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Toxicity of Copper (Cu) on Abnormalities and Mortality of Tilapia Fish (*Oreochromis niloticus* L.) Prolarvae

Wa Ode Harlis[1*)], Desti Yulia Saputri [1)], Nurhayu Malik[1)], Andi Septiana[1)], Resman[2)]

[1)] **Department of Biology, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, Indonesia**
[2)] **Department of Soil Science, Faculty of Agriculture, Halu Oleo University, Kendari, Indonesia**
*Corresponding author: waodeharlis@gmail.com

ABSTRACT

Contamination of the heavy metal Copper (Cu) in waters can inhibit fish growth by reducing the level of gonad maturity and closing the gill membranes. changes in activity, morphological abnormalities of the prolarvae and even death. This study aims to assess the toxicity of Copper (Cu) to tilapia fish prolarvae (*Oreochromis niloticus* L.). This research is a type of experimental research structured based on a Completely Randomized Design (CRD) pattern. A total of 240 three-day-old tilapia prolarvae were divided into four treatment containers, namely Control, P1(2.0 mg/L), P2(2.5 mg/L), P3(3.0 mg/L), and each treatment consisted of three replications. Six hours after administration of copper, mortality parameters were observed, while prolarval abnormalities were observed after 48 hours. Data were analyzed using one way ANOVA (Analysis of Varience) α=0.05. The results of the study showed that the average percentage of mortality for controls was (0%), P1(6.67%), P2(23.33%), P3(36.67%) while the forms of abnormalities found were kyphosis, lordosis, scoliosis and tail sticking. The research results show that copper (Cu) has a toxic effect because it increases the percentage of mortality and causes abnormalities in tilapia prolarvae (*Oreochromis niloticus* L.).

Keywords: Copper (Cu); *Oreochromis niloticus*; prolarvae; toxicity

Toksisitas Tembaga (Cu) Terhadap Abnormalitas dan Mortalitas Prolarva Ikan Nila (*Oreochromis niloticus* L.)

ABSTRAK

Pencemaran logam berat Tembaga (Cu) pada perairan dapat menghambat pertumbuhan ikan dengan menurunkan tingkat kematangan gonad dan menutup membran insang. perubahan aktivitas, abnormalitas morfologi prolarva bahkan kematian. Penelitian ini bertujuan untuk mengkaji toksisitas Tembaga (Cu) terhadap prolarva ikan nila (*Oreochromis niloticus* L.). Penelitian ini merupakan jenis penelitian eksperimental yang disusun berdasarkan pola Rancangan Acak Lengkap (RAL). Sejumlah 240 ekor prolarva ikan nila yang berumur tiga hari dibagi kedalam empat wadah perlakuan yaitu Kontrol, P1(2,0 mg/L), P2(2,5 mg/L), P3(3,0 mg/L), dan setiap perlakuan terdiri dari tiga ulangan. Enam jam setelah pemberian Tembaga dilakukan pengamatan parameter mortalitas, sedangkan pengamatan abnormalitas prolarva dilakukan setelah 48 jam. Data dianalisis menggunakan one way ANOVA (*Analysis of Variance*) α=0.05. Hasil penelitian menunjukkan bahwa rata-rata persentase mortalitas yaitu kontrol sebesar (0%), P1(6,67%), P2(23,33%), P3(36,67%) sedangkan bentuk abnormalitas yang ditemukan adalah kiposis, lordosis, skoliosis dan ekor menempel. Hasil penelitian diketahui bahwa Tembaga (Cu) memiliki efek toksik karena meningkatkan persentase mortalitas dan menyebabkan abnormalitas pada prolarva ikan nila (*Oreochromis niloticus* L.).

Kata Kunci: Prolarva; *Oreochromis niloticus;* tembaga (Cu); toksisitas

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INTRODUCTION

Pollution that occurs in waters due to the presence of heavy metals has become a very important issue in the environmental and health fields (Das *et al.*, 2023). Heavy metals are one of the toxic pollutants that can cause death (lethal) and non-death (sublethal) such as disruption of growth, behavior and morphological characteristics of various aquatic organisms (Karunia *et al.*, 2024). The heavy metal commonly found in waters is copper (Cu). Copper (Cu) is a reddish powder that settles in water (Elkhatat *et al.*, 2021). High levels of Copper (Cu) in the aquatic environment can damage the life of aquatic organisms (Mebane, 2023). Based on the quality standards for Copper (Cu) in the aquatic environment according to government regulations No. 20 of 1990 of 0.2 mg/L which is permitted to exist in waters (Ibrahim & Elbastamy, 2023).

Contamination of the heavy metal Copper (Cu) is generally caused by various types of waste, both domestic, industrial, agricultural and mining (Chileshe *et al.*, 2020). Based on the report by Tuoyo and Ikechukwu (2025), which revealed that the presence of Copper (Cu) metal comes from household waste such as cleaning materials and pesticides used in agricultural activities which enter river water through surface flow. This is also based on Umi *et al.* (2023) that Copper (Cu) enters the waters as a result of erosion of mineral rocks and through Copper (Cu) compounds in the atmosphere carried by rainwater. Water contaminated with the heavy metal Copper (Cu) will affect aquatic organisms such as fish. According to Tuoyo & Ikechukwu (2025), Copper (Cu) in water can accumulate in the fish's body directly through the gills and skin. The impact of heavy metal accumulation in fish can, among other things, reduce the level of gonad maturity, close the gill membranes, so that the fish will lack oxygen and inhibit growth, besides that the fish meat will become unsafe for consumption (Ahmad *et al.*, 2023).

Fish can be used as a bioindicator of environmental pollution, including heavy metal contamination (Wójtowicz *et al.*, 2022). Fish can show reactions to the presence of pollution in the waters within certain concentration limits, such as changes in activity, effects on abnormal growth and even death (Canosa & Bertucci, 2023). Tilapia belongs to the genus Oreochromis and is known to have good tolerance to environmental stress, is easy to breed and has a fast weight growth rate (Abd *et al.*, 2022). This fish is also classified as a euryhaline type of fish based on its way of life, namely a fish that is able to live with high salinity tolerance so that its distribution is quite wide, including rivers, lakes, reservoirs, swamps and also brackish water (Jahan *et al.*, 2025).

The development of tilapia embryos before entering the larval phase consists of three phases, namely prolarvae, postlarvae and juveniles (Abu *et al.*, 2023). The larval phase is a phase where the body organs are not yet complete and the larva's life is completely dependent on food sources or energy reserves that have been prepared by the mother (Hastenreiter *et al.*, 2020). The prolarval phase is a very sensitive phase because it has not been able to adapt well to the environment and its body organs are not as complete as the parent (Harlis and Malik, 2025). This also makes the prolarvae very susceptible to heavy metal Copper (Cu) pollution in the water.

Toxic stress from the environment such as exposure to copper (Cu) can have an impact on fish larvae, namely death and abnormalities in the larvae. According to Harlis *et al.* (2025)) tilapia (*Oreochromis niloticus* L.) is a type of fish that has been proven to be susceptible to abnormalities in larvae. Copper research on tilapia has been reported by several researchers. Research conducted by Jahan *et al.* (2025) shows that high concentrations of Copper (Cu) in fish can damage the fish's gills, liver, kidneys and nervous system. This is also supported by research conducted by Harlis *et al.* (2025) that copper levels at a concentration of 2.3-2.5 mg/L can kill fish and will cause damage to the mucous membranes. According to Jahan *et al.* (2025), if fish live in water and are exposed to heavy metals for a long time, the Cu ion content can be absorbed into the fish's body and cause toxic effects. Copper (Cu) toxicity testing on prolarval stage tilapia has not been widely reported so this research is important to carry out. The expected aim of this research is to provide information to the public and government regarding the negative impacts of heavy metal pollution in waters for aquatic organisms and human health, so that it can become a consideration and establish regulations to maintain the health of the aquatic environment.

RESEARCH METHOD

Study area and time

This research was carried out in June-July 2024 at the Fisheries Cultivation, Hatchery and Production Laboratory, Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Southeast Sulawesi.

Animals and materials

The materials used in this research included: Three month old male and female tilapia (*Oreochromis niloticus* L.), tilapia prolarvae, clean water, label paper, tissue, copper (Cu) and fish food. The tools used include: basins, analytical scales, vials, blowers, stationery, cameras, spatulas, jars, water quality testers, light microscopes and small filters.

Research design

The method in this research is the Completely Randomized Design (CRD) method which consists of one control and three treatments, namely control/without addition of Copper (K0); Copper concentration 2.0 mg/L (P1); Copper concentration 2.5mg/L (P2) and Copper 3.0 mg/L (P3) (Pratiwi, 2020).

The treatment containers in this study used jars with a water volume of 1 L containing 20 tilapia prolarvae per container (Yoanda *et al.*, 2023). Based on the grouping pattern, there are three groups, namely K0, P1, P2 and P3, each consisting of 3 replications. The research design can be seen in Figure 1 below.

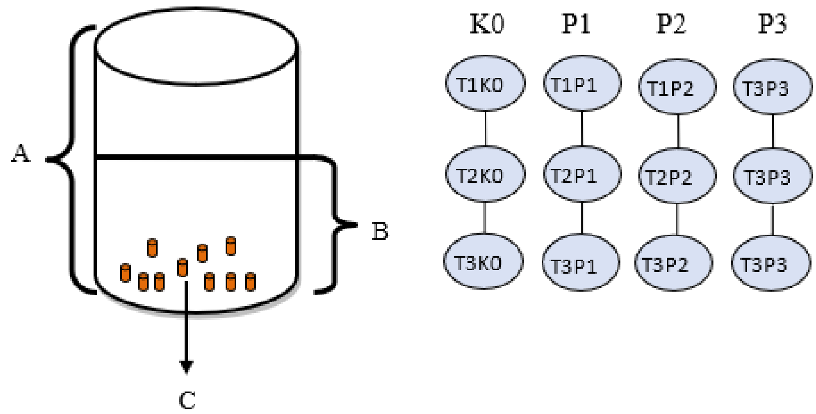

Figure 1. Treatment container design. (T) Test prolarval container; (A) Treatment container; (B) Water volume; (C) Tilapia prolarvae.

Preparation of prolarvae

The female tilapia fish used first have their mouths checked to ensure there are fertilized eggs in them. Checking fish eggs is carried out periodically for 2 days. After the eggs hatch and turn into prolarvae, they are then removed from the mother's mouth and placed in a treatment container that has been treated with copper. Observations for mortality were observed for 6 hours, while observations for abnormalities were observed for 48 hours (Harlis & Malik, 2025). The size of the observation container used was 2 liters with a water volume of 1 liter. The total amount of copper used was calculated using the formula: volume of water x concentration of copper (Cu) (Harlis *et al.*, 2025).

Treatment

Tilapia fish prolarvae were put into a treatment container containing 1 liter of water that had been exposed to copper (Cu) with a concentration of 2.0 mg/L, 2.5 mg/L and 3.0 mg/L in each container, then given a blower to control oxygen levels. The treatment containers were divided into 3 treatments and 1 control, then the observation stage was carried out on the 3rd day after the tilapia eggs hatched and entered the prolarva stage. The observation variables are the percentage of prolarval abnormalities and the percentage of prolarvae mortality (Harlis *et al.*, 2025).

Data collection

The percentage of prolarval abnormalities was measured by the Degree of Larval Abnormality (DA) in each treatment using the equation Harlis *et al.* (2025) as follows:

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

Where, DA : Degree of abnormality; $\sum$: Total number

Prolarval mortality is known using the equation Harlis *et al.*, (2025) as follows:

$$\text{Mortality} = \frac{a}{b} \text{x } 100\%$$

Where,

a: Number of dead tilapia prolarvae; b: Total number of tilapia prolarvae tested

Data analysis

Data analysis in this research was carried out using the Statistical Product Services Solution (SPSS) application program. Data on abnormal parameters and prolarval mortality were analyzed using Analysis of Variance (ANOVA) with a confidence level of 95% and the Least Significant Difference (LSD) further test with a confidence level of 95% (Sallam *et al.*, 2025).

RESULTS AND DISCUSSION

Percentage of Abnormalities and Percentage of Mortality of Tilapia Prolarvae

Prolarva abnormalities are abnormalities that occur in the prolarva after administration of toxic substances. Prolarval mortality is the death rate that occurs after administration of toxic substances. The results of observing the percentage of abnormalities and the percentage of mortality after administering copper metal (Cu) to tilapia prolarvae are presented in Table 1.

Table 1. Average Abnormal Percentage and Mortality of Tilapia Prolarvae after Giving Copper (Cu).

Treatment	Abnormal Percentage (Mean ± Standard Deviation)	Mortality Percentage (Mean ± Standard Deviation)
K0	(0.00 ± 0.00^{a})	(0.00 ± 0.00^{a})
P1	(43.33 ± 1.53^{b})	(6.67 ± 0.58^{a})
P2	(63.33 ± 2.08^{bc})	(23.33 ± 0.58^{b})
P3	(86.67 ± 1.53^{c})	(36.67 ± 0.58^{c})

Note: Numbers followed by different notations indicate significant differences in the LSD test α=0.05

Based on Table 1, it can be seen that there is a significant mean difference between treatments. The average number of abnormalities in each treatment was, K0 no abnormal prolarva were found, in treatments P1, P2 and P3 there was an increase in the average abnormality. The mean number of abnormalities between treatments K0 and P1 was significantly different in the LSD test (α=0.05), as well as in P2 and P3 as indicated by the mean values marked with different letters. Table 1 shows that the administration of copper (Cu) concentrations can have a toxic effect on abnormalities in fish prolarvae as the copper concentration administered increases. K0 treatment has an abnormal percentage of 0%. This percentage shows that the K0 prolarvae are in normal condition, whereas when treated with concentrations of 2.0 mg/L, 2.5 mg/L and 3.0 mg/L, there was an abnormal increase in tilapia larvae. This increase occurs because the copper concentration level in the exposure container is increasing, causing absorption to increase and resulting in many abnormalities being formed. Exposure to Copper (Cu) in fish prolarvae, through water contaminated with copper, can cause developmental and health problems in the early stages of fish life.

The average percentage of mortality in each treatment was, at K0 no dead prolarva were found. This makes it possible for the prolarva to suit the environment in the treatment container for its development. In line with research conducted by Farias *et al.*, (2025) which stated that the control treatment had no effect on the survival rate and heart rate of tilapia larvae, while in the treatment the average percentage of deaths increased, namely 6.67% (P1), 23.33% (P2) and 36.67% (P3). This increase shows that copper concentration has a toxic effect on fish prolarvae, causing death.

Exposure to copper in fish larvae causes an osmotic imbalance and changes the ion exchange regulatory system, which ultimately reduces blood pH and erythrocyte volume and then lowers hematocrit (Yulianto *et al.*, 2023). Copper powder does not have polarity which makes it insoluble in water. Copper powder can enter the bodies of aquatic organisms such as fish through the process of digestion or absorption by the gills and skin. This is exacerbated by the toxic impact of Cu which damages the fish's olfactory sensory system so that they have difficulty responding to food. In line with research conducted by Jahan *et al.*, (2025) that an excessive increase in copper concentration causes toxic effects, namely it can damage the gills, liver, kidneys and nervous system of fish, leading to death

Abnormal Forms of Tilapia Prolarvae

The abnormal form of tilapia prolarvae can be seen in Figure 2.

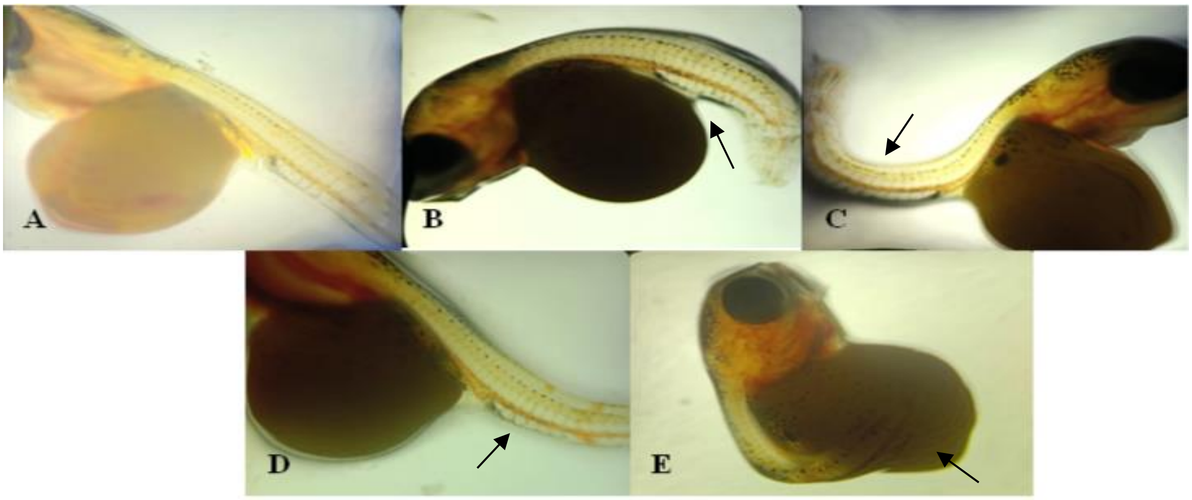

Figure 2. Various abnormal shapes of tilapia fish prolarvae (Oreochromis niloticus L.) after administration of copper (Cu) at varying concentrations. (A) normal prolarvae; (B) prolarvae experiencing kyposis; (C) prolarva undergoes lordosis; (D) prolarva has scoliosis; (E) prolarva has tail attached.

Based on Figure 2, it shows that administration of copper causes abnormalities in tilapia prolarvae. Treatment with a concentration of 2.0 ml/L (P1) found abnormal lordosis and kyposis prolarvae. Meanwhile, treatment with a dose of 2.5 ml/L (P2) and 3.0 ml/L (P3) found abnormal types of lordosis, kyposis, scoliosis and tail sticking. This is supported by the statement of (Wang *et al.*, 2020) that increasing copper levels can result in toxicity in fish because it disrupts their physiological functions. Copper that accumulates in the bodies of fish larvae can increase the production of Reactive Oxygen (ROS) in cells. This is in line with research by (Jahan *et al.*, 2025) which states that excessive ROS will cause oxidative stress and disrupt cellular function through lipids and proteins which can damage the structure and function of the spine. Velisek *et al.* (2022) stated that giving a dose of 1.0; 2.0

and 4.0mg/L in larvae indicate developmental abnormalities. Overall copper exposure has developmental abnormalities, especially those related to the spine (kyphosis and lordosis) and the cardiovascular system (abnormal position and looping of the heart).

High copper levels in exposure containers not only increase the percentage of abnormalities in larvae, but there are also other side effects that arise due to this exposure, namely, more types of abnormalities are formed. Another abnormality found in this study was a sticking tail. The cause of the high level of damage or abnormality is due to the excessive amount of concentration used, resulting in a sticking tail abnormality. Velisek *et al.* (2022) said that increasing waterborne copper levels can affect the development of larvae that are more sensitive to toxicity. This is supported by Yulianto *et al.* (2023) that exposure to copper can cause increased abnormalities in larval morphology, such as delayed development and spinal and craniofacial deformities.

Calcium is closely related to skeletal development, bone maintenance and mineralization. Calcium has a very important role in the fish's body, including in bone formation. Copper in high concentrations can change the structure and function of the calcium-ATPase enzyme. Copper can bind to sulfhydryl (SH) groups on enzymes, thereby replacing metal ions that play a role in enzyme activity and inhibiting their function, thereby reducing the efficiency of calcium-ATPase, which results in disturbed regulation of calcium in cells (Harlis *et al.*, 2025)

Measurement of Water Quality in Prolarva Rearing Containers

Water quality is an indicator that greatly influences the success of fish hatcheries. Water quality measurements aim to monitor and ensure that the treatment container remains appropriate for the development process of fish larvae. The water quality parameters observed in this study were temperature and pH. The results of the water quality analysis can be seen in Table 2 below.

Table 2. Average Measurement of Water Quality in Prolarva Rearing Containers.

Treatment	Temperature (°C)	pH
K0	27	7.7
P1	27	7.66
P2	27	7.53
P3	27	7.53

Water quality is an indicator that greatly influences the success of fish hatcheries. Water quality measurements aim to monitor and ensure that the treatment container remains appropriate for the development process of fish larvae. The water quality parameters observed in this study were temperature and pH. Based on water quality measurements in each treatment container, the average temperature obtained in the treatment container was 27°C, this temperature is a good temperature for the development process of fish larvae. This is in line with research conducted by Fadila *et al*. (2023) that a good temperature for raising tilapia fry is between 25-32 °C. Another parameter in this research is pH. The average results obtained from pH measurements were 7.7; 7.66; 7.53 and 7.53. The average pH obtained is a suitable pH for rearing tilapia larvae, and includes the optimum standard for hatching and development of tilapia eggs.

Wulansari *et al*. (2022) said that increasing temperatures do not always kill fish but can also cause health problems, the body becomes weak, and behavior becomes abnormal. When the temperature drops it allows the water to contain more oxygen, but low temperatures can also cause the fish to become stressed, weakening the respiratory rate and heart rate which can lead to the fish fainting, resulting in a weakened immune system. Masruroh & Purnomo (2023) also said that low water pH causes the bioaccumulation of the heavy metal copper to become higher and if the pH value of the water is high it causes low bioaccumulation of the heavy metal copper. Based on established quality standards, the presence of copper in water that can be tolerated is 0.2 mg/L based on Republic of Indonesia Government Regulation No. 82 of 2001 (Ariq *et al*., 2022).

CONCLUSION

The heavy metal Copper (Cu) in all treatments caused an increase in the percentage of mortality and caused abnormalities in the prolarvae. The types of abnormalities found include lordosis, kyphosis, scoliosis and sticking tail. The heavy metal copper (Cu) is toxic to aquatic organisms, so there needs to be efforts from the community and government to prevent heavy metal copper pollution in waters.

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GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies

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