Toxic effect of lead (Pb) on hatching rate and larvae abnormalities of Nile Tilapia (*Oreochromis niloticus*)

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**Abstract.** Like other fish, Nile tilapia (*Oreochromis niloticus*) is one species that potentially exposed to pollutants, including lead (Pb). However, the impact of Pb exposure on hatching rate and larvae development of Nile tilapia is still not investigated. Therefore, this study aimed to examine the toxic effect of Pb exposure on hatching rate and larvae abnormalities of Nile tilapia. A total of 2,400 fertilized eggs of Nile tilapia was distributed to control and 3 treatment group namely: treatment A (0.21 mg/L PbCl2), treatment B (0.42 mg/L PbCl2) and treatment C (0.63 mg/L PbCl2) with triplicate. The exposure period lasted for ten days. Cumulative hatching rate, survival rate, malformation rate, heart rate, body length, total lead content and deformities of larvae were analyzed. The results showed that increasing Pb concentration significantly increased malformation rate, heart rate, and total Pb content in Nile tilapia larvae. The highest malformation rate, heart rate and total lead content observed in treatment C were 3.4%, 115.6 beats/minute and 4.80 mg Pb/kg, respectively. Furthermore, Pb was affect several deformities of Nile tilapia larvae including lordosis, kyphosis, and curved tail. Otherwise, exposed to lead up to concentration 0.63 mg/L PbCl2 have no significant effect on cumulative hatching rate, survival rate and body length in Nile tilapia.

1. **Introduction**

Lead (Pb) is one of the heavy metal components known used for the production process of various metal products, batteries, pesticides, and ceramics [1]. Pb enters the aquatic ecosystem through the disposal of...
industrial waste, soil erosion, aerial deposition, and mining activity [2]. Previous results revealed a Pb contamination in several Indonesian waters, including the Tondano River, North Sulawesi [3], Lake Balang Tojong, South Sulawesi [4], and Batang Hari River, West Sumatra [5].

Nile tilapia (Oreochromis niloticus, Linnaeus 1758) is one common fish species widely distributed in Indonesian waters, being this species is vulnerable to pollutant exposure, including Pb. Exposure to various types of pollutants has reported affecting death and physiological disorders in Nile tilapia [6, 7]. Muliari et al. (2018) documented the histopathological damage in gill tissue in fish exposed to palm oil mill effluent, for instance, hyperplasia, lamella fusion, hemorrhage, and atrophy [8]. Moreover, the damage has led to a disruption in the respiratory performance of fish.

It is well known that exposure to pollutants can present a dangerous risk to the fish reproduction system [9]. Pb exposure was proven to disrupt the fish spawning process, which indicated by the increase of morphological anomalies and sperm mortality [10]. Pb diffuses passively into the yolk and inhibits the activity of the embryo’s growth enzyme [11]. In addition to, Pb displaced the ions in bone, causing several deformities in fish larvae such as scoliosis, lordosis, and kyphosis [11]. A study by Jeziorska et al. (2008) showed that Pb exposure disrupted the embryogenesis process of carp (Cyprinus carpio) characterized by blastula, shortening of the spine, and cardiac abnormalities [12].

The effects of pollutant exposures on hatchability and larval abnormalities in various fish species including zebrafish (Danio rerio) [13], carp (Cyprinus carpio) [12] and catfish (Clarias gariepinus) [10] have been recorded. However, studies underlying the effects of Pb exposure on hatching and abnormalities of tilapia larvae still not reported. Therefore, this present study aimed to investigate the effect of Pb exposure on the hatchability and abnormalities of Nile tilapia larvae.

2. Materials and Methods

2.1 Chemical and fish eggs specimen
Lead chloride (PbCl₂) (Pudak Scientific, Indonesia) was purchased from the Mathematics and Natural Sciences, Laboratory of Chemistry, Syiah Kuala University. A total of 2,400 fertilized Nile tilapia eggs were obtained from Brackish Aquaculture Fisheries Center (BPBAP) Ujong Batee, Aceh Besar Regency, Province of Aceh, Indonesia. The stages of exposures and observations on hatchability and abnormalities of larvae were conducted at BPBAP. Measurements of Pb contents in fish larvae were conducted at the Laboratory of Research and Industrial Standardization Laboratory, Banda Aceh, while data analysis was carried out at the Biology Laboratory of the Faculty of Science and Technology, Ar-Raniry State Islamic University of Banda Aceh.

2.2 Experimental design
The experiments were conducted with four treatments, each with three replications. Concentration of Pb used in each treatment was selected based on environmentally realistic concentrations of Pb in several Indonesia waters that exceeded the government regulatory limit for Pb, i.e. greater than 0.03 mg/L (PP No. 82 of 2001), for instance, Tondano River (0.14 mgPb/L) [3], Lake Balang Tojong (0.49 mgPb/L) [4], and Lhokseumawe waters (0.10 mgPb/L) [14]. In detail, Pb concentrations in each treatment are as follows: Control (0 mg/L of PbCl₂), Treatment A (0.15 mgPb/L, equivalent to 0.21 mg/L of PbCl₂), Treatment B (0.30 mgPb/L equivalent to 0.42 mg/L of PbCl₂), and Treatment C (0.45 mgPb/L equivalent to 0.63 mg/L of PbCl₂).

The hatching process of Nile tilapia eggs carried out in a funnel filled with 90 liters of brackish water (12-13 ppt) equipped with aeration. 200 Nile tilapia fertilized eggs were randomly distributed to each treatment funnel. Pb exposure period lasted for 10 days. Several parameters such as hatching rate, survival, heart rate, malformation rate, abnormalities of larvae, Pb content in larvae, bioconcentration factors, and daily accumulation rates of Pb were examined. Heart rate was measured on the fifth day, while survival rate, larval length, malformation rate, larval deformities, and Pb content in larvae were determined at the end of the exposure period. Heart rate was calculated by counting the number of heartbeat in 60 s using video recording. Larval body length measurement was conducted by measuring the total length of 30 larvae in each treatment and control by using a stereo microscope (Olympus, Japan).
equipped with a micrometer. The deformities in both dead and live larvae were observed and photographed using a stereo microscope (Olympus, Japan) connected to a camera and computer at the end of the exposure period.

2.3 Data analysis
Data were presented as mean and standard deviations. Hatching rate, survival rate, malformation rate, heart rate, and larval length between treatments were analyzed using a one-way analysis of variance (one-way-ANOVA) at 95% of confidence level. Data related to larval deformities were examined descriptively. Statistical analysis was performed using SPSS software version 22 (IBM SPSS Statistics, IBM, Chicago, USA, Macintosh Version).

3. Results and Discussion
3.1 Hatching rate, survival rate, total length, and heart rate
Nile tilapia eggs began to hatch on the second day or the third day of the exposure period. The total length of larvae at the end of the exposure period ranged from 11.33 to 11.40 mm. The statistical analysis showed that there were no significant differences in the parameters of cumulative hatching rate, survival rate, and larval length between treatments (p > 0.05). Conversely, there was a significant increase in heart rate in treatment C compared to control (p < 0.05). The heart rate of tilapia larvae in control was 91.37 ± 7.61 beats/minute while in treatment C, it increased by 115.6 ± 10.65 beats/minute (Table 1).

These results tend to be different from other related studies on the impact of the pollutants on fish larvae. For example, Pb exposure with a concentration of 6.86 mg/L has caused a decrease in survival rates in juvenile tiger grouper (Epinephelus fuscoguttatus) [15]. No toxicological impact of Pb on the survival rate and total length of Nile tilapia larvae in this study might be related to the low concentration of Pb in exposure media. A similar result was also reported by Triayani et al. where Pb exposure with a concentration of 0.05 mg/L did not show a significant effect on the survival rate of duck grouper fish (Cromileptes altivelis) [16]. Pb exposure with a concentration of 0.006 mg/L showed no effect on the survival of tilapia (Oreochromis niloticus), but at a concentration of 0.18 mg/L Pb could reduce the survival rate [17]. Other related research results also explained that at a low concentration with a short exposure period, exposure to Pb also did not affect the growth lengths of larval of zebrafish (Danio rerio) and tilapia (Oreochromis niloticus) [11].

| Parameter               | Control      | Treatment A   | Treatment B   | Treatment C   |
|-------------------------|--------------|---------------|---------------|---------------|
| Hatching rate (%)       | 59.83 ± 14.02a | 49.17 ± 5.77a | 43.00 ± 7.00a | 43.33 ± 7.82a |
| Survival (%)            | 91.37 ± 4.10a | 85.98 ± 6.04a | 85.94 ± 5.62a | 85.39 ± 14.89a |
| Total length (cm)       | 11.40 ± 0.18a | 11.40 ± 0.16a | 11.32 ± 0.17a | 11.33 ± 0.07a |
| Heart rate (bpm)        | 91.37 ± 7.61a | 102.40 ± 10.94a | 110 ± 7.20a   | 115.60 ± 10.25b |

Values in a row with different alphabetical superscripts are significantly different (p < 0.05).

Although it has no significantly reduced hatching rate, survival rate, and a total length of Nile tilapia larvae, exposure to Pb negatively impacts the heart rate and malformation rate of Nile tilapia larvae. Larval heart rate has begun to be used as a bioindicator to assess the negative impact of pollutant exposure [18]. The heart rate can describe blood flow, metabolic processes, respiration, and stress levels in fish [19]. The results showed that exposure to lead chloride with a concentration of 0.63 mg/L was significantly increased the heart rate of Nile tilapia larvae. Similar results were also observed in zebrafish (Danio rerio) exposed to monosodium glutamate [20]. Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) has caused a significant increase in heart rate of zebrafish larvae up to 140-180 beats per minute [21]. An increased heart rate of fish larvae due to exposure to pollutants was an effort to support the performance of detoxification of pollutants and other body metabolisms [22]. In addition, damage to fish gill tissue due to Pb exposure had an impact on decreasing the amount of oxygen that can be induced...
into the body [16]. This has an impact on reducing oxygen supply to support the body's metabolic processes. Therefore, the fish heart seeks to respond by increasing the heart rate so that the frequency of oxygen binding increases.

3.2 Larvae abnormality and malformation rate

There were three forms of larval abnormalities observed in this study namely lordosis, kyphosis, and curved tail (Figure 2). Lordosis was the most common form of abnormality, while the curved tail was the least which were 54% and 15%, respectively (Figure 3). The malformation rate of larval tended to increase with the increasing Pb concentration in the exposure media. The highest larval malformation rate was found in treatment C while the lowest one was found in the control treatment, were 3.4% and 0.33%, respectively. Based on the statistical analysis, there were significant increased of larvae malformation rates in treatment C compared to controls ($p < 0.05$) (Figure 3). The rate of tilapia malformation tended to increase with the increasing Pb concentration in the exposure media. Lordosis is the most common type of malformation observed in Nile tilapia larvae exposed to Pb. Several other studies revealed that lordosis was also found in larvae of African catfish (Clarias gariepinus) exposed to Pb [23], in zebrafish (Danio rario) exposed to mercury [13] and in tilapia (Oreochromis niloticus) exposed to insecticides [24].

![Figure 1](image1.png)

**Figure 1.** Frequency of abnormality and malformation rate of nile tilapia larvae. Values with different alphabetical superscripts are significantly different ($p < 0.05$).

3.3 Pb Content, bioconcentration factors, and daily accumulation rates

The highest Pb content in Nile tilapia larvae was observed in treatment C while the lowest one was observed in the control were 4.80 mg/kg and 0 mg/kg, respectively. The bioconcentration factors and the daily rate accumulation of Pb tended to increase as well as increasing concentration of Pb in exposure media and Nile tilapia larvae. The highest of bioconcentration factors and daily accumulation rates were found in treatment C (8.01 and 0.48 mgPb/day, respectively) (Table 2).

**Table 2.** Pb content, bioconcentration factors, and daily accumulation rates of Pb in Nile tilapia larvae at the end of the exposure period.

| Treatment   | Pb content in media (mg/l) | Pb content in larvae (mg/ kg) | Bioconcentration Factor | Daily Accumulation Rate (mgPb / day) |
|-------------|--------------------------|-------------------------------|-------------------------|-------------------------------------|
| Control     | 0.00 ± 0.00              | 0.00 ± 0.00                   | 0.00 ± 0.00             | 0.00 ± 0.00                         |
| Treatment A | 0.20 ± 0.00              | 1.54 ± 1.53                   | 7.36 ± 0.95             | 0.15 ± 5.98                         |
| Treatment B | 0.40 ± 0.00              | 1.35 ± 2.35                   | 3.39 ± 0.86             | 0.13 ± 6.27                         |
| Treatment C | 0.60 ± 0.00              | 4.80 ± 4.27                   | 8.01 ± 2.97             | 0.48 ± 3.68                         |
Pb tends to accumulate in the bone tissue of the larva resulted in impaired bone development [25]. The results showed a positive correlation between increasing Pb content in the media and increasing Pb content in fish larvae. Pb accumulation in fish larvae results from the presence of metallothionein (sulfhydryl-SH) and amines (nitrogen-NH) that bind covalently to Pb. The Pb will enter the cells and be distributed by the blood throughout the whole body so that it can accumulate in other organs. Pb exposure was reported to disrupt the process of calcium ion exchange in bone tissue, resulting in fish bones experiencing abnormalities in the form of lordosis, kyphosis, and curved tail [11]. Pb ions have the ability to replace the presence of calcium ions contained in bone tissue, causing skeleton malformations in fish embryos [26]. Malformed larvae were at risk of slow growth and difficulty in obtaining food and avoiding predation [27].

Figure 2. Malformations observed in Nile tilapia larva exposed to different concentrations of PbCl\(_2\). (A) Normal appearance of Nile tilapia larvae after six days of exposure to 0 mg/L PbCl\(_2\). (B) Larvae with curved tails after four days of exposure to 0.20 mg/L PbCl\(_2\). (C) Larvae with kyphosis after four days of exposure to 0.40 mg/L PbCl\(_2\). (D) Larvae with Lordosis after nine days of exposure to 0.60 mg/L 1 PbCl\(_2\).

4. Conclusion
Exposure to Pb chloride with a concentration higher than 0.63 mg/L, has caused an increase in heart rate and malformation rate of tilapia larvae. The malformation of tilapia exposed to Pb includes lordosis, kiposis, and tail formation.

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