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Toxicity of the Heavy Metal Lead (Pb) on the Development of Tilapia Fish Prolarvae (*Oreochromis niloticus* L.)

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Abstract: The heavy metal lead (Pb) entering the water has a negative impact on aquatic animals, one of which is tilapia (*Oreochromis niloticus* L.). The accumulation of lead (Pb) can disrupt the spawning process, cause morphological anomalies, and increase the mortality of tilapia sperm. This study was conducted to determine the toxicity of Lead (Pb) metal on the development of prolarvae of tilapia (*Oreochromis niloticus* L.). This research is a type of experimental research that is structured based on a Randomized Block Design (RBD) pattern, totalling 6 tilapia fish consisting of 3 male tilapia and 3 female tilapias. Tilapia eggs are obtained by injecting male and female broodstock using the hormone Ovaprim twice to stimulate gonadal maturity, after which fertilization is carried out, namely the merger between fish sperm cells and eggs in the container. After the eggs and sperm cells are fertilized, all eggs are divided into 5 treatments, and each treatment is filled with 20 eggs. The treatments consisted of control and 4 concentrations of Lead (Pb), namely 0.15 mg/L (P1), 0.30 mg/L (P2), 0.45 mg/L (P3), 0.50 mg/L (P4). Exposure to Lead (Pb) was given for 3 days, and observations were made after the eggs entered the prolarva stage to determine the degree of abnormality and various forms of abnormality. The observation parameter was the formation of abnormal types at the prolarvae stage. Data were analyzed using one-way ANOVA (Analysis of Variance) with a 95% confidence level and an LSD further test. The research results showed that the percentage of abnormalities in treatment P1 was 26.67%, P2 was 45%, P3 was 73.33%, and P4 was 83.33%. The abnormalities found were lordosis, kyphosis, scoliosis, yolk deformation accompanied by lordosis of the tail tip, and enlarged anterior yolk sac. Based on the results of the study, it is concluded that exposure to Lead (Pb) is toxic to tilapia larvae. It is hoped that the results of this research will provide input for the government and society regarding the importance of maintaining water quality from pollution

Keywords: Lead (Pb); *Oreochromis niloticus*; Prolarvae; Toxicity.

Introduction

Water pollutants caused by heavy metals have become a serious concern in the field of environmental and public health. [1] stated that one of the most common heavy metals found in the aquatic environment is lead (Pb). Lead pollution comes from various sources, such as coal-burning industrial activities, smoke from factories, printing, glass, polyvinyl, plastics, the use of insecticides (Pb arsenate) and organic and inorganic waste as a source of toxicants [2]. These activities can lead to accumulation in the environment [3].

Lead is accumulated in waters through human activities such as waste disposal, which then settles as sediment [4]. In addition, lead also crystallizes in the air and dissolves in rainwater naturally. High rainfall causes heavy metals to erode from the soil and be carried along with soil sediments containing lead to the sea through rivers [5]. This leads the waters to be the last place to accumulate lead pollution. Water contaminated with lead (Pb) hurts aquatic organisms, especially fish that are sensitive to lead [6]. Fish will absorb lead in a polluted environment through outer membranes such as skin and gills passively, resulting in the accumulation of lead and an increase in lead concentration in the fish body [7].

The presence of heavy metals, particularly lead, can negatively affect the development, health, and physiological systems of tilapia. Research by [8] indicated that lead exposure can disrupt the spawning process, cause morphological anomalies, and increase the mortality of tilapia sperm. Lead that passively diffuses into the egg yolk can also inhibit the work of enzymes that play a role in the growth and development of tilapia embryos. Tilapia (*Oreochromis niloticus* L.) is one type of freshwater fish that has high economic value and is the main target in aquaculture. Tilapia is widely cultivated because of its fast growth, high reproduction, and tolerance to various environmental conditions [9].

The stages of fish embryo development when entering the larval phase have several stages, including prolarvae, postlarvae, and juveniles. The prolarvae stage is a critical period in the life cycle of fish where their organs are not yet complete like their parents, so they are very vulnerable to environmental changes. The effects of toxic substances such as lead on the development of fish larvae include body shape abnormalities. Lead will replace ions in the larval bones, resulting in abnormalities such as scoliosis, lordosis, and kyphosis [10]. Abnormalities that occur in larvae due to lead can reduce the survival and abundance of fish in the aquatic environment. These

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abnormalities reduce resistance and survival to environmental conditions and cause an increase in larval mortality of up to 90% [11].

The effect of lead on fish embryo development has been reported by several researchers. Research conducted by [12], stated that tilapia eggs contaminated with Pb will cause changes in the potential of the egg membrane. Research conducted by [10], exposure to lead chloride with concentrations greater than 0.42 mg/L decreased egg hatchability and fish larval abnormalities. Research conducted by [13] showed that lead exposure can cause changes in body shape, such as kyphosis, in catfish (*Clarias gariepinus*) embryos. Research on lead toxicity to the development of tilapia prolarvae has not been widely reported so the results of this study are expected to provide information on the toxicity of lead (Pb) to the development of tilapia prolarvae (*Oreochromis niloticus* L.).

Research Methods

This research was conducted in April-June 2023 at the Biology Laboratory of the Wetland Ecology Unit and continued at the Biology Laboratory of the Zoology Unit, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, Southeast Sulawesi. The tools used in this research are an aquarium, beaker, petri dish, microscope, camera, plastic container, small net, stationery, dropper pipette, water quality meter, analytical balance, aerator, spatula and vial bottle. The materials used in this study are male and female tilapia (*Oreochromis niloticus* L.), tilapia eggs, clean water (FMIPA UHO borehole), fish feed (Platelled commercial), label paper, tissue, NaCl 0.9%, ovaprim hormone, chicken feathers, and 70% alcohol.

The method and design of this research used a Completely Randomized Design (CRD), namely that the quality of the aquarium as a treatment container was adjusted to achieve optimum conditions. The study consisted of 1 control and 3 lead (Pb) concentration treatments.

Research Procedure

Test Animal Preparation

The animals used in this study were tilapia (*Oreochromis niloticus* L.) obtained at BBI (fish seed center) Rahandouna, Poasia sub-district, Kendari city. Fish that had been obtained were then acclimatized. Acclimatization is done by immersing the plastic-containing fish obtained from BBI for 10-15 minutes in the broodstock aquarium until the fish comes out of the plastic itself. The criteria for tilapia that are used as broodstock for the spawning process are gonadally mature tilapia, both male and female fish.

The male and female tilapia with mature gonads were then injected with the hormone Ovaprim to obtain more seeds and a fast-spawning process. The injection of each hormone is done once at 18.00 WITA intramuscularly in male and female parents at a dose of 0.8 ml/kg female body weight, while for males, 0.3 ml/kg body weight [15]. After 5-6 hours from the injection, ovulation is checked by stripping the fish. If the parent fish has not shown signs of ovulation, then check once an hour until the parent ovulates. If the mother fish has not shown signs of ovulation, then check once an hour until the mother ovulates.

The next step is the preparation of hatchery ponds to be exposed to lead (Pb) with different concentrations. The size of the pond used was a bottle container with a volume of 1 liter, and an aerator was added to help the eggs not stick together while maintaining oxygen conditions in the water. The total amount of lead used in each pond was calculated using the formula: total volume of water in the aquarium multiplied by the amount of lead (Pb) concentration.

The semi-artificial spawning process with stripping technique after injection and lead exposure pool preparation. Fertilization is carried out by combining eggs and sperm and adding a 0.9% NaCl solution as a physiological solution, as well as diluting fish sperm. When it has been mixed, it will be homogenised using fine chicken feathers to maintain the condition of the fish eggs. The last stage is to put the egg sample into each treatment pond.

Aquarium Quality Measurements

The measurement of water quality parameters is carried out to determine the levels of temperature, pH, and DO. Measurement of aquarium temperature using a water quality meter, where the optimal temperature of water for tilapia fish ranges around 25-30°C. Measurement of the pH of the aquarium using a water quality meter, a suitable pH of good water for tilapia cultivation is between 6 and 8.5, with an optimum range of 7-8. DO (Dissolved Oxygen) or dissolved oxygen is measured using a DO meter. Good, dissolved oxygen (DO) is between 5-7 ppm [14].

Determination of Lead (Pb) Concentration

The concentration calculation of lead (Pb) in this study refers to the effective concentration dose obtained from research conducted by [14] using lead (Pb) concentrations of 0.15 mg/L, 0.30 mg/L, 0.45 mg/L, and 0.50 mg/L for the calculation of heavy metal concentrations of lead (Pb) based on the amount of water volume used in the study using the formula Total volume of water in the aquarium multiplied by the amount of lead (Pb) concentration.

Animal Test Treatment

Tilapia eggs that have been fertilized, put into a treatment container containing water with a volume of 1.5L that has been given exposure to lead (Pb) with a concentration of (Pb) 0.15 mg/L, 0.30 mg/L, 0.45 mg/L, and 0.50 mg/L, and then put into an aerator bottle to control oxygen levels in the water and keep the eggs from sticking together. The collection was carried out on day 3 after the eggs had hatched and entered the prolarvae stage. Taking the prolarvae using a spoon was done carefully to maintain the condition of the larvae. The characteristic found when the eggs have hatched and entered the prolarvae stage is when the hatching larvae are found floating to the surface or larvae at the bottom of the hatching pond. Larvae will be taken when the larvae have just entered the prolarvae phase,

with a total of 300 larvae, so each treatment and control will take 20 larvae.

Observation of Tilapia Prolarvae Abnormality

All live larvae in each treatment were examined for abnormalities using a 40 x 10 magnification microscope to calculate the percentage in each treatment. Parameters that are categorized as prolarvae experiencing abnormality are characterized by abnormal development, such as abnormal body shape. The degree of larval abnormality (DA) in each treatment was measured using the equation [16] below:

$$DA=\frac{\sum \text{abnormal larvae}}{\sum \text{larvae total}} \text{ x } 100\ \%$$

Description:
DA: Degree of abnormality
∑: Total quantity

Data Analysis

Data analysis in this study used the Statistical Product Solution (SPSS) version 26.0 application. Data on abnormal prolarvae parameters were analyzed using one-way ANOVA and continued with an LSD (Least Significant Difference) test at a 95% confidence level to see which treatment gave different effects.

Results and Discussion

Water Quality Measurement

Water quality measurements aim to monitor and ensure that the pond environment meets the needs for the hatching process of fish eggs and larvae. The results of the hatchery aquarium water quality measurements can be seen in Table 1.

Table 1. Hatchery aquarium water quality measurements

Treatment	Hatchery aquarium water quality measurement		
	Mean Temperature (°C)	Mean pH	Mean DO (mg/L)
K	28	7.76	5.3
P_1	28	7.7	5.3
P_2	28	7.66	5.26
P_3	28	7.63	5.26
P_4	28	7.63	5.26

Based on Table 1, the average measurement of the hatching aquarium temperature of all treatments is 28°C. This temperature is a suitable condition for the development process of fish embryos to the larval stage. In line with research conducted by [18], the temperature range for hatching tilapia eggs is in the range of 27-33°C, and the optimum temperature for egg hatching is ± 29°C. The average pH measurements in K, P_1, P_2, P_3, and P_4 are 7.76; 7.7; 7.66; 7.63, and 7.63 treatments also meet the pH standard suitable for hatching which is 7.84 this figure shows the optimum standard for hatching and development of tilapia eggs and the suitable pH range is 6.5-8.5 [17]. The dissolved oxygen content in the hatching funnel is quite good, which is 5.26-5.3 mg/L, as the dissolved oxygen content in suitable hatchery aquarium media is> 5 mg/L.

The results of the aquarium quality measurements carried out show that all aspects of aquarium quality fulfil the hatching process and the development of tilapia embryos. The absence of the influence of quality makes Lead exposure one of the main factors in the process of disrupting the prolarvae stage embryo development in tilapia, which results in the formation of abnormalities due to Lead exposure.

Percentage of Abnormal Prolarvae of Tilapia (*Oreochromis niloticus* L.)

The results of observations on the percentage of tilapia prolarvae abnormalities are presented in Table 2.

Table 2. Percentage of Abnormal Tilapia Prolarvae after 3 days of lead application.

Treatment	Percentage of Abnormal Tilapia Prolarvae (%) ± SD
K	0.00 ± 0.00^a
P_1	26.67 ± 7.64^b
P_2	45 ± 10.00^c
P_3	73.33 ± 7.64^d
P_4	83.33 ± 12.50^d

Note: Different letters indicate significant differences in the BNT test at a 95% confidence level

Based on Table 2, there is a significant mean difference between treatments. The mean number of abnormalities in each treatment, namely in the control treatment (K) was 0.00 lead concentration 0.15 mg/L (P1) was 26.67%; lead concentration 0.30 mg/L (P2) was 45%; lead concentration 0.45 mg/L (P3) was 73.33% and lead concentration 0.50 mg/L (P4) was 83.33%. The mean number of abnormalities between treatments K, P1, P2, and P3 showed a significant difference in each treatment ($P < 0.05$) this refers to the different letters that appear after the BNT test, while in the treatment of P3 and P4 is not significantly different ($P>0.05$) this is indicated by the same letter because the lead concentration between treatments only differs by 0.05 mg/L difference causing no significant abnormal increase. In contrast to the previous treatment, it was different to 0.15 mg/L.

The increase in the mean of the ANOVA test results shows that the provision of lead concentration affects the presence of abnormalities in prolarvae. This occurs because there is a significant influence between the independent variable and the dependent variable. The percentage difference in the mean abnormality is presented in Figure 1 below.

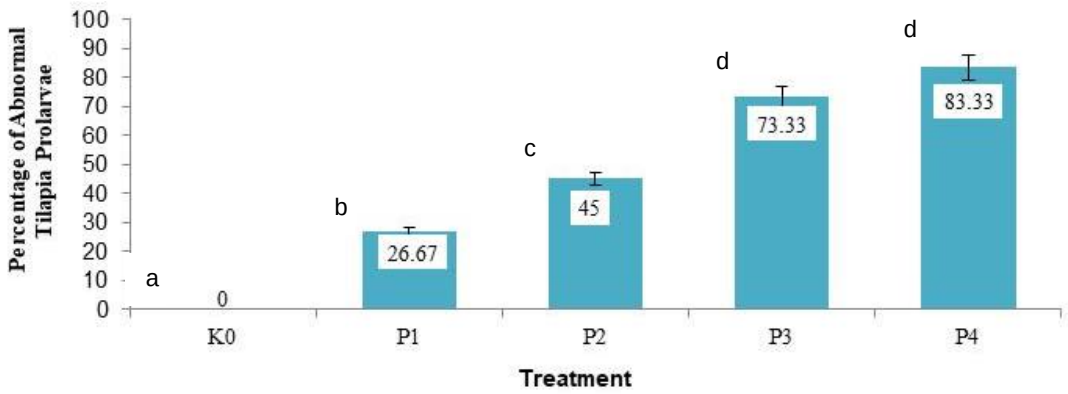

Figure 1. Histogram of the mean percentage of abnormal larvae of tilapia (*Oreochromis niloticus* L.) between control and treatment.

Based on the abnormal percentage histogram, Figure 1 shows that the control treatment (K) has an abnormal percentage of 0%. This percentage indicates that the control larvae (K) are in normal condition. [18] stated that the control treatment did not affect the survival rate and heart rate of tilapia larvae (*Oreochromis niloticus* L.). While in the treatment of lead administration, there was a marked increase in the histogram.

The increase in abnormal percentage occurred directly proportional to the increase in lead concentration given. This occurs because of the concentration of lead in the exposure container, which causes absorption to increase, causing many abnormalities to form. Exposure of larvae to lead through lead-contaminated water can cause developmental and health problems in the early stages of fish life, including larvae or prolarvae [19]. This can result in an increased percentage of deformed prolarvae. The higher the concentration of lead in the water, the greater the likelihood of larvae absorbing more lead, resulting in an increased percentage of abnormalities as the percentage of lead increases. This statement is supported by the research of [12], which states that the abnormality rate of tilapia larvae tends to increase as the concentration of lead in the exposure site increases.

Abnormal Morphology of Tilapia Prolarvae

Another side effect of lead exposure is that there are more varied abnormal shapes, or many types of abnormalities formed. The following types of fish prolarvae abnormalities can be seen in Figure 2.

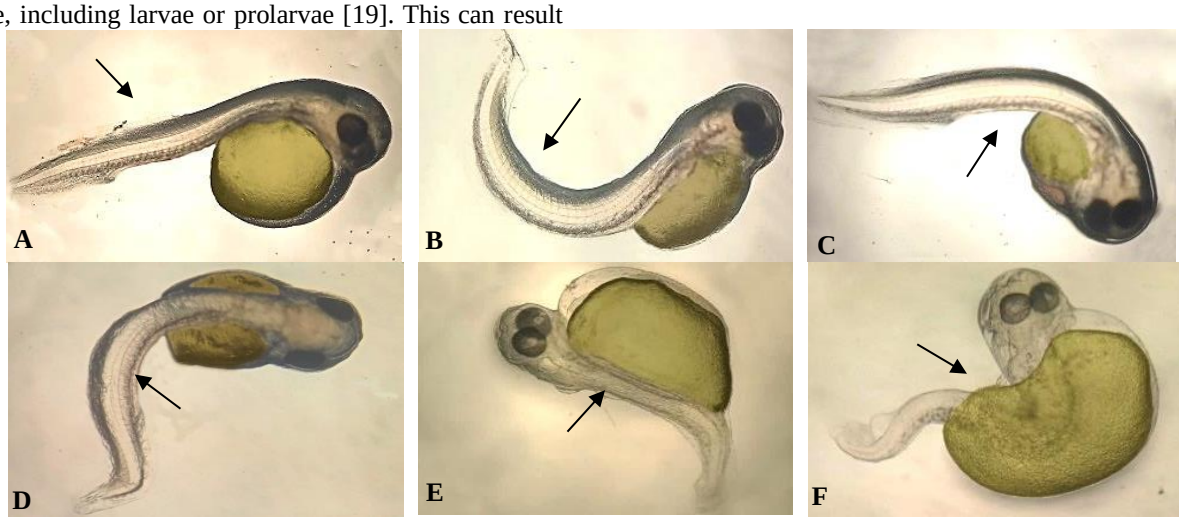

Figure 2. Various abnormal prolarvae of tilapia (*Oreochromis niloticus* L.) after lead (Pb) administration with varying concentrations for 3 days, magnification 100X

Description:

A. The prolarvae was normal.
B. Prolarvae with lordosis.
C. Prolarvae with kiposis.
D. Prolarvae with skoliosis.
E. Yolk deformation, with lordosis of the tail tip.
F. Yolk deformation, anterior yolk sac enlarged.

Figure 2 shows that the high level of lead (Pb) in the exposure media not only affects the increase in abnormalities in larvae, but also causes other side effects, such as more varied abnormal forms or many types of abnormalities formed. The abnormal forms of prolarvae found in this study include lordosis, kyphosis, yolk deformation accompanied by lordosis of the tail tip, and enlarged anterior yolk sac. The most common types of abnormalities found in this study were kyphosis, lordosis, and scoliosis. This is because lead is a toxic

substance that can affect the nervous system, bone development, and organs of the body. The nervous system and bone development are related to the spine in fish larvae, so abnormalities such as kyphosis, lordosis, and scoliosis are commonly found.

Types of kyphosis, lordosis and scoliosis disorders occur because reciprocity is a toxic substance that can affect the nervous system, bone development and body organs. The nervous system and bone development are related to the formation of spines in fish prolarvae. This is because the pond is exposed to lead during the egg phase, resulting in the lead contained in the pond being able to diffuse and be passively absorbed into the egg [20]. Lead that enters the body will accumulate in the tissue and damage it, especially bones. After lead enters the bone tissue, ion exchange occurs between the metal ions and the calcium mineral forming in the bones, as a result, it can disrupt the growth of bone tissue [23].

Calcium ions are very important in the development of bone tissue at the prolarval stage. Calcium is one of the main minerals used by organisms to build and strengthen bone and skeletal structures during development [12]. In the prolarvae stage of fish, calcium ions act as one of the main components in the bone matrix, which helps in the deposition of minerals such as calcium phosphate to form hard and strong bones; however, due to exposure to lead, bone development is disrupted. This happens because lead will replace calcium molecules. This condition can occur because there are several similarities between lead (Pb) and calcium (Ca) in a chemical context [24].

This is in line with the statement put forward by [20] that the most frequent abnormalities are those in the spine, especially lordosis (V-shaped dorsal-ventral curvature), kyphosis (Y-shaped dorsal-ventral curvature) and scoliosis (lateral curvature). Some studies also revealed that kyphosis, lordosis, and scoliosis were also experienced by zebrafish (Danio rerio) embryos [9] and kyphosis was also found in dumbo catfish (*Clarias gariepinus*) larvae exposed to Lead [22].

Other prolarvae abnormalities found in this study were yolk deformation accompanied by lordosis of the tail tip and enlarged anterior yolk sac. Yolk deformation at the prolarvae stage of fish refers to the condition in which the absorption and utilization of the yolk sac are abnormal during the early stages of fish larval development. In this study, deformation was found at the highest concentrations of P3 and P4. Research conducted by [19] also proved that exposure to Lead at a concentration of 0.40 mg/L caused six types of yolk deformation, followed by other abnormalities. Increased exposure to toxic substances, such as Lead or other pollutants, can cause yolk deformation in fish larvae. These toxic substances can interfere with the absorption and use of nutrients in the yolk sac, resulting in abnormalities in the early stages of larval development, as a result, fish larvae experience nutritional deficiencies that can cause yolk deformation [20].

Abnormalities that occur at the pro-larvae stage are caused by ponds that are exposed to lead during the egg phase, resulting in lead contained in the pond being able to passively diffuse into the eggs. This statement is supported by [10], which leads diffuses passively into the yolk. Incoming lead can accumulate in tissues and damage these tissues, especially bone. After lead enters bone tissue, ion exchange occurs between metal ions and calcium mineral formation in bone, which can interfere with the growth of bone tissue [21].

Lead replaces important metals in eggs and inhibits enzyme function in using proteins for embryonic growth. Inhibition of enzyme activity occurs through the formation of compounds between heavy metals and sulfhydryl (S-H) groups [23]. Enzymes that have S-H groups are the most easily affected enzyme groups. This happens because the S-H group easily interacts with heavy metal ions that enter the body. As a result, the enzyme's working power is significantly reduced or even stopped altogether, causing physiology and metabolism in the pro-larva stage to be disrupted [15].

Conclusion

Based on the results of this study, it can be concluded that Lead is toxic to tilapia prolarvae development. This is evidenced by the administration of Lead with various concentrations of 0.15 mg/L, 0.30 mg/L, 0.45 mg/L, and 0.50 mg/L, causing an increase in the percentage of abnormal cells along with the increase in Lead concentration. The increase in Lead also affects the types of abnormalities formed, such as lordosis, kyphosis, scoliosis, yolk deformation accompanied by lordosis of the tail tip, and enlarged anterior yolk sac. The heavy metal lead (Pb) has been proven to be toxic, so serious attention is needed in maintaining water quality from heavy metal contamination.

Author's Contribution

The research team's contributions to this research are: Wa Ode Harlis: contributed to data collection, data analysis and writing of the paper. Nurhayu Malik: contribution was data analysis and revision of the paper draft. Febrianto: contribution is data collection and data analysis. Resman: contribution is revision according to the review correction

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