 1074.02 µg/g. Followed by metals Zn and Pb have lowest concentration (35.76 µg/g) in fresh sample. According to ANOVA test, there is no significant different ($p>0.05$) between the condition of fresh and acclimatized sample in all metals.

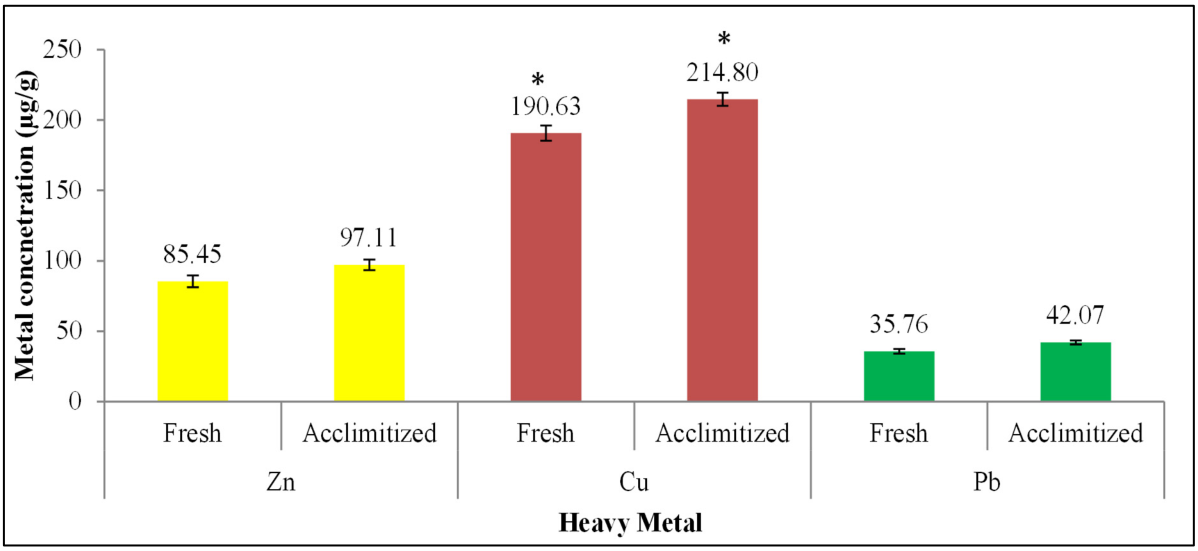

Figure 3: Distribution of the concentration (mean ± standard error; µg/g dry weight) of Zn, Cu and Pb between different conditions.(Remark: * is the actual values need to be multiply with 5).

The different conditions of fresh and acclimatized could demonstrate that concentration of metal in acclimatized fish sample is not significantly higher as compared to the fresh sample. This is may be due to the simple reabsorption of metal through skin or through gills from the acclimatized water (Alex *et al*, 2013). Thus, the conclusion from this parameter is there is no significant if we consumed the fish from acclimatized condition with the fresh fish sample. The acclimatized condition should be regularly checked and the water needed to be replaced with clean water regularly. So, that the metal leaked out in the water will not be reabsorbed back into the body through gills or skins.

3.3 Distribution of Heavy Metals between Organs

Figure 4 shows the comparison of metal concentration (mean ± standard error) in *O. niloticus* fish with different organ; muscle and liver. Based on the graph, metals concentration is higher in liver organ compare to muscle tissues. Metal Cu has 99.71% higher concentration in liver organ compared to muscle tissues. While, metal Zn concentration in liver has 71.78% higher in liver organ. However, metal Pb has 42.72% higher concentration in muscle tissues than liver. There are significant different ($p<0.05$) in metal concentration between organ muscle and liver.

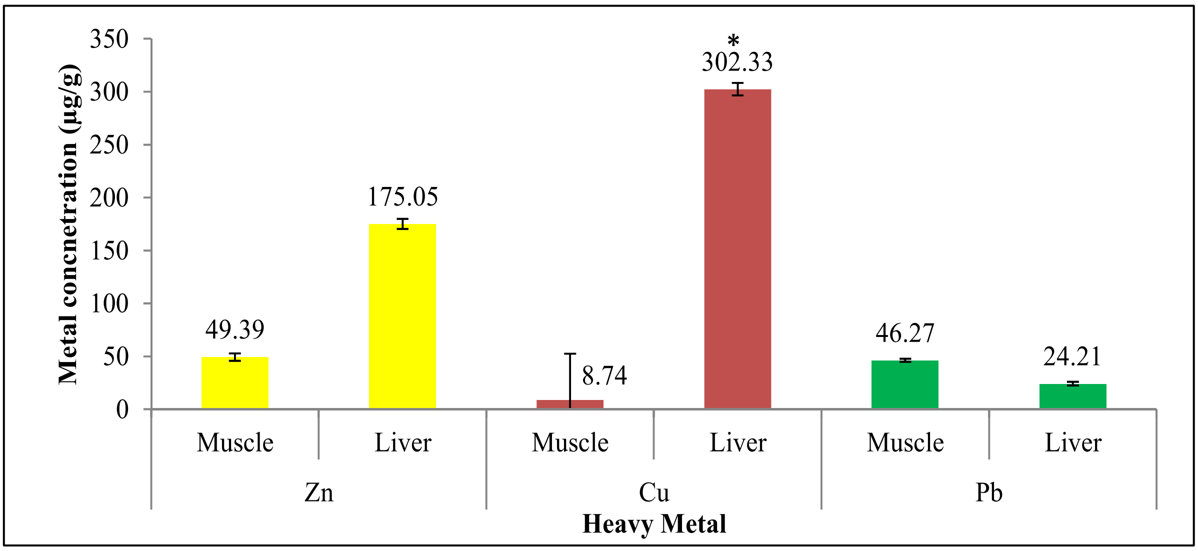

Figure 4: Distribution of the concentration (mean ± standard error; µg/g dry weight) of Zn, Cu and Pb between organs of muscle tissues and liver organs. (Remark: * is the actual values need to be multiply with 10).

In general, the level of heavy metal is lower in muscle tissues as compared to liver organ. Similar results have been reported with sample genus of fish *Oreochromis* and other species of fish. According to [1], the concentration of heavy metals Zn, Cu, Pb and Ni in liver organ was higher than muscle tissues of *O.niloticus* in Zambia's lake. Also, [12] also reported higher concentration of metals in liver organ than muscle tissues in various species of fish. Although muscle tissues have low concentration of metal as compare to liver, this study is focus on the fish muscle since it is the part which people consumed.

Liver is an important organ with many essential functions in storage of nutrients, metabolism function, immunity, detoxification or transformation of metals (Evans *et al*, 1993). Concentration of Zn and Cu in liver organ at four different sampling sites is high as related to the natural binding protein in hepatic tissues such as metallothioneins (MT). The MT is a ubiquitous protein that has high affinity to essential metals, Zn and Cu and stored it for other function such as enzymatic and metabolic demand (Salkusak, 2012). Their synthesis is one of the best known biochemical responses to metal exposure where they involve in the regulation of the essential metals and in the detoxification of non-essential metals (Buhari and Ismail, 2017), antioxidant activity (Atif *et al*, 2006) and radical scavenging (Wright *et al*, 2000) and helps in metal ion homeostasis in a cell (Shariati and Shariati, 2011). Future studies could be conducted to study in details the functions and behaviour of MT in various aquatic animals.

4 CONCLUSION

In conclusion, present study determined that Cu in *O. niloticus* from Malacca River was significantly higher than other studies metal. Concentration of heavy metals may vary depending on locally anthropogenic activities. Concentration of heavy metals may not reduce after short time acclimatization. Liver contain significant amount of heavy metals. The use of *O. niloticus* as a biomonitoring agent is a good way to determine the water pollution in order to manage the aquatic ecosystem.

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REFERENCES

Shouta,MMN., Yoshinori, I., Kaampwe, M., Kennedy, C., Balazs, O., Hiroki, T., Naoharu,M., Mayumi, I., 2010.Heavy Metal Accumulation in Lake Sediments, Fish (*Oreochromisniloticus* and *Serranochromisthumbergi*), and Crayfish (*Cheraxquadricarinatus*) in Lake Itezhi-tezhi and Lake Kariba, Zambia.*Achieve of Environment Contamination Toxicology*59: 291-300.

Zulkifli, SZ.,Mohamat-Yusuff, F., Arai, T., Ismail, A., Miyazaki, N., 2010. An Assessment of Selected Trace Elements in Intertidal Surface Sediments Collected from the Peninsular Malaysia. *Environmental Monitoring and Assessment* 169(1-4): 457-472.

Naji, A., Ismail, A., 2012. Sediment Quality Assessment of Klang Estuary, Malaysia. *Aquatic Ecosystem Health & Management*15(3): 287-293.

Ashraf,MA.,Maah, MJ., Yusoff, I., 2012.Bioaccumulation of Heavy metals in Fish Species Collected from Former Tin Mining Catchments.*International Journal Environment Res*earch 6: 209-218.

Cogun,HY.,Yuzereroglu, TA., Kargin, F., 2003.Accumulation of Copper and Cadmium in Small and Large Nile Tilapia *Oreochromisniloticus*.*Bulletin of Environmental Contamination and Toxicology*71: 1265-1271.

Batvari, BPD, Kamala-Kannan S, Shanthi K, Krishnamoorthy R, Lee K J, Jayaprakash M 2007 Heavy Metals in Two Fish Species (*Carangoidelmalabaricus* and *Belone stronglurus*) from Pulicat Lake, North of Chennai, Southeast Coast of India. *Environmental Monitoring and Assessment* 145: 167-175

Popma T, Masser M 1999 *Tilapia Life Story and Biology* (Stoneville, MS: Southern Regional Aquaculture Center Publication)

Zulkifli, SZ.,Mohamat-Yusuff, F., Mukhtar, A., Ismail, A., Miyazaki, N., 2014. Determination of Food Web in Intertidal Mudflat of Tropical Mangrove Ecosystem using Stable Isotope Markers: A Preliminary Study. *Life Science Journal* 11(3): 427-431.

Zulkifli,SZ.,Mohamat-Yusuff, F., Mukhtar, A., Ismail, A., Miyazaki, N., 2016.Biomagnification of Selected Toxic Trace Elements (Cr, As, Cd, Pb) in a Mangrove Ecosystem: Insights from Stable Isotope Analysis. *Pollution Research*35(2): 211-219.

Iavicoli, I., Fontana, L., Bergamaschi, A., 2009.The Effects of Metals as Endocrine Disruptors. *Journal of Toxicology and Environmental Health, Part B* 12(3): 206-223.

Ismail, A., Ramli, R., 1997. Trace Metals in Sediments and Molluscs from an Estuary Receiving Pig Farms Effluent. *Environmental Technology*18: 509-515.

Ahmad,AK.,Suhaimi, MO., 2010. Heavy Metal Concentration in Sediments and Fishes from Lake Chini, Pahang, Malaysia. *Journal of Biological Science*10: 93-100.

Alex E, Ezemonye L I, Frances A 2013 Heavy Metals Concentration in Surface Water and Bioaccumulation in Fish (*Clariasgariepinus*) of River Owan, Edo State, Nigeria. *European International Journal of Science and Technology* 2: 31-39.

Evans,DW.,Dodoo, DK., Hanson, PJ., 1993. Trace Element Concentrations in Fish Livers: Implications of Variations with Fish Size in Pollution Monitoring. *Marine Pollution Bulletin*26: 329-334.

Salkusak, N., 2012.Metallothionein: An Overview on its Metal Homeostatic Regulation in Mammals. *International Journal Morphology*30: 1007-1012.

Buhari, TRI., Ismail, A., 2017. Evaluation of Metallothionein Content in Giant Mudskipper *Periophthalmodonschlosseri* collected from West Coast of Peninsular Malaysia to Assess Environmental Metal Pollution. *Journal of Coastal Life Medicine* 5(7): 279-287.

Atif, F., Kaur, M., Yousuf S, Raisuddin S 2006 *In Vitro*Free Radical Scavenging Activity of Hepatic Metallothionein Induced in an Indian Freshwater Fish, *Channapunctata* Bloch. *Chemico-Biological Interactions* 162(2): 172-180.

Wright, J., George, S., Martinez-Lara, E., Carpene, E., Kindt, M., 2000.Levels of Cellular Glutathione and Metallothionein Affect the Toxicity of Oxidative Stressors in an Established Carp Cell Line. *Marine Environmental Research* 50(1-5): 503-508.

Shariati, F., Shariati, S., 2011. Review on Methods for Determination of Metallothioneins in Aquatic Organisms. *Biological Trace Element Research* 141(1-3):340-436.