Biological test of the laundry industry toxicity of detergents and concentration of hemoglobin in tilapia (*Oreochromis niloticus*)

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Abstract. The detergent from the laundry industry that continues to flow directly into the river will affect the oxygen supply of the aquatic biota, due to the disruption of the respiratory system. The objective is to determine the level of toxicity and hemoglobin concentration of tilapia (*Oreochromis niloticus*) exposed to detergent residues from the laundry industry. This research method uses a randomized block design. This test was performed with five treatments and control with three repetitions. Each treatment was filled with detergent residues from the laundry industry with varying levels of concentration, namely 0%, 1%, 2%, 3%, 4% and 5% in each aquarium. The results showed that the highest mortality of tilapia occurred at a concentration of 5% up to 8 tails. While the lowest concentration of hemoglobin on day 10 and day 20 each occurred at a concentration level of 4% in (8.37% gr and 7.65% gr), while on day 30, the lowest concentration of hemoglobin was found in Treatment concentration level of 5% for (6.42 gr%). It can be concluded that residues from the washing industry can affect the oxygen supply in fish, which is characterized by a reduced concentration of hemoglobin in tilapia from the normal concentration of hemoglobin.

1. Introduction

The growing number of the human population makes the use of detergents in the community increase along with the improvement of income. The detergents that are used daily are usually in the form of detergents that function as cleaning clothes from dirt. The use of detergent will produce waste that flows into the water. The detergent is one of the waste originated in the home and in the laundry industry. The laundry industry is highly developed in large cities, especially in the vicinity of student pensions that want an instant washing process. The working process of the laundry industry is very simple, that is, dissolving detergents in water because detergents have a better hardness than soap [1].

These detergent residues from the laundry industry will cause a decrease in water quality. The negative effect of the detergent on the physical and chemical conditions of the water flowing through the waste can occur directly or indirectly. One of the effects of detergent residues on the environment is the decrease in dissolved oxygen levels in the water. Tilapia (*Oreochromis niloticus*) is one of the test animals that is used as a bioindicator of pressure changes in the environment, especially in water [2]. Tilapia (O. niloticus) is one of the aquatic biota recommended by the USEPA (Environmental
Protection Agency of the USA), as a test animal for toxicology and easy to maintain in the laboratory [3].

The greater the accumulation of detergent flowing in the water, the lower the supply of dissolved oxygen in the water. This causes the interruption of the breathing process in the fish. So that causes death in the fish.

The death that occurs due to a physiological deviation of the fish that causes the components of the blood to change. A component of blood in fish is the concentration of hemoglobin. Physically, hemoglobin has an important relationship with oxygen. When erythrocytes pass through human capillaries, hemoglobin binds oxygen to form oxyhemoglobin. Conversely, when it passes through the systemic capillary, hemoglobin will release oxygen to the tissue, and it will be converted back into hemoglobin [4].

The impact of water pollution caused by detergents is not only detrimental to marine biota and the environment but can also endanger human health or even cause death, reduce or damage the aesthetic value of the coastal environment, and it can be detrimental to socioeconomic conditions [5].

Hemoglobin works to bind the oxygen used in the catabolism process to produce energy. The low oxygen supply in the water will reduce the oxygen transfer in the body of the fish. If the need for oxygen in the body of the fish decreases, the concentration of hemoglobin in the fish will be affected. Therefore, the level of toxicity and concentration of tilapia hemoglobin in the detergent residues of the laundry industry can be used as an indicator to detect and determine the health level of the fish [6]. Based on this background, the researcher must investigate with the title "Biological test of the toxicity of detergents in the laundry industry and the concentration of hemoglobin in tilapia (Oreochromis niloticus)."

2. Methods

This research was carried out in Wundulako Fish Seed Hall, Wundulako Subdistrict, Kolaka Regency, Southeast Sulawesi Province in August-October 2018. The research was conducted for 40 days. This research is an experimental study. The design of this study uses the control design only with subsequent testing because it was not previously tested with the sample before treatment. The experimental design used was RAL with three repetitions and 6 treatments (0%, 1%, 2%, 3%, 4% and 5%).

2.1 Population and samples

The test animals used in this study were tilapia (Oreochromis niloticus). Homogeneous samples of up to 90 tilapia were taken with an average age of 2-2.5 months, size 10-16 cm, and a weight of 15-20 g / head. The sampling technique used is intentional sampling, with characteristics of bright fish color (not opaque), active movements and without physical changes in the body, active movement of the fins and the body. Sampling was carried out at the Wundulako Fish Seed Center in Kolaka Regency.

2.2 Tools and Materials

The tools used in this study include: aquarium, aeration, hose, fishing net, analytical scales, oximeter, pH meter, lead, jrigen, Hb Sahli, Syiling of 1 ml, Itherma vacuum tube of 4 ml, pipette, fabric, cotton, ruler, latex gloves, masks, scissors, label paper, stationery and cameras. While the materials used in this study were tilapia, fish feed, wash water, tap water, and greasy water.

2.3 Preparation of Test Fish

The research preparation initially prepared 90 healthy and homogeneous tilapia, prepared fish pellets, adapted test for the maintenance tank for 1 week in order to adjust to water conditions in the laboratory and feed. Feeding fish during the adaptation process 2 times a day, measuring environmental conditions, namely DO, temperature (°C) and pH as the initial data in the aquarium and cleaning the remaining feed and feces every morning and evening.
2.4 Preparing the aquarium
The preparation of the aquarium with a size (40 cm x 30 cm x 30 cm) of 18 aquariums is clean, placing the aquarium at random, organizing the aeration in each aquarium. Each aquarium is filled with a mixture of seawater and freshwater that has been deposited for 24 hours. The sea water is obtained from the Kolaka Regency culinary tourism beach. Fill each aquarium with a mixture of sea water and fresh water with a certain volume. Prepare the washing of industrial wastewater from one of the "X" laundry places in Kolaka Regency. Wastewater from the laundry industry is diluted with several concentration levels, namely 0%, 1%, 2%, 3%, 4% and 5% using the dilution formula V1 x M1 = V2 x M2.

2.5 Implementation of the investigation
Insert the test fish into a maintenance aquarium with a density of 5 fish/aquarium. Clean remaining foods and feces every morning and evening, measure OD, temperature (°C) and pH every ten days, 20 days, and 30 days. Count the number of dead fish every ten days, 20 days, and 30 days. The blood samples are taken every ten days, 20 days and 30 days. Before taking a blood sample, the fish faints first with dry ice. After the fish fainted, blood was drawn with a 1 ml syringe injected into the heart of the fish. Blood obtained from fish samples for hemoglobin concentration tests is placed in the EDTA tube. All blood samples are labeled. Next, observe the concentration of hemoglobin using the Sahli method.

2.6 Concentration of hemoglobin
The observation of the hemoglobin concentration after treatment with exposure to detergent residues from the laundry industry can be measured using the Sahli method. The working system of this method is to observe the similarity of the color of the treated sample with the standard color in the sahli device. To observe the hemoglobin concentration, tilapia blood samples were first aspirated from each treatment group with a sahli pipette on a 20 mm3 scale. The tip of the pipette that is used is cleaned of the blood remnants with tissue paper. The blood is transferred to the sahli tube which has been filled with 0.1 N HCl solution to the number 10. The liquid is then shaken until it is homogeneous. The sahli tube is placed between 2 tubes that contain standard colors. Gradually add distilled water to the sahli tube with a pipette, until the color is the same as the standard color, and the result is expressed in g% [7].

2.7 Data analysis
The data obtained in this study were analyzed statistically. To determine the effect of detergent residues from the laundry industry on the state of tilapia hemoglobin concentration, an analysis of variance (ANOVA) was performed. If the analysis shows a significant effect or Fcount> Ftable, continue with the Smaller Significant Difference Test (BNT) with a confidence level of 95%.

3. Results and discussion

3.1 Mortality from tilapia
Test animals in the form of tilapia were treated in the form of detergent residues from the washing industry that differed in concentration so that the level of toxicity of detergent residues in the washing industry and the response of the detergent were known. Body at the concentration of hemoglobin. In this study, support data were also measured, namely, the environmental conditions (means) of the tilapia in the form of OD, temperature (°C), and pH.
Table 1. Mortality by tilapia during the research

| Concentration/Pool | Σ Early Fish | Σ Mortality (Day) | Average (%) |
|--------------------|--------------|-------------------|-------------|
|                    |              | 0-10  | 11-20 | 21-30 |   |
| 0%                 | 5            | 0     | 0     | 0     | 0  |
| 1%                 | 5            | 0     | 0     | 0     | 0  |
| 2%                 | 5            | 0     | 1     | 0     | 6.66 |
| 3%                 | 5            | 1     | 1     | 1     | 20  |
| 4%                 | 5            | 1     | 1     | 2     | 26.66 |
| 5%                 | 5            | 2     | 3     | 3     | 53.33 |

According to table 2, the lowest mortality of fish in concentrations of 0 and 1%. While the highest mortality was at a concentration of 5%, that is, 8 tails. The influence of detergent residues from the laundry industry can affect fish mortality rates according to statistical 1-way ANOVA tests. The higher the waste concentration, the higher the mortality rate of the tilapia during the study. This is caused by toxic substances in detergents that can not be tolerated by fish that damage the body's respiratory system in fish. Fish whose body physiology can tolerate these toxic substances can survive until the end of such research, otherwise. The concentrations of 0% and 1% can be tolerated by all populations in each aquarium.

The observations of tilapia mortality are carried out every day, the average percentage of tilapia deaths is high enough to reach 50% more in the observations at the end of the study. This is due to several environmental factors of detergent waste from the laundry industry. For example, at concentrations of 4% and 5% in each aquarium, the behavior of the tilapia tends to be aggressive and attack each other. This is a reasonable behavior because tilapia has cannibalism. The act of killing each other and consuming all or most of individuals that have the same species. Cannibalism events can occur due to several things, the unequal size or age of fish, species and environmental conditions, which can cause the death of fish [8].

In the treatment of the laundry industry, the concentration of detergent residues in 2% and 3% of the death of tilapia is influenced by toxic substances of detergents that can not be tolerated by fish, thus damaging The respiratory system in the body of the fish and indirectly causes biochemical changes in the body of the fish and can deactivate various types of enzymes that is necessary for the cells in the body of the tilapia, due to the changes in metabolism and metabolic imbalances that they are too big because tilapia cannot survive and die.

3.2 Concentration of hemoglobin
Hemoglobin (Hb) is a red pigment that carries oxygen in red blood cells, which is an iron-rich protein [9]. The increase and decrease in the concentration of hemoglobin in fish depend on the supply of nutrients consumed [10]. Most industrial detergent residues contain toxins because they cause the bloodstream to circulate nutrients and oxygen to be disrupted, thereby reducing the ability of hemoglobin to bind oxygen. Hemoglobin works to bind oxygen and is then used in the catabolism process to produce energy. The ability of blood to carry oxygen depends on the level of hemoglobin in the blood [11].
Table 2. The average concentration of tilapia hemoglobin exposed to detergents in the laundry industry on days 0, 10, 20, and 30.

| Concentration/Pool | Observation Time (Days) |       |       |       |
|--------------------|-------------------------|-------|-------|-------|
|                    |                         | 0     | 10    | 20    | 30    |
| 0%                 | 10.87±1.1               | 12.77±0.70 | 12.94±0.63 | 12.25±1.51 |
| 1%                 | 11.64±1.61              | 11.36±0.13 | 10.31±1.03 | 10.46±2.50 |
| 2%                 | 11.29±0.47              | 10.61±1.73 | 9.23±0.56  | 8.64±0.87  |
| 3%                 | 11.73±1.77              | 9.88±1.28  | 9.76±1.12  | 9.33±0.99  |
| 4%                 | 11.66±1.51              | 8.37±1.19  | 7.65±0.50  | 6.84±1.35  |
| 5%                 | 11.35±1.28              | 9.49±1.33  | 8.74±0.55  | 6.42±1.41  |

Based on table 2 shows that the observation of the concentration of hemoglobin of fish on day 0 in all treatment concentrations (0%, 1%, 2%, 3%, 4%, and 5%) almost had the same concentration that is still in the concentration range of normal fish hemoglobin. The concentration of hemoglobin in normal fish ranges between 10 and 14 g%. In the treatment of 0% concentration (control), the hemoglobin concentration of the fish is still in the normal range, even on days 10 and 20 tends to increase [12]. It is supposedly due to adequate aeration so that the tilapia has a large oxygen supply that increases the concentration of hemoglobin.

In the treatment of the concentration of 1% detergent in the washing industry of all observations of the concentration of hemoglobin in fish, a decrease in the concentration of hemoglobin, but still in the range of the concentration of hemoglobin, has not been observed normally. However, in the 2% -5% treatment, detergent concentrations in the laundry industry at all observations (day 10, day 20 and day 30) showed that the concentration of tilapia hemoglobin decreased significantly below the range of hemoglobin concentration. Normal fish The higher the concentration of detergent residues in the laundry industry, the faster the decrease in the concentration of hemoglobin in tilapia, for example, in the treatment of the 5% concentration of detergent residues from the washing industry, where the decrease in the concentration of hemoglobin in fish was very significant at 30 days of observation below 7 g.

![Figure 1. Average Percentage of Tilapia Mortality](image-url)
The results of the statistical analysis showed that the hemoglobin levels in the treatment controls (0%) were significantly different ($P <0.05$) with the treatment of all the treatments ($1\%$, $2\%$, $3\%$, $4\%$, and $5\%$). The $1\%$ concentration treatment was not significantly different from the $2\%$ concentration treatment but was significantly different from the other treatments. The $2\%$ concentration treatment was not significantly different from the $3\%$ concentration treatment but was significantly different from the $4\%$ and $5\%$ concentrations. The concentration treatment of $3\%$ was significantly different from the $4\%$ and $5\%$ concentrations. The concentration treatment of $4\%$ was significantly different from the $5\%$ concentration treatment. This fact shows that the effect of detergent residues from the washing industry in high concentrations can significantly reduce the concentration of hemoglobin in the blood of tilapia.

The results of this study indicate that detergent residues from the washing industry greatly influence the hemoglobin concentration of tilapia. Physiologically, hemoglobin determines the level of resilience of the body of fish due to its close relationship with the presence of the power of oxygen binding by blood. The decrease in hemoglobin concentration of tilapia after exposure to detergent residues from the laundry industry showed a decrease in the appetite of the fish, which caused the amount of dead fish to reach more than 50\%.

The observation of the hemoglobin concentration can be used as initial information for tilapia producers that detergent residues in the laundry industry can affect the concentration of hemoglobin in the blood so that it can reduce the survival of the fish that will affect the decrease in crop yields. Therefore, the river estuary from the residential area to the location of the fish far should be a concern of farmers. The results of this study can also be used as information material for other researchers relevant to this study.

This research requires additional research to observe the maximum effect of detergent residues from the washing industry, which is required to observe the hematological parameters of tilapia, including leukocytes, erythrocytes, hematocrits, and differential leukocytes.

4. Conclusion
It can be concluded that industrial laundry residues can affect the supply of oxygen in fish, which is characterized by high mortality rates and a reduced concentration of hemoglobin in tilapia from normal concentrations of hemoglobin.

5. Reference
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