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Median Lethal Concentration (LS-50) of Lead and the Effect on Osmoregulation of the "Red Tilapia" Fish (*Oreochromis* sp.)

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Abstract. The aims of this study are analyzing the score of median lethal concentration (LC_{50}) of lead on the red tilapia fish and also test the influence of Pb exposure on osmoregulation of the red tilapia. This study consists of two steps. The first step is finding out the values of median lethal concentration (LC_{50}) of lead on the red tilapia. The second step is finding out the influence of toxicity of lead metal exposure by giving the metal to the red tilapia maintained in the fresh water for 60 days. The lead concentration used are sub-lethal concentration 0 mg Pb/L, 1.7 mg Pb/L, 2.9 mg Pb/L, 4.9 mg Pb/L, 8.3 mg Pb/L and 14.1 mg Pb/L. The influence of lead metal exposure observed in this observation are the mortality of red tilapia fish and osmoregulation. The data of mortality are analyzed by Trimmed Spearman Karber (TSK) version 1.5 from EPA, the data of osmoregulation are analyzed by variant analysis with SPSS version 16. The result of the first step study was the values of LC_{50} due to the exposure of Pb metal was 13.12 mg Pb/L. The result of the second step study showed that the exposure of Pb metal also caused the disturbance of osmoregulation of red tilapia fish shown by the increase of osmotic pressure blood of red tilapia fish.

Introduction

Heavy metal pollution is very dangerous for the environment. Many reports describe the dangers of water pollution due to heavy metals. The dangers was caused by consuming water and biota that living in the polluted waters [1]. One of heavy metals which contaminate waters is lead (Pb).

Lead is a metal that can accumulate in the tissues of organisms. Presence of lead in the tissues of organisms increase in accordance with its concentration in the water and the time of the organism is in contaminated waters Pb. This is because the aquatic organisms are not able to regulate heavy metals Pb that enter to their body. The maximum level of lead in water for fishing activities is equal to 0.03 mg/L [2].

Red Tilapia is the aquatic biota that can accumulate lead. It is a fish that can live in bad conditions of water quality. Red Tilapia generally, is a fish require a spesific quality and quantity of water to sustain life. Changes in quality and quantity of water will disturb the fish in completing its life cycle. Characteristic of disruption due to the changes in the quality and quantity of water is a reduction species of fish in the population. Sudden changes in water quality will cause mass fish deaths. Heavy metals in water that could contaminate fish is an important study because it triggers the change of quality standard in these water.

Based on the reason above, it is important to do research on the effect of heavy metals (Pb) with different concentrations on mortality and osmoregulation of red tilapia.

Material and Method

Material of study. The animal used in this study was red tilapia. That is measuring 5-7 cm. The animal used must comply the requirement of the test animals for acute toxicity test according to the criteria of the EPA [3,4].

The material used in this study was lead compound (II) acetate, $Pb(CH3COO)_2.3H_2O$ (Merck). That is a powder with a molecular weight of 379.3 and Pb atomic weight of 207.2. Furthermore, the material is made into a solution of 1,000 ppm of lead.

The container used in toxicity test is an aquarium which contained 15 liters of test solution with a density of 10 fish. Before being used for the acute toxicity test, the containers are cleaned in accordance with the procedure of APHA [5]. The equipment used for measuring water quality such as thermometer , pH meter and DO meter

Method of acute toxicity test. This study was conducted in several stages i.e. a preliminary test or the range finding test, the main test or definitive test and the impact test.

The range finding test is being done to determine the below threshold concentration (LC_0 - 48 hours), which is the highest concentration at which all the animals still alive within 48 hours. And the upper threshold concentration (LC_{100}- 24 hours) which is the lowest concentration at which all the animals died in the interval 24 hours [6].

Lower and upper threshold value is used to determine the concentration of the test on the definitive test, using formula 1 below[6] :

$$log\frac{N}{n} = K\ log\left(\frac{a}{n}\right) \quad (1)$$

$$\frac{a}{b} = \frac{b}{a} = \frac{c}{b} = \frac{d}{c} = \frac{e}{d} \quad (2)$$

Where :
N = The upper threshold concentration
n = The lower threshold concentration
K = Number of the test concentration
a = The smallest concentration
a, b, c, d, e = The desired concentration

Experiments is conducted using completely randomized design with three (3) replicates. Mortality data for 96 hours were analyzed by using a software Trimmed Spearman Karber (TSK) version 1.5 from the US-EPA to get LC_{50} (Median Lethal Concentration), with the values of the interval at the 95% confidence limit.

Method of Impact Test. Based on the value of the Sub Lethal Concentration or < LC_{50} then the impact is being tested to determine the impact of osmoregulation . Osmoregulation data are tabulated in the table and calculated the average and standard deviation. To determine the normality of data distribution, we used Kolmogorov - Smirnov test (Test Distribution) and when the data has a normal distribution then followed by one-way ANOVA test . When the ANOVA test showed a significant result (significantly different or very significantly different) then continued with LSD test.

Water quality data observed every 24 hours during the study. Substitution test medium performed every 48 hours. Substitution is done by substituting the test solution or move the animal into a container which contain the new test media [7].

Results and Discussion

Mortality. Mortality percentage of the red tilapia fish that exposure of various concentrations of lead are presented in Table 1 and Figure 1.

Table 1. Mortality percentage of red tilapia fish after exposure 96h at various concentrations of lead (mg Pb/L)

Pb concentrations (mg Pb/L)	Mortality Persentage (%)
	Red Tilapia Fish
1.7	6.67
2.9	40
4.9	20
8.3	26.67
14.1	53.33

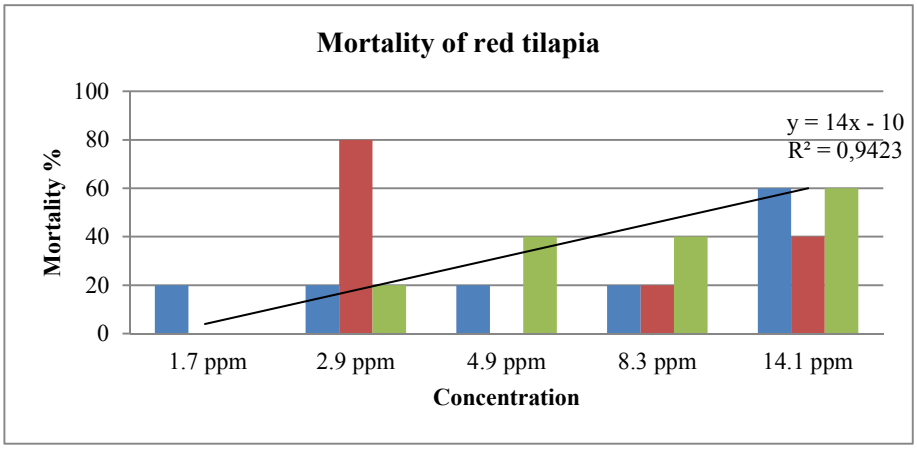

Figure 1. Mortality percentage of red tilapia fish after 96 hours exposure at various concentrations of lead (mg Pb/L)

Determination of median lethal concentration (LC_{50}) value of lead is done by analyzing the mortality percentages above using the software Trimmed Spearman Karber (TSK) version 1.5 from the US-EPA.

Table 2. Value of LC_{50}-96 hours of lead at red tilapia fish

Fish	LC_{50}	Convidence Interval 95 %	Description
Red Tilapia	13,12	8,06 < x < 21,35	LC_{50} – 96 h

Table 1 and Figure 1 show the higher concentration of lead exposured at the red tilapia fish. Followed by mortality percentage to be higher as well. From calculation using the software Trimmed Spearman Karber (TSK) version 1.5 from the US-EPA, we obtained value of LC_{50}-96 hour is 13.12 mg Pb/L.

Mortality of red tilapia fish caused by the toxic effects of lead were stronger due to the high absorption of lead from the environment. Increasing concentrations of lead in the environment causes increased absorption by the fish. According to [8], a lethal effects of pollutants on organism is a response that occurs when physics or chemistry substances disrupt cellular or sub-cellular processes in living organisms up to a limit that cause death directly.

Metal ions enter the cell by penetration into the lipid layer. Lead is a metal class B is involved in the processes of enzyme function normally. Lead is the highly reactive metal to ligand bond with sulfur and nitrogen, so it is very important in systems that disrupt the function metaloenzim (toxic) against cell metabolism [9].

Based on toxicity criteria from Pesticide Commission Department of Agriculture [6], Value LC_{50}-96 hours of lead is the high category.

Osmoregulation. Based on the value of the median lethal concentration (LC_{50}-96 hours) of lead is 13.12 mg Pb/L, therefore the new treatment was made. There are sub lethal concentration of lead as follows: 0 mg Pb/L, 2 mg Pb/L, 4 mg Pb/L, 6 mg Pb/L, 8 mg Pb/L and 10 mg Pb/L with the objective to determine the effect of lead exposure to osmoregulation of red tilapia fish.

Table 3. Osmoregulation of red tilapia fish

Treatment (mg Pb/L)	Osmotic Pressure (mOsm/Kg)	
	Water	Blood
0	27.33	266.67
2	28.67	332.67
4	28.67	337.33
6	29	355.67
8	32	366
10	34	413.67

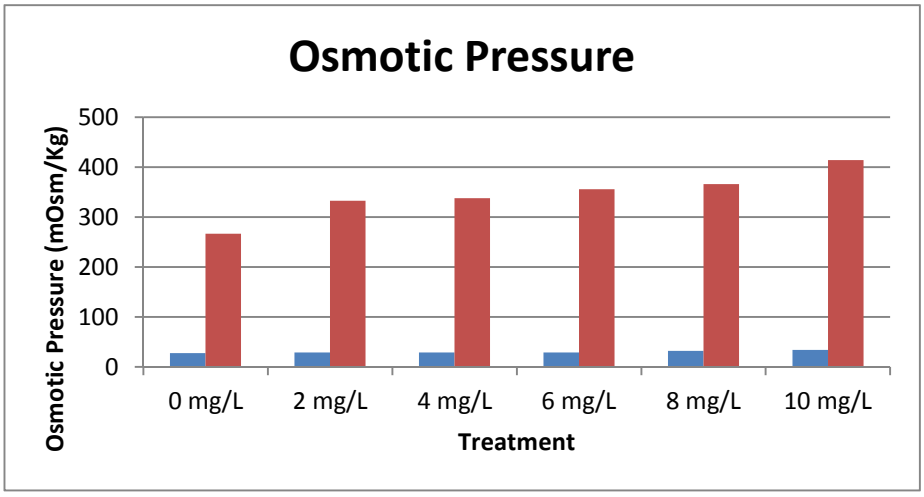

Figure 2. Blood osmotic pressure of red tilapia fish after 96 hours exposure at various concentrations of Pb metals (mg Pb/L)

From Table 3 and Figure 2 above shows that increasing lead (Pb) exposure will cause increase the osmotic pressure of the water and the osmotic pressure of blood of red tilapia *Oreochromis sp.* The red line in Figure 2 shows blood and the blue one is water. This is in accordance with the opinion of [10] which says that the presence of dissolved metals in the media will increase the osmotic pressure of the media. That is cause to stop the movement of solvent molecules into the solution through a membrane semi-permeable (osmosis process), which resulted in the osmotic pressure of the blood of the fish also increase.

Conclusion

Based on the results of this study, we concluded that:

1. Value LC_{50}-96 hour of Pb in a red tilapia was 13.12 mg Pb/L.
2. Increasing concentrations of lead will increase osmotic pressure of media (water).
3. Increasing concentrations of lead will increase osmotic pressure of blood of red tilapia.

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