The Sublethal Effects of Tannery Wastewater Exposure on Oxygen Consumption Levels and Operculum Movement of Indonesia Mahseer Fish

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Abstract. This study aimed to analyze the effect of tannery wastewater exposure of Padang Panjang, Indonesia, on the level of oxygen consumption (OC) and the operculum movement of the Mahseer fish (Tor tambra CV). Mahseer is an endemic fish in the Batang Anai River in West Sumatra Province. The study is started by determining the acute toxicity value of LC₅₀-96h using a static method within 96 hours of observation. Based on the results of the Probit Method, the LC₅₀-96h amount of mahseer was 15.41%. Furthermore, variations in the wastewater concentration were 0%, 10%, 20%, and 30% of the LC₅₀-96h value. Observations were carried out for 30 days in triplicate. Based on OC value, the concentration of 10%, 20% and 30% has decreased by 0.13 mg O₂/g.hours; 0.18 mg O₂/g.hours, and 0.27 mg O₂/g.hours consecutively otherwise in control, treatment increased by 0.05 mg O₂/g. While the operculum movement, the concentration of 10%, 20%, and 30% have increased by 90 times/minute, 108 times/minute, and 116 times/minute, respectively. In control, treatment has risen by 44 times/minute. OC's correlation value and mahseer's operculum movement ranged from 0.94 to 0.97, which means the correlation was reliable. The significance value obtained p <0.05, which means that there were significant differences in OC values and movements of the mahseer's operculum.

1. Introduction
The tannery industry is an industry that treats raw leather into ready-made leather by using chemicals in the tanning process. In the processing, natural leather was carried out by biological, chemical, and physical processes. The by-product of the tannery process were wastewater and solid waste. Usually, wastewater can still be recycled or directly discard into the river or water body[1].

One of the leather tanning industries was XYZ Inc at Padang Panjang City, West Sumatra Province, Indonesia. XYZ Inc used a tanning agent in Chromium (Cr), with the raw materials used are animal skins (cows, buffaloes, goats), mainly from slaughterhouses. The XYZ Inc produced leather processing ± 500 kg in one production and produced ± 12-16 m³ of wastewater that could pollute the environment. The outlet of Wastewater Treatment Plant heading to Batang Anai River, Padang Panjang City, West Sumatra.
Mahseer fish (Tor Tambra CV) is a type of endemic fish in the BatangAnai River, Padang Panjang City, West Sumatra. Mahseer has the local name "garing" (garing means crispy). It raises the potential of heavy metal contamination because of the outlet of XYZ Inc's wastewater plant. Besides, Mahseer fish is a type of fish that becomes a favorite food for West Sumatra[2,3]. Consuming fish contaminated with heavy metals can cause diseases to humans, such as kidney failure, liver damage, and dermatitis. Fish, as an indicator, has a significant role in determining water pollution because it has a high sensitivity to changes in the water environment [4].

Sublethal influences in fish body organs cause interference with these organisms' physiology and histology [5]. One of the physiology of fish includes metabolic processes[6]. The metabolic process of living things could be estimated by measuring the amount of oxygen consumed by living things per unit of time (also known as the level of oxygen consumption). If the fish's metabolic process were disrupted, it would reduce the fish's ability to consume oxygen[7,8].

Fish operculum was the process of fish swallowing water with its mouth and pressing it through the gills and then out through the hole under the operculum. The movement of the operculum was related to the level of oxygen consumption. If the fish is exposed to wastewater, it will damage the gills, so the fish's ability to consume oxygen would be reduced, and the movement of the operculate in the fish will increase[8–10].

This study aimed to analyze the acute toxicity and sublethal toxicity of mahseer fish in tannery wastewater exposure. Fish used are the wild fish and cultured fish in the BatangAnai River, Padang Panjang City, Indonesia.

2. Materials and methods
This study used 12 glass tanks with 35 x 30 x 30 cm for control and three variations of concentration. The study used mahseer fish (Tor tambra CV) As a test animal with an average size of 5-7 cm with a weight of 2-4 grams each. The acute toxicity test and the sublethal test used 60 fish for each test. One tank contains 16-18 liters of water for five fish (one liter of water for a fish weight of 0.8 grams) [11–14].

At the time of acclimation, the test animals were given food in the form of fish pellets and given oxygen through an aerator. Mahseer fish were fed pellets every 2-3 times a day, i.e., morning, afternoon, and evening. The amount of feed given is 2-3% of the weight of fish per day[15]. Replacement of test water was also carried out every four days [16]. Acclimatization was carried out for seven days [17]. Environmental factors measured during acclimatization were dissolved oxygen levels, temperatures, and pH levels, measured using Lutron type DO-5510 and pH meter PH-009 (1) A. During the acclimation period, the aquarium's environmental conditions must be adjusted to the ecological needs suitable for maintaining mahseer fish.

Wastewater samples in this study were taken and analyzed to determine the characteristics of liquid waste. Sampling was conducted on weekdays at the outlet point of wastewater treatment. The components of wastewater tested are Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), Chrome (Cr), Oil, and Grease, Nitrogen, Ammonia, Sulfide, and pH. The results of the wastewater characteristics showed that XYZ Inc's wastewater had exceeded the quality standard according to the Indonesian Ministry of Environment Regulation No. 5 of 2014 concerning Wastewater Quality Standards.

2.1. The acute toxicity test
The preliminary test aimed to determine the limits of the range of crisis concentrations of the test material used for the determination of LC50-96h. This experiment's treatment was carried out with five dilution variations of the tannery industrial liquid waste and control. United States Environmental Protection Agency (USEPA) recommends the concentration of pollutants (wastewater), among others, 100%, 50%, 25%, 12.5%, 6.25%, and one as a control. Experiment with three repetitions or triplicate. During the experiment, the test animals were not fed. Observations were made at 0, 24, 48, 72, 96 hours.
The primary test was carried out on the tannery industrial wastewater with variations in concentration in the range that refers to the preliminary test. The primary analysis was carried out to obtain a more precise LC50-96h value with a concentration range close to 50% of test animals (LC50). The basic test concentration range was obtained from the preliminary test LC50 output value on Probit, which was then varied to five concentrations based on the upper threshold concentration. The lower threshold concentration was obtained [14].

2.2. The sublethal toxicity test
The implementation of sublethal toxicity tests in the study for 30 days [18, 19]. Research by repeating three times to each concentration. During the investigation, Mahseer fish were fed pellets every 2-3 times a day, i.e., in the morning, afternoon, and evening [20, 21]. The amount of feed given is 2-3% of the weight of fish per day [15]. The parameters observed were the level of oxygen consumption and the operculum movement in mahseer fish. The operculum's move followed the oxygen consumption level in this study using a counter and a camera. During the trial period, the parameter measurement data used in the regression and correlation analysis are the data on day 0, 10th, 20th, and 30th.

2.3. The level of oxygen consumption
The level of oxygen consumption was calculated using the formula [22]:

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OC = \frac{(V) \times (DOo - DOT)}{W \times T}
\]

OC = level of oxygen consumption (mgO₂/g.hour)
V = volume of water in the container (l)
DOo = concentration of dissolved oxygen at the beginning of the observation (mgO₂/l)
DOT = dissolved oxygen concentration at time t (mgO₂/l)
W = weight of test fish (g)
T = observation period (hours)

2.4. The operculum movement
Calculation of the operculum movement was using a counter and a video camera. During one hour of video capture three times. The video results were applied to the Movie Maker tools; the footage was slowed down so that the operculum movements could be calculated using a counter.

3. Results and discussion

3.1. The acute toxicity test
Acclimatization was carried out for seven days. Environmental parameters measured during acclimatization were dissolved oxygen levels and temperatures measured using a DO meter and pH levels measured with a pH meter. During the acclimation period, DO measurements ranged from 7.2 to 8.3 mg/l, pH ranged from 7.5 to 8.15, and temperatures ranged from 25.5 to 26.75 °C. According to USEPA [17], the allowable DO range was > 4 mg O₂/l; the pH was around 6-9, and the temperature was 25-30 °C. So, it could be concluded that all parameters obtained were still within the allowed range at the time of all parameters. This condition was still eligible for the maintenance of mahseer fish.

The preliminary test was carried out for 96 hours of observation time by measuring pH, DO, and temperature as well as the data of dead test animals for each observation. At the preliminary tests of pH values ranged from 7.28 - 8.78, DO levels covered between 6.3 - 7.7 mg/l, and the temperature ranged between 24.6 - 26.6°C. pH values obtained still meet the range of fish rearing, where fish experience optimal growth in the pH range of 6.0 - 9.0 [17]. The USEPA[17] explained that the dissolved oxygen content was suitable for freshwater fish life in waters is> 4 mg/l. This temperature
was still within the temperature tolerance for mahseer's life, where fish could live with temperatures ranging from 25-30˚C [17].

![Figure 1](image1.png)

**Figure 1.** The preliminary test.

Based on figure 1, it could be concluded that in the preliminary test for 96 hours, the mahseer fish did not experience death at a concentration of 0%, but the fish died at a level of 6.25%, 12.5%, 25%, 50%, and 100%. Based on the study results, the tannery wastewater concentration was directly proportional to the percentage of test animal mortality. Based on data on test animals' death, there were two or more concentrations that experience death, so the LC$_{50}$-96h value can be determined by the probit method[17]. The LC$_{50}$-96h value was 13.403% (the highest concentration was 16.210%, the lowest was 11.060%). Then, this lowest and highest concentration limit was used for attention in the primary test.

After a preliminary test, the primary test was carried out using a range of waste concentrations that caused 50% fish mortality based on a preliminary test conducted on the sample. The concentration range used were between 11,060% -16,210%. In order to facilitate the dilution of waste, concentrations of 0% (control), 9%, 11.25% were chosen; 13.5%; 15.75%; 16% and 18% as the concentration of wastewater in the primary test. The basic test was carried out for 96 hours. Parameters that affected the life of fish were pH, DO, and temperature.

![Figure 2](image2.png)

**Figure 2.** The primary test.
The water temperature ranged between 25 - 26.3°C, DO levels varied from 6.3 mg/L to 7.8 mg/L, and the pH value ranged between 7.33 - 8.77. This condition was still within the tolerance of temperature for the life of fish [17]. The increased pH value indicates the possibility of organic decomposition. This pH value still meets the range of fish maintenance, where the fish experienced optimal growth in the pH range of 6.0 - 9.0 [17].

Figure 2 shows that the higher the concentration of tannin wastewater added to the tank, the more mahseer fish deaths, and vice versa. This observation resulted in the LC50-96 h value was 15.410%.

3.2. The sublethal test
During the acclimation period, DO measurements ranged from 8.10-8.60 mg/l, pH ranged from 7.20-8.10, and temperatures ranged from 26.30-27.20°C. According to USEPA [17], the allowable DO range was > 4 mg O2/l, the pH was around 6-9, and the temperature was 25-30°C. It can be said that at the time of acclimation, DO values, pH, and temperature obtained is still in the range that is allowed so that it still meets the requirements for the maintenance of fish.

A sublethal toxicity test was carried out for 30 days. The concentration used was below the LC50-96h value of 15.41%, which varied to 1.5% (0.1 from 15.41%); 3% (0.2 from 15.41%); and 4.6% (0.3 of 15.41%). During the observations, fish were given the same treatment as the acclimatization treatment. This treatment includes cleaning up impurities, feeding, replacing water, measuring pH, DO, temperature, and aeration.

During the sublethal test, DO measurements ranged from 8.70 to 9.80 mg O2/l; pH measurements ranged from 7.03 to 8.73; the temperature ranged from 25.93-26.87°C. According to USEPA [17], during the observation of DO values, pH, and temperature obtained was still within the allowed range.

3.3. Oxygen consumption (OC) level
The measurement of OC levels was calculated based on the ratio of dissolved oxygen (at the beginning and end of the observation) using iodometric titration. The value of the level of OC in mahseer fish was obtained based on equation 1. The graph of the relationship between the level of oxygen consumption of mahseer fish and the observation time can be seen in figure 3.

![Figure 3](image)

Figure 3. The relationship between the OC level and the observation time.

3.4. Movement of operculum
Based on figure 4, it can be seen that there was an increase in operculum movement in mahseer fish caused by exposure to wastewater, from observations from day 0 to day 30. In control, the movement...
of the operculum increased from 125 to 169 times/min. At a 10% concentration, the fish operculum movement also experienced a significant increase ranging from 130 times/min to 220 times/min. Whereas at 20% also increased from 137 to 245 times/min, 30% increased from 155 to 271 times/min. Between these variables, at the concentration of 0%, 10%, 20%, and 30% has a correlation value of 0.99, 0.98, 0.97 and 0.97 respectively. Based on those correlation values, it means that the duration of exposure and concentration of the wastewater to the operculum movement has a solid relationship.

![Figure 4](image)

**Figure 4.** The relationship between the operculum movement and the observation time

3.5. *The relationship between OC level and operculum movement*

The OC level and the movement of the operculum were related to each other. Both are related to breathing in fish. The relationship of OC level with the operculum movement can be seen in figure 5.

![Figure 5](image)

**Figure 5.** The relationship between OC level and operculum movement

Based on the exposure with a concentration of 0%, 10%, 20% and 30% obtained a correlation value of 0.97, 0.97, 0.94 and 0.97, respectively. The lower level of oxygen consumption causes this; the operculum movement has increased from the beginning of observation (day 0) to the end of observation (30th day).
Based on figure 5, a concentration of 0% the OC level of mahseer fish on day 0 to day 30 increased from 0.85 mg O_2/g.hour to 0.90 mg O_2/g.hour. At a 10% concentration, the OC level of fish decreased by 0.83 mg O_2/g.hour to 0.70 mg O_2/g.hour. Likewise, with a 20% concentration, the OC level also decreased from 0.88 mg O_2/g.hour to 0.65 mg O_2/g.hour. The same happened at a 30% concentration, decreased from 0.83 mg O_2/g.hour to 0.54 mg O_2/g.hour. At the concentration of 0%; 10%, 20%, and 30% wastewater has a correlation value of 0.97; 0.99; 0.99 and 0.98 respectively. So the concentration of XYZ Inc's wastewater exposure affected the OC of mahseer fish with a solid relationship. The decrease in oxygen consumption level occurred after the fish were exposed to XYZ Inc's wastewater exposure. This situation was because the metabolic processes in fish were disrupted, which causes the reduced ability of fish to consume oxygen caused by gills in fish that were damaged due to the influence of wastewater [23–25].

Fish take dissolved oxygen in water by filtering water that enters through the mouth and carries oxygen dissolved in water using gills. Breathing will begin to be disrupted if the water used as a place for living animals contains toxic substances. That would continuously enter the gills because of damage to the gills. The dissolved oxygen absorbed through the gills was reduced and caused the fish's operculum to work so that the respiration process keeps it running [26–28]

4. Conclusions
The LC_{50}-96h value of mahseer fish was 15.410%. The OC level of fish from day 0 to day 30, between concentration 10 - 30% decreased ranged from 0.83 mg O_2/g.hour to 0.56 mg O_2/g.hour. Movement of the operculum for 30 days of observation ranged between 125 to 271 times/minute. Correlation values of OC levels and operculum movement on the exposure time and wastewater concentration based on 0%, 10%, 20%, and 30% obtained correlation values of 0.97, 0.97. 0.94 and 0.97, respectively. The longer the time and the higher the concentration of tannery wastewater exposure, the OC level will decrease, and the operculum movement increases.

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