Histologic structure of red Nile tilapia fish (*Oreochromis niloticus* Var.) gill which is exposed to lead acetate

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**Abstract:** Fish is a water biota commonly used as the bioindicator of water pollution level. One of the animals which are recommended by EPA as the test species is red Nile Tilapia fish (*Oreochromis niloticus* var). The purpose of this research is to get the value of LC50-96 hours in the toxicity test toward the fishes which are exposed to lead acetate with some details identification of the gills damage by gill histologic structure examination together with the determination of the lead concentration which caused the destruction. Sixty fish were used in the preliminary test to detect the threshold concentration (LC0-48 h); whereas 120 fish were used in the toxicity test to get the mortality level of fish up to 50% in 96 h. Finally, for treatment test, there were 80 fish which were exposed to lead acetate in the concentration of 0 ppm, 259.51 ppm, 291.94 ppm and 324.38 ppm. All the treatment tests were given for four weeks. The data were collected at the end of the 4th week, and then, the description of the fish gill histology structure was done. The histology observation of the fishes gill detected some damages in the form of edema (0% - 25%), lamellar fusion (1% - 75%), hyperplasia (0% - 50%), epithelial lifting (0% - 50%), and necrosis (0% - 50%). The results indicate that administration of lead acetate accelerates moderate damage to the red Nile tilapia fish gill structure. The greatest level of damage is lamellar fusion, while the other decline damages are edema, hyperplasia, epithelial lifting, and necrosis. Furthermore, it can be concluded that LC50-96 hours value in the lead acetate toxicity test of red Nile tilapia fish is 324,38 ppm with some histologic structure damage in the gill the fishes.

1. **Introduction**

Lead (Pb) is a dangerous and often detectable sea pollutant. It is hard to be degraded and can not be destroyed by living organisms, which resulted in the accumulation of lead in the environment. The accumulation is mainly in the sediment part of some aquatic environments settling and forming a complex composition along with organic and inorganic materials [1]. The high concentration of lead can kill some types of aquatic biota. Based on the research conducted by [2] on the test of lead toxicity to the gill of Tilapia fish resulted in LC50 equal to 247.51 mg/l. The results showed that fish gills suffered some edema damage, epithelial removal, hyperplasia, lamellar fusion, and necrosis. Fish is one of the bioindicators of water pollution level. Therefore, if the fish is already contained high levels of metal and exceed the normal threshold that has been determined, it can be used as an indicator of a
pollution in the environment [3]. The objectives of this research are to determine the value of LC50-96 hours on toxicity test of Tilapia fish exposed to Pb Acetate, to explore the damage of gill histology structure, and to measure the lead concentration causing damage of histology structure of Tilapia fish gill.

The aquatic environment pollution will cause a gill damage that can be categorized based on changes in secondary lamella anatomy and gill filaments [4]. According to [5] gill damage is categorized as follows: Edema is a condition in which there is an increase in the amount of fluid in the tissues. Hyperplasia is the addition of a part of the body or organ due to an increase in the number of new cells; whereas Lamellar fusion is due to an increase in the number of mucous cells in the lamella base. Moreover, the epithelial lifting is edema by removing a flat epithelium covering the secondary lamella that serves as a defense mechanism, since secondary lamellar epithelial separation will increase the distance of pollutants contained in the water. Therefore, water must diffuse to reach the bloodstream [6], and Necrosis, the cell deaths that occur due to hyperplasia and excessive secondary lamellar fusion, which causes the inactivity of gill tissue. Most of the aquatic biota obtain oxygen through the gills from the outer membrane by the diffusion mechanism. The intake of oxygen of the microorganisms are obtained from the body tissues through the surface part; whereas the bigger living things such as fish, the intake of oxygen and nutritive compounds are diffused into the circulatory fluid.

According to Palar [7], the process of lead intoxication through the gills is helped by the other metal ions and food which have accumulated by lead and then it will form fat-soluble ions. The ions can penetrate the gill cell membrane; then it will trigger the cell fluid regulation.

Lead can cause a gill lamella damage, especially the gill epithelial cells which occurs due to mucus binding to the leads passing through the lamella. In larger compositions, it can block the process of gas and ions exchange in the lamella resulting in the fish respiration system inhibition and causing severe symptoms of death [8].

The lead exposure to the gills is larger than the other organs. The lamellar epithelial layer of the gill will react with dissolved lead and creates an osmoregulation imbalance. The longer exposure will result in physiological changes or necrosis. These physiological changes may include secondary lamella edema, hyperplasia, and lamellar fusion and may even cause deaths because the exchange of gases in the gills becomes impaired [9].

2. Methods
The study was conducted through 3 stages of the test, i.e., preliminary test; toxicity test; and treatment test. The preliminary test used six treatments with one control, toxicity test used five treatments with one control, and the treatment test used three treatments and three replications with one control. As the main test on the treatment used LC50: 324.38 ppm, LC50-10%: 291.94 ppm, and LC50-20%: 259.51 ppm.

The preliminary test referred to the procedure [10], as many as 60 animals were used as test animals, obtained by LC90 48 h of 100 ppm and LC100 24 h of 1000 ppm. The toxicity test employed five concentrations at 147.9 ppm, 323.4 ppm, 478.2 ppm, and 707.1 ppm. A total of 120 tilapia fish were divided into six groups (1 control group, five treatment groups). Data were analyzed using Probit Method from Finney (1978) referred to [10]. Toxicity test was performed for four days, and LC50-96 h was obtained at 324.38 ppm.

The treatment test aimed to know the effect of lead acetate on microanatomy structure of tilapia fish gill at various concentrations of lead at the limit of LC50. This research used 3 concentration levels, i.e. concentration LC50 = 324.38 ppm, LC50-10% = 291.94 ppm, LC50-20% = 259.51 ppm and control. The treatment test used 80 fish samples which were divided into four treatment groups. The organ damage observed was in the form of edema, lamellar fusion, hyperplasia, epithelial lifting, and necrosis.
3. Results and discussion

3.1. Toxicity test and the abiotic factors
Tilapia LC50 is a median lethal concentration of lead acetate causing 50% mortality (death) of test animals. Toxicity test was performed for 96 h to determine the toxic level of a toxic substance [10]. Calculation of probit analysis obtained Tilapia LC50-96 hours at 324.38 ppm. This result is not much different from those of [2] which obtained LC50 of tilapia at 247.51 ppm. Therefore, it can be said that tilapia fish have high enough resistance to the environmental change and have a low mortality rate.

Abiotic test results (water quality) indicated that all parameters, i.e., water temperature, water pH, soluble O2 and CO2 are at a safe threshold since they do not exceed the specified threshold. Thus, fish can live in the test water.

3.2. Gill histologic structure
The result of the observation showed that there is damage to the histologic structure of the gills caused by lead at various concentrations. The damage was edema (0% -25%), lamellar fusion (1% -75%), hyperplasia (0% -50%), epithelial lifting (0% -50%), and necrosis (0% -50%). The histologic structure of Tilapia fish gills exposed to lead is presented in Figure 1.

![Figure 1. The histologic structure of Tilapia fish gills exposed to lead](image)

There are three main mechanisms of a substance enters the organism, i.e., through the process of absorption, distribution, and accumulation. In the respiratory channel, the lead acetate can cause damage to the gill and gill-related organs. The intoxication of heavy metals (lead acetate) will result in damage to gill tissue or even tissue death. Gill damage due to pollutants (heavy metals) begins with edema, then hyperplasia, lamellar fusion, epithelial lifting, and necrosis [5].

The results of fish gill observation showed that there was tissue damage after lead exposure in the treatment test for four weeks. The damage of edema was at 0-25%, the lamellar fusion was at 1-75%, hyperplasia was at 0-50%, epithelial lifting was at 0-50%, and necrosis 0-50%. The results obtained can be concluded as the lead acetate can cause the middle level of damage on the fish gill histologic structure of tilapia fish. The severe damage was lamellar fusion followed by edema, hyperplasia, epithelial lifting, and necrosis.

Edema occurring in all three treatments was not different, it was under 25% and occurred almost evenly in all fish samples. Edema can be caused by the introduction of lead acetate into the gills that cause cells to be irritated resulting in cell swelling [5]. Edema often results from exposure to pollutants derived from chemicals, such as heavy metals [11].

Hyperplasia also occurred in this study in groups of 259.51 ppm and 291.94 ppm with damage average below 50%. Hyperplasia occurred at a lower rate of irritation and usually accompanied by an increase in the number of mucus cells in the lamella base which over time will lead to lamellar fusion. The interlammatic space which is the mucous ducts and mucus production space may become blocked by epithelial cell hyperplasia from the primary filaments. In the end, the entire space of interlamellar is
filled by new cells. Hyperplasia results in the thickening of epithelial tissue at the tip of the filament which exhibits forms such as a baseball stick (clubbing distal) or thickening of epithelium tissue located near the base of lamella [12]. Hyperplasia occurs due to the addition of a body part or organ due to an increase in the number of new cells caused by irritant pollutants [6]. In this case is lead acetate. At the treatment of concentration of 324.38 ppm, it was not found damage to hyperplasia because it has become a lamellar fusion.

Damage to lamellar fusion was observed in all three treatments and for the treatment of 324.38 ppm was the highest percentage. The occurrence of lamellar fusion resulting from mucus cells located at the base of lamella increased in number resulting in the intermediate merging of secondary lamellae. The fusion of lamella is the attachment of two parts of the secondary lamella [12]. Also, lamellar fusion is also caused by excessive mucus in the gills. Thus, it will close the secondary lamella. This excess mucus is one of the responses of the mucus gland to protect the gills from lead entering the ion form into the gills. Excess mucus will result in inhibition of oxygen uptake from water.

Epithelial lifting damage was found in all treatments, especially at lead concentrations of 324.38 ppm, although with a low percentage. Epithelial lifting is a mild swelling of gill cells due to the entry of lead ions, resulting in the rise of a secondary lamellar flat epithelium enveloping the secondary lamella that serves as a defense mechanism. The elevation of these secondary lamellar epithelial flats is a form of defense to increase the distance of pollutants in the form of lead ions contained in water so that water must diffuse to reach the bloodstream [6].

Necrotic damage was found randomly to all three treatments with a low percentage of 25%. Necrosis occurs due to hyperplasia and excessive secondary lamellar fusion. Thus, the gill tissue is not intact anymore. It is because of the concentration of lead in water is too high, and there is the continuous absorption of lead ions into the gill tissue. In this incident, lamella suffered severe damage and was included in the category of heavy pollution [5].

Damage to the histologic structure of the gills causes the fish to be difficult to breathe and cause the oxygen content in the blood to be reduced. Therefore, there are Ha difficulties in binding oxygen and hypoxia as a result of secondary lamella damage from the gills [3].

4. Conclusion
Based on the research results, it can be concluded that; (1) tilapia LC50-96 h on toxicity test of tilapia fish (Oreochromis niloticus Var.) exposed to lead that was at 324.38 ppm; (2) the exposure of lead resulted in the change of the histologic structure of the fish gill; (3) At concentration of 259.51 ppm; 291.94 ppm; 324.38 ppm lead causes a change in the histologic structure of tilapia fish gill. The change was in the form of edema; lamellar fusion; hyperplasia; epithelial lifting, and necrosis. The researcher suggests that further investigation on various concentrations to get the safe level of lead pollution threshold since 259.1 ppm was still causing the gill damage.

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