The effect of volcanic ash from the eruption of Mount Sinabung on the survival and tissue structure of Mahseer fish (Tor tambroides)

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Abstract. Volcanic ash of Mount Sinabung enters the Lau Borus River. One of the biota in the river that is affected by volcanic ash is the Mahseer fish. This study aims to determine the LC50 from the exposure of Mount Sinabung volcanic ash to Mahseer fish, water quality, and tissue histology of Mahseer fish. The concentrations of volcanic ash for toxicity tests were 0 mg/L, 66,000 mg/L, 87,000 mg/L, 115,000 mg/L, and 152,000 mg/L. The results of the probit analysis showed that the LC50 values at the exposure time at 24, 48, 72, and 96 hours were 135,249 mg/L, 106,741 mg/L, 72,210 mg/L, and 68,798 mg/L, respectively. The higher concentration of volcanic ash given will accelerate the time of death of Mahseer fish, followed by damage to the skin, gill and intestinal tissue. There is muscle necrosis and the epidermis is thinning in the skin. In the gills there is hyperplasia, hypertrophy, fusion, and necrosis of cells. Hyperplasia and hypertrophy of goblet cells, and necrosis of the intestinal villi in the intestine.

1. Introduction
Mount Sinabung is a volcano in the highlands of Karo Regency, North Sumatra, which is located at the coordinates 3º10'LU 98º23'E with an altitude of 2,460 meters above sea level. This mountain first erupted in 1600, then reactivated on August 27th, 2010. In November 2013, May-June 2015, and May 2016 the mountain erupted again. Then the last eruption occurred on 19 February-April 2018 and continues to erupt until now [1]. The eruption of Mount Sinabung emitted volcanic ash that covered residential areas around the slope of Mount Sinabung. Volcanic ash at the foot of the mountain will enter the waters including the rivers around it. One of the rivers in Karo Regency, North Sumatra and which receives the flow of volcanic ash is the Lau Borus River. Volcanic ash will increase water turbidity and affect the aquatic ecosystem, especially the life of Mahseer fish (Tor tambroides) that live in the river. Mahseer fish (T. tambroides) is a type of endemic freshwater fish from North Sumatra which has a large enough potential for germplasm [2]. This Mahseer fish is often called the Batak Fish by the local community.

The effect of exposure to volcanic ash needs to be studied further to find out at what concentration fish died by determining the LC50 value and the extent of tissue damage to Mahseer fish. The purpose of this study was to determine the toxicity of volcanic ash resulting from the eruption of Mount Sinabung for Mahseer fish, clinical symptoms due to exposure to volcanic ash, water quality and to...
see the damage that occurs in the skin tissue, gills and intestines of Mahseer fish.

2. Materials and methods
The research was carried out from August to September 2019 at the Hatchery Laboratory of the Faculty of Agriculture, Malikussaleh University. Meanwhile, histological preparations were made at the Anatomical Pathology Laboratory, Faculty of Medicine, University of North Sumatra. The materials used were Mahseer fish measuring 10-12 cm, Mount Sinabung volcanic ash, 10% BNF as a preservative for fish samples and materials used for making histological preparations consisting of alcohol 70%, 80%, 90%, 96%, and alcohol. absolute, xylol, paraffin, glycercin albumin, hematoxylin-eosin dye, and canada balsam. The tool used is an aquarium with a size of 30 x 20 x 30 cm 3 and a water quality measuring instrument.

The research method used is an experimental method with 2 stages, namely the preliminary test and the toxicity test to see how much the LC_{50} value as a result of exposure of volcanic ash to Mahseer fish. The preliminary test aims to find the upper and lower threshold values. This test was carried out using 5 treatments and 3 replications. Each of the 10 fish were kept in an aquarium with concentrations were used 0 mg / L, 50,000 mg / L, 100,000 mg / L 150,000 mg / L, and 200,000 mg / L for 48 hours. Then count the number of fish that died in each aquarium. The upper limit value was obtained at a concentration of 200,000 mg / l and the lower limit value was at a concentration of 50,000 mg / l. Then this value were used to determine of concentration and will be used in the toxicity test using the calculation formula according to [3]. Furthermore, the concentrations to be used in the toxicity test were 66,000 mg / l, 87,000 mg / l, 115,000 mg / l and 152,000 mg / l, respectively.

The fish were used of 10 fish to toxicity test per aquarium. Then the volcanic ash was put in according to the predetermined concentration of 3 replications. The toxicity test was carried out for 96 hours. The parameters observed were the number of fish that died, the clinical symptoms experienced by the fish after exposure to volcanic ash and the water quality of media. The fish that died in the LC_{50}-96 hours toxicity test were taken for their skin, gill, and intestinal tissues. Then the histological preparations were made using the paraffin embedded method which have been modified from the procedure specified by [4].

2.1. Data analysis
To be able to determine the value of the LC_{50} concentration, a probit regression analysis was carried out. In addition, observations of histological preparations were analyzed descriptively to describe changes in the tissue structure of Mahseer fish at each concentration of toxicity tests. Analysis data is displayed in tables and figures.

3. Results and discussion

3.1. Toxicity test
Observation of the mortality of Mahseer fish in the 96-hours toxicity test showed that there was a difference in the number of mortality of Mahseer fish at each concentration The mortality value of the toxicity test for 96 hours showed an increase in line with the increasing exposure to the concentration used. This is in accordance with the statement [5], which states that the higher the concentration, the higher the number of fish deaths, while the lower the concentration, the less the number of fish deaths.

The observation results of the mortality of Mahseer fish in the toxicity test were analyzed using the regression method probit analysis to obtain the LC_{50} value at the exposure time of 24, 48, 72, and 96 hours. The LC_{50} values at exposure times of 24, 48, 72 and 96 hours were 134,249 mg / l, 106,741 mg / l, 72,210 mg / l and 68,798 mg / l, respectively (figure 1). The results of the toxicity test showed that the LC_{50} value would decrease as the length of time the volcanic ash was exposed to Mahseer fish. [6] stated that the longer the exposure time, the lower the LC_{50} value. Furthermore, the acute toxicity potential of the test compound at concentrations of 5,000–15,000 mg / L was classified as nearly non-toxic [7]. Observation of clinical symptoms experienced by
Mahseer fish after exposure to volcanic ash with different concentrations had an effect on behavioral and physical changes in Mahseer fish bodies (Table 1). The fish that were exposed to volcanic ash showed clinical symptoms such as irregular swimming, weak movement, loose scales and bleeding on the skin. In contrast, control fish looked healthy and swam agile and in groups. Furthermore, the color of the fish scales is bright and there is no loose or bleeding on the skin.

![Figure 1](image.png)

**Figure 1.** The LC\textsubscript{50} values 24, 48, 72 and 96 hours of volcanic ash toxicity test for Mahseer fish.

**Table 1.** Clinical symptoms that occurred during the volcanic ash toxicity test for Mahseer fish.

| Concentrations (mg/L) | Condition and activity of test animals |
|-----------------------|---------------------------------------|
| 0                     | Agile movements, shiny scales, swimming in the middle of the aquarium in groups. |
| 66,000                | Jumping onto the surface of the water, swimming irregularly, and experiencing death. |
| 87,000                | Fish are seen gathering in a corner of the aquarium nearing aeration, gradually dying. |
| 115,000               | Fish swim slowly, loose scales, until they experience death |
| 152,000               | Irregular swimming, slow motion, loose scales causing bleeding and death. |

Volcanic ash also causes a decrease in the quality of water for the maintenance media, such as increasing turbidity. The turbidity value that exceeds the optimal limit in the toxicity test occurs at a concentration of 152,000 mg/l with turbidity values ranging from 25.6 - 27.7 NTU. Mahseer fish are fish that live in clear waters which would be disturbed by high turbidity due to exposure to volcanic ash. [8] stated that the optimal turbidity value for fish farming is less than 20 NTU. The turbidity condition that is not optimal causes the fish to experience stress, it have been seen from the irregular movement of the fish so that there is friction with other fish which causes the scales to peel off. Water quality during the toxicity test can be seen in the following table.
Table 2. Range of water quality during the toxicity test.

| Treatment (mg/l) | Water quality parameters |
|------------------|--------------------------|
|                  | Temperature (ºC) | pH  | DO (ppm) | Turbidity (NTU) |
| A (control)      | 25 - 28           | 6.9 - 7.2     | 4.6 - 5.8     | 0.7 - 1.5       |
| B (66,000)       | 25 - 27           | 6.8 - 7.1     | 3.2 - 6.5     | 8.7 - 9.9       |
| C (87,000)       | 25 - 28           | 6.8 - 7.1     | 4.5 - 6.2     | 11.4 - 13.1     |
| D (115,000)      | 27 - 28           | 6.7 - 7.1     | 3.3 - 6.3     | 16.2 - 17.8     |
| E (152,000)      | 27 - 27           | 6.6 - 7.2     | 3.2 - 5.7     | 25.6 - 27.7     |

The water temperature at the time of the toxicity test ranged from 25 - 28 ºC, pH ranged from 6.6 - 7.2, DO ranged from 3.2 - 6.5 ppm and turbidity ranged from 0.7 - 27.7 NTU. Tor fish live in clear and rocky waters, moderate to swift currents with dissolved oxygen value > 5 ppm, air temperatures water temperatures ranging from 25-26 ºC, and pH ranging from 6-7 [9]. Furthermore, [10] requires a suitable pH for freshwater aquaculture activities belonging to the class III category ranging from 6 - 9 and good dissolved oxygen (DO) for fisheries activities at least 3 mg / l. Turbidity describes the optical properties of water which is determined based on the amount of light absorbed and emitted by the materials present in the water [11]. According to [12] that there is no evidence that suspended solids concentrations less than 25 mg / l have any harmful effects on fisheries, and that good to moderate fisheries can be maintained in waters with 25 - 80 mg / l suspended solid.

3.2. Analysis of the tissues structure of Mahseer fish (Tor tambroides) after exposure to volcanic ash on toxicity tests

![Figure 2. Analysis of the skin tissue structure of the fish, description: 1. epidermis layer; 2. dermis layer; 3. muscle tissue; 4. muscle tissue necrosis.](image)

Based on the results of the research that has been done, it can be seen on the skin in treatment A (control), the skin structure of Mahseer fish still looks normal where the visible parts of the skin are
the epidermis layer, dermis layer, pigment cells, and muscle tissue. In treatment B (66,000 mg / L), C (87,000 mg / L), and D (115,000 mg / L), respectively, there was damage in the form of muscle necrosis. This is due to the lysis of muscle cells due to stopping blood supply to the muscle tissue, making the muscle cells look hollow. However, treatment D (115,000 mg / L) showed the worst muscle necrosis where the cells were seen to melt (lysis) due to the high concentration of volcanic ash and the length of time of exposure.

Volcanic ash also affects the thickness of the epidermal layer of the skin, where each treatment has decreased the level of the epidermal layer of the skin (figure 2). In treatment B (66,000 mg / L), C (87,000 mg / L), and D (115,000 mg / L) the thickness of the epidermal layer continued to decrease due to exposure to volcanic ash for a period of 96 hours. This is because the skin continues to have continuous contact with the fine volcanic ash material for a long time even though the dose given is less than treatment E (152,000 mg / L). In treatment E (152,000 mg / L) the high concentration of volcanic ash given caused less severe damage because the fish died at 48 hours.

In treatment A (control) it can be seen from the gills consist of primary lamellae consisting of many secondary lamellae and cartilage bone. The gill damage in treatment B (66,000 mg / L) was found the same as treatment C (87,000 mg / L), namely mucosal cell hypertrophy, chloride cell hyperplasia, and mucosal cell hyperplasia (table 2). However, in treatment C (87,000 mg / L), more mucosal cell hyperplasia occurred than treatment B (66,000 mg / L). Meanwhile, in treatment D (115,000 mg / L) with a higher concentration, it caused gill damage in the form of cell necrosis, where the death of epithelial cells, mucosal cells, and chloride cells almost all of the secondary lamellae and there was secondary lamella fusion. In treatment E (152,000 mg / L), the gill structure was damaged, such as mucosal cell hypertrophy, chloride cell hyperplasia, secondary lamella fusion, and cell necrosis (epithelial cells and mucosal cells). Lamella fusion occurs more due to mucosal cell hyperplasia where mucosal cells produce mucus in response to protect the gills so that it will interfere with the rate of oxygen uptake by fish. The higher concentration of volcanic ash causes more damage from the gills so that the fish die within 48 hours. According to [13] that the occurrence of lamella
fusion, secondary lamella hyperplasia is a chronic impact caused by parasites, bacterial effects, and irritation of foreign materials. The results of this study are in line with research [14] which states that volcanic ash affects the structure of the gill tissue, namely the occurrence of secondary hyperplasia and fusion of lamellae.

Based on the results of observations on the fish intestine, it can be seen that in treatment A (control) the intestine is still intact and its structure is still normal. The damage in treatment B (66,000 mg / L) was the same as treatment C (87,000 mg / L), namely goblet cell hyperplasia. However, in treatment C (87,000 mg / L) there was hyperplasia of goblet cells in almost all intestinal villi. Goblet cells function to produce mucus which helps the digestive process and the intestinal response to the entry of foreign materials to protect the villi. [15] stated that the result of exposure to volcanic ash affects the goblet cells in the fish intestines to increase. So that the fish are still able to survive even though they are exposed to volcanic ash for 96 hours. However, in treatment D (115,000 mg / L) goblet cell hypertrophy occurs because the fluid outside the cell enters the cell so that the goblet cells enlarge. Intestinal villi necrosis also occurred in treatment D (115,000 mg / L) and E (152,000 mg / L). However, treatment D (115,000 mg / L) was the worst damage to the intestinal villi, but it could be still tolerated by the fish so that the fish could survive until the end of the observation, which was 96 hours. Damage to the villi will disrupt the absorption of nutrients which can lead to fish mortality [15]. In a previous study conducted by [14], volcanic ash affected the intestinal villi resulting in necrosis and altered its structure, as well as affecting the large number of goblet cells in the intestine due to stress in fish. According to [13] that in chronic conditions the entry of foreign material into the intestine can cause hyperplasia of the goblet cells where the number will continue to increase and goblet cell hypertrophy in the intestine can also occur.

![Figure 4](image-url)

**Figure 4.** Analysis of mahseer fish intestinal tissue structure, description: 1. outer muscular layer; 2. deep muscular layer; 3. intestinal villi; 4. hyperplasia of cells in the intestinal villi; 5. cell hypertrophy in the intestinal villi; 6. necrosis of the intestinal villi.

### 4. Conclusions

The LC₅₀ values in the volcanic ash toxicity test for Mahseer fish were 24 hours LC₅₀ 135,249 mg / l, 48 hours LC₅₀ values 106,741 mg / l, 72,210 mg / l LC₅₀ values, and 96 hours LC₅₀ values 68,798 mg / l.
The higher the concentration of volcanic ash given, the faster the fish will experience death and the higher the turbidity value. The worst damage to skin organs, gills and intestines of fish occurred in exposure to volcanic ash with a concentration of 115,000 mg / L (treatment D) with an exposure time of 96 hours. However, at a concentration of 152,000 mg / L (treatment E), Mahseer fish experienced a faster death, namely at the 48th hour so that the damage to the skin organs, gills, and intestines of the fish was not too severe.

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