ATOMIC ABSORPTION SPECTROSCOPIC STUDY OF HEAVY METAL LEAD AND ITS TOXICITY ON TELEOST FISH *Oreochromis niloticus*

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Abstract

Lead is a poisonous metal, exhibiting neurotoxicity in mammals and a host of physiological and behavioural changes in fish. Chronic lead exposure results in decreased production of delta-aminolevulinic acid (ALA-D), a critical enzyme in the blood, altered osmoregulation, and severe physical abnormalities in fish such as black tails, lordoscoliosis, muscular atrophy, caudal fin degeneration, and paralysis. Neurological damage and behavioural changes include increased prey spitting, hyperactivity, and decreased reaction capabilities. While fish may be able to adapt to chronic exposures of other metals, for example copper, by producing certain proteins such as metallothioneins, studies have shown that fish do not produce these same physiological adaptive responses to lead, and instead tend to simply increase their body burden of lead[1]. Selenium and vitamin E are very effective antioxidant agents which play important roles in improving and development of aquaculture sector. This study was conducted to determine the protective and treatment effects of vitamin E and selenium against lead toxicity. Administration of both vitamin E and selenium ameliorated the adverse effects of lead acetate toxicity through significant increase in haemoglobin, packed cell volume, RBC count, WBC count, and lymphocytes compared to lead acetate-exposed groups especially after the 10th week. Also, it is revealed that severe decrease of total protein, calcium, phosphorous, and magnesium in lead acetate intoxicated group. On contrary, significant increase of blood parameters upon addition of vitamin E and selenium combined with/without lead [2]. Metallic lead occurs in nature but is rare, and usually found in ore with copper and occasionally zinc and silver (Samans 1949). Lead pollution in waterways

is a consequence of anthropogenic activities, deposited by industrial effluents and mining. It is one of the metals of highest concern in terms of its effects on physiology (Heath 1995)[1]. Insignificant decreases of sAST, sALT, urea, and creatinine in group fed on vitamin E and selenium, while increase in lead acetate intoxicated group. Lead acetate caused increasing of lipid peroxidation level (malondialdehyde) and decreasing of superoxide dismutase activity and reduced glutathione level. From these results, it is concluded that exposure to lead acetate is considered as hepatotoxic environmental pollutant. Exposure to lead acetate induced significant effects on antioxidant status. Antioxidants (vitamin E and selenium) showed important roles to protect body against lipid peroxidation, which considered as the first step of cell membrane damage, in addition to the improvement of the endogenous antioxidant enzyme activities [2].

Keywords: *Neurotoxicity, Delta-aminolevulinic, Hyperactivity, Selenium, Aquaculture sector, Osmoregulation, Peroxidation, Hepatotoxic, Oreochromis niloticus.*

Introduction

Lead (Pb) is a highly toxic metal in aquatic environments. Fish are at the top of the food chain in most aquatic environments, and are the most susceptible to the toxic effects of Pb exposure. In addition, fish are one of the most abundant vertebrates, and they can directly affect humans through food intake; therefore, fish can be used to assess the extent of environmental pollution in an aquatic environment. Pb-induced toxicity in fish exposed to toxicants is primarily induced by bioaccumulation in specific tissues, and the accumulation mechanisms vary depending on water habitat (freshwater or seawater) and pathway (waterborne or dietary exposure). Pb accumulation in fish tissues causes oxidative stress due to excessive ROS production. Oxidative stress by Pb exposure induces synaptic damage and neurotransmitter malfunction in fish as neurotoxicity. Moreover, Pb exposure influences immune responses in fish as an immune-toxicant. Therefore, the purpose of this review was to examine the various toxic effects of Pb exposure, including bioaccumulation, oxidative stress, neurotoxicity, and immune responses, and to identify indicators to evaluate the extent of Pb toxicity by based on the level of Pb exposure [3]. Pb and Cd concentrations in fish organs were measured using dry ashing-acid digestion method and analysed by atomic absorption spectroscopy (AAS). The highest

concentrations of Pb were detected in the gills (0.151 ± 0.12 mg/g) followed by bones (0.108 ± 0.09 mg/g) and the least in muscle tissues (0.078 ± 0.05 mg/g)[4].

Heavy metals are common in rocks but due to economic and developing processes, heavy metals currently can be easily found in urban soils yielded from anthropogenic sources such as industrial, urban development and transport activities [5]. These human activities have increased concentrations of heavy metals above normal levels in the environment contributing to serious and widespread environmental issues leading to chronic toxicity. Recently, heavy metal pollutions in aquatic ecosystem are becoming critical issue. In Malaysia, many ex-mining and exlandfill areas that are reclaimed for other purposes such as for agriculture activities, freshwater fish farming area, recreational area, housing area and industrial area have become potential settlement for heavy metals[6]. Bioaccumulation of heavy metals in fish can be biomagnified into human consumption leading to more adverse health effects [7]. Heavy metals poisoning that involves lead (Pb) and cadmium (Cd) are quite common. Many studies have been conducted in Malaysia to assess exposure of Pb as in biological, food or toys samples [8]. Pb has a very long biological half-life, spanning from months to years and chronic exposure could lead to long-term health effects [9].

Methodology

Experimental fish: 64 black Tilapia are taken for experiment. Their body weight ranged from 150 to 160 g and 3 to 4 months ages. All fish were subjected to acclimatization for 2 weeks in prepared glass aquarium [2]. The collected fish was measured for the physical appearance; weight and size to ensure that each fish has similar characteristics. The fish were then placed in clean polyethylene bags and put into the icebox. The samples were immediately taken to laboratory where the samples were deep-frozen at -20 degrees C until further analysis [4].

Sample analysis: Muscle tissues, gills and bones of each fish were collected by skinning and filleting method. The collected organs were then mixed separately using commercial grade food grinder to produce homogeneous samples. Twenty-five (25) g of wet muscle tissues, gills and bones for each fish sample were weighed and placed into a crucible and was transferred into a furnace with a temperature between 450 degrees C -500 degrees C for overnight. Samples were removed after the furnace process and were left to cool down

at room temperature. Wet dilution was then performed by adding 2 mL of concentrated nitric acid (65%) and 20 mL dilution of hydrochloric acid (10%) to digest 25 g of muscle tissues, 1 g of bones and 1 g of gills in polytetrafluoroethylene (PTFE) beaker followed by heating on a hot plate at 80 degrees C. The mixture was then swirled until a yellowish solution appeared and left to cool at room temperature prior to filtration. The mixtures were filtered using 0.45 lm pore size filter paper to make 50 mL sample solution. Pb was then determined by using atomic absorption spectroscopy (AAS) AA 800 (Perkin Elmer, Foster City, CA, USA).

Human health risk estimation: Various approaches are available to predict the potential risks to human health regarding the consumption of heavy metals in fish. In this study, hazard quotient (HQ) estimation was applied to provide indication level of the human health risk due to Pb exposure [10].

$$HQ = \frac{EF\ X\ ED\ X\ FIR\ X\ C}{RfD\ X\ WAB\ X\ TA}$$ where, EF is exposure frequency; ED is the exposure duration FIR is the fish ingestion rate;

C is the metal concentration in tilapia fish muscle (mg/g); RfD is the oral reference dose; WAB is the average body weight; and TA is the average exposure time for non-carcinogens.

Statistical analysis: SPSS v21 (SPSS Inc., Chicago, IL, USA) was used for data analysis using independent t-test and one-way analysis of variance (ANOVA) followed by post-hoc Tukey test.

Results and Discussion: The low concentration of Pb in the fish muscles, Pb exposure can still be harmful to aquatic animals (e.g. tilapia fish) due to the bioaccumulation process in other parts of the fish. It is because Pb accumulation in fish can cause hypocalcemia by inhibiting the basolateral transport mechanisms of ionocytes in the gill epithelium of the fish. This will disrupt the electrochemical gradient and ion regulation leading to fish death (Lee et al., 2019). Pertaining to that, our results of Pb concentration in gills and bones showed approximately 90% and 40% higher than Oreochromis niloticus muscle tissues (Table-1).

Fish Organ	Pb concentration (mg/g) ±SD
Muscle tissues	0.078± 0.05
Gills	0.151±0.12
Bones	0.108±0.09

<Table:1;Distribution of Pb concentrations in different parts of Oreochromis niloticus(n=32)[4].

ANOVA analysis indicated the level of Pb were statistically different in different fish organ ($p < 0.05$) indicating the importance of fish organ as the bioindicator to measure Pb level in the fish. The highest of Pb in the gills was speculated because of its large surface area which is an essential route of Pb from the surrounding water. The adsorption of Pb is likely to be facilitated into the gills during respiration and osmoregulation processes (Fernandes, 2019). On a second note, metallothionein proteins, a biomarker for heavy metals are normally found in fish gills, thus contributing to the bioaccumulation of Pb in the gills [11].According to report fish's hard tissues were prone to higher accumulation of heavy metal compared to soft tissues and this is in agreement with our finding of higher Pb level in the bones than muscles of Oreochromis niloticus.

Table-2[4], ANOVA on the concentration of lead

Metal	Treatment	Sum of Squares	Degree of freedom.	Mean Square	Factor	Significance
Pb	Between samples within samples.	0.054 0.737 0.790	2 93 95	0.027 0.008	3.383	0.038(at a level of 0.05)

Table-3[4]; Health risk estimation for lead ingestion from tilapia fish muscle tissue consumption. HQ=Hazard Quotient=

Pb(once in a week)	Pb (7 times in a week)
0.01	0.10

In the context of heavy metal contamination in the fish, this study has demonstrated that the metal distribution within an organism is a complex issue due to different affinity of

various metals to the different organ of fish. One-way ANOVA indicated statistically significant difference ($p < 0.05$) in the variation of Pb.

Conclusion

When fish are exposed to lead, the ALA-D enzyme is inhibited. Studies show that a 60-day chronic exposures of a freshwater fish lead resulted in a severe reduction of red-blood cell counts, hematocrit (the percentage of blood volume occupied by red blood cells), haemoglobin concentration, and mean corpuscular volume [2].However, a review of biomonitoring studies of ALA-D levels in fish show that organic lead has little effect on the enzyme, and only inorganic lead is able to inhibit ALA-D (Hodson et al. 1984). Lead negatively effects osmoregulation in fish. An experiment that exposed various concentrations of lead to rainbow trout in slightly hypotonic brackish water for 30 days showed a dose-dependent elevation in plasma potassium (Haux and Larson 1992). A study conducted on fish from a lead-contaminated lake showed a decrease in plasma sodium but no change in plasma potassium (Haux et al. 1985). It is possible that previous or chronic lead exposure has led to physiological adaptations over the generations of fish who have lived in this contaminated environment (Heath 1995). The toxicity of waterborne lead, however, is also affected by the chemical and physical conditions of the water, as well as whether the lead is in dissolved form. Studies have shown that pH, water hardness, and the partial pressure of CO2 (gaseous) largely affect the toxicity of lead. Water that is lower in pH (more acidic) increases the uptake of lead, because acidification increases lead's availability (Campbell and Stokes 1985). Water hardness has a large effect on lead's toxicity (Spehar & Flandt YEAR); lead was found to be more toxic in soft water over hard water.

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REFERENCES

1. Authors: Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47906 https://tippingpointplanner.org/white-papers/171-tipping-points-and-indicators-fact-sheet-lead.
2. Lead poisoning in Nile tilapia (Oreochromis niloticus):oxidant and antioxidant relationship- by MdTanekhy. Received 9July2014/Accepted:19February215,Springer International Publishing Switzerland 2015.Environ Monit Assess(2015)187:154;DOI 10.1007/s10661-015-4387-8.
3. Toxic effect of lead exposure on bioaccumulation, oxidativestress, neurotoxicity, and immune responses in fish: A review by Ju-WookLee,Hoon Choi,Un-Ki Hwang, Ju-Chan Kang, Yue Jai Kang,Kwang Il Kim, Jun-Hwan Kim. Envirn Toxicol Pharmacol.2019May;68:101-108.doi:10.1016/j.etap.2019.03.010. Epub2019 Mar 8. PMID:30884452.
4. Determination of lead and cadmium in Tilapiafish (Oreochromis niloticus) from selected areas in Kuala Lampur by Ahmad Razali Ishak, Md Shahrir Zuhdi, Md Yusmaidie Aziz. Egyptian Journal of Aquatic Research .Article history: Received 26 February 2020 Revised 6 June 2020 Accepted 6 June 2020 Available online 25 June 2020
5. Gall, Boyd, & Rajakaruna, 2015; Tian et al., 2015.Transfer of heavy metals through terrestrial food webs;A review.Environmental Monitoring and Assessment 187.201.
6. Hashim et al., 2018; Kusin, Azani, Hasan, & Sulong, 2018; Sakai & Yoneda, 2018.An Analysis of Heavy Metals in lakes of Former Tin MiningSites in the city of Ipoh,Perak ,Malaysia. International Journal of Academic Research in Business and Social Sciences 8, 687-697.
7. Bosch, O'Neill, Sigge, Kerwath, & Hoffman, 2016; Heavy metals in marine fish meat and consume health:A review journal of the Science of food and agriculture 96,32-48.
8. Hassim et al., 2018; Iyen, Ab Wahab, Mohamad, Mohamad, & Erejuwa, 2016; Shamsudin, Marzuki, Jeffree, & Lukman, 2017; Tan & Sze, 2017.
9. Alli, 2015 Blood level of cadmium and lead in occupationally exposed persons in Gwagwalada, Abuja, Nigeria. Interdisciplinary Toxicology 8,146-150.;

Bernhoft,2013, Cadmium toxicity and treatment. The scientific World Journal 2013.

10. Taweel,A.Shuhaimi-Othman,M.Ahmad,A.2013Assessment of heavy metals in tilapia fish (Oreochromis niloticus) from the Largat River and Engineering Lake in Bangi,Malaysia, and evaluation of the health risk from tilapia consumption .Ecotoxicology and Environmental Safety 93,45-51.
11. Naji.A. Ismail, A. Kamrani,E.Sohrabi.T,.2014Correlationof MT levels in livers and gillswith heavy metals in wild tilapiafromthe Klang River. Malaysia.Bull Environ Contam Toxicol 92,674-679.