Histopathology overview of tilapia (*Oreochromis mossambicus*) liver organs contaminated by lead metal (Pb) in Lake Tempe, Wajo Regency

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Abstract. Tilapia fish (*Oreochromis mossambicus*) is one of the most popular freshwater fish in the community because it is easy to breed and its survival is high and group. The purpose of this study was to determine the histopathology of the liver in tilapia fish contaminated with lead heavy metal (Pb) in Lake Tempe, Wajo Regency. This research was conducted in March to June 2020. The number of fish samples used in this study was 21 fish. Measurement of lead heavy metal content (Pb) contained in the liver of tilapia fish (*Oreochromis mossambicus*). Measurement of heavy metal content was carried out with Atomic Absorption Spectrophotometer and obtained lead metal content (Pb) <0.14-2.8 µg / g with an average of 0.82 ± 0.83 µg / g. Preparation of liver organ fixation using 10% neutral buffered formalin (NFB), multilevel alcohol is used for dehydration, embedding using paraffin and haematoxylin-eosin staining. Analysis of the data used is descriptive qualitative. This research shows that lead metal (Pb) causes damage to the liver of tilapia fish. Damage to the liver in the form of inflammatory cell infiltration, fat degeneration, haemorrhage and necrosis. Damage that occurred as a result exposure to heavy metals, dissolved in the aquatic ecosystem that passed the threshold limit.

1. Introduction
Tempe Lake is located in Wajo, Sidrap, and Soppeng Regencies, South Sulawesi [1]. The main source of Lake Tempe water comes from the river. These rivers are a place for various community activities, both as a means of transportation and as a place for bathing, washing and other activities. The direct interaction between the community and the river that enters the lake causes the waters of Lake Tempe to become polluted. Therefore, the source of lake Tempe pollution comes from domestic waste, agriculture, and household industrial waste. The results of water quality measurements and analysis of Lake Tempe water quality status conducted by BLHD (Regional environmental regency) South Sulawesi showed that several water quality parameters were beyond the feasible threshold, one of which was the heavy metal lead (Pb) [2].

The dominant fish species caught in Tempe Lake are tilapia (*Oreochromis mossambicus*) and Tawes (*Barbonymus gonionotus*) [3]. Tempe Lake Wajo Regency is one of the habitats for tilapia fish that is
not free from heavy metal contamination of lead (Pb). The toxicity of lead metal (Pb) to aquatic organisms can cause tissue damage, especially in sensitive organs such as gills and intestines and then to internal tissues such as the liver where the metal accumulates [4].

2. Materials and method
The livers of 21 samples of tilapia fish suspected of being contaminated with lead from Lake Tempe were tested for metal content and made histological preparations. The procedure for measuring heavy metals is based on the method carried out at the Makassar Health Laboratory Center, where 2 g of each sample is inserted into a block tube then the sample is put into 0.5 ml distilled water. To avoid air splashing and to facilitate rapid reaction with acids, the sample to which water was added was digested with 10 ml of HNO₃ concentration carried out at ambient temperature of 100°C for approximately 2 hours. After cooling for about 15 minutes, as much as 0.5 ml of perchlorate (HClO₄) was added to the solution little by little then the solution was heated again in the digestion block for about 1 hour and added 50 ml of distilled water, filtered using Whatman filter paper no. 42. Filter results are ready for analysis. Standard lead minerals were analyzed using AAS, and heavy metals were analyzed.

The preparation of histopathological preparations has several stages, namely fixation, trimming, processing and embedding, cutting, and staining. Observation and image taking were carried out using an advanced optical microscope camera. The histological preparations of tilapia fish livers were then observed.

3. Results and discussion

3.1. Content of lead heavy metal (Pb) in livers of tilapia fish

Table 1. The results of the observation of the average content of heavy metal (Pb) in tilapia fish liver samples using the atomic absorption spectrophotometer method

| No. | Sample Code | Body Weight (g) | Liver Weight (g) | HSI (%) | Body Length (mm) | Lead Levels (µg.g⁻¹) |
|-----|-------------|----------------|-----------------|---------|------------------|---------------------|
|     |             |                |                 |         |                  |                     |
|     | Sta. 1      |                |                 |         |                  |                     |
| 1.  | H. S1. 1 (Pb)| 232.47         | 3.81            | 1.64    | 230              | 0.16                |
| 2.  | H. S1. 2 (Pb)| 192.78         | 2.37            | 1.23    | 203              | 0.92                |
| 3.  | H. S1. 3 (Pb)| 116.23         | 1.60            | 1.38    | 177              | 1.08                |
| 4.  | H. S1. 4 (Pb)| 121.9          | 1.55            | 1.27    | 190              | 0.45                |
| 5.  | H. S1. 5 (Pb)| 226.8          | 2.75            | 1.21    | 222              | 0.21                |
| 6.  | H. S1. 6 (Pb)| 172.93         | 2.70            | 1.56    | 216              | 0.2                 |
| 7.  | H. S1. 7 (Pb)| 119.07         | 1.93            | 1.62    | 170              | 0.39                |
|     | Range       | 116.23-232.47  | 1.55-3.81       | 1.21-1.64 | 170-230         | 0.16-1.08           |
|     | Average ± SE| 168.88±19.18   | 2.39±0.30       | 1.41±0.07 | 201.14± 8.69    | 0.49±0.14           |
|     | Sta. 2      |                |                 |         |                  |                     |
| 1.  | H. S2. 1 (Pb)| 187.11         | 2.03            | 1.08    | 215              | 0.43                |
| 2.  | H. S2. 2 (Pb)| 150.25         | 1.92            | 1.28    | 196              | 0.76                |
| 3.  | H. S2. 3 (Pb)| 147.42         | 3.25            | 2.2     | 191              | 0.41                |
| 4.  | H. S2. 4 (Pb)| 127.57         | 2.09            | 1.64    | 170              | 1.62                |
| 5.  | H. S2. 5 (Pb)| 136.08         | 1.47            | 1.08    | 192              | 0.3                 |
| 6.  | H. S2. 6 (Pb)| 184.27         | 2.17            | 1.18    | 210              | 2.8                 |
| 7.  | H. S2. 7 (Pb)| 110.56         | 1.38            | 1.25    | 169              | 1.65                |
|     | Range       | 110.56-187.11  | 1.38-3.25       | 1.08-2.2 | 169-215         | 0.3-2.8             |
Based on the table above, it can be seen that the liver contains heavy metal Pb, from the Atomic Absorption Spectrophotometer test results with a total of 21 liver samples then averaged as in Table 1 shows the difference in lead levels (Pb) in each liver. In the liver of tilapia fish contaminated with lead (Pb), namely <0.14-2.8 µg / g with a mean of 0.82 ± 0.83. The result of the value obtained is that the Pb concentration in the liver is very high and exceeds the predetermined quality standard. According to SNI 7387: 2009, it is 0.3 mg / kg in the liver (fish and their processed products).

**Table 2.** The regression equation of the relationship between lead content and body weight and between lead content and body length of tilapia fish (*Oreochromis mossambicus*) at each station and all stations combined

| Station | Parameter | n  | Regression equation          | R²   |
|---------|-----------|----|------------------------------|------|
| 1       | Weight    | 7  | Pb = 1.6694e-0.009W          | 0.3459 |
|         | Length    | 7  | Pb = 39.573e-0.023L          | 0.4944 |
| 2       | Weight    | 7  | Pb = 0.0007W2 - 0.2074W + 16.235 | 0.2477 |
|         | Length    | 7  | Pb = 0.0017L2 - 0.642L + 62.428 | 0.2167 |
| 3       | Weight    | 7  | Pb = -0.0003W2 + 0.1116W - 7.2476 | 0.4111 |
|         | Length    | 7  | Pb = -0.0012L2 + 0.4753L - 46.117 | 0.5653 |
| Combined| Weight    | 21 | Pb = -0.0002W2 + 0.0515W - 2.9184 | 0.1303 |
|         | Length    | 21 | Pb = -0.0007L2 + 0.2605L - 24.259   | 0.1344 |

Table 2 shows the relationship between lead content and body weight as well as between lead content and body length following polynomial regression, except at Station 1 for the relationship between lead content and body length. Based on the value of the coefficient of determination (R²) obtained, it is better to predict the lead content in fish livers using the fish body length parameter.
Table 3. The regression equation of the relationship between lead content and the Hepatosomatic Index (HSI) of tilapia (*Oreochromis mossambicus*) of each station and parameter

| Station | Parameter | N | Regression equation          | R²   |
|---------|-----------|---|------------------------------|------|
| 1       | HIS       | 7 | \( y = -12.169x^2 + 33.898x - 22.744 \) | 0.4665 |
| 2       | HIS       | 7 | \( y = -3.9629x^2 + 12.559x - 8.0984 \) | 0.289  |
| 3       | HIS       | 7 | \( y = -5.6629x^2 + 12.479x - 5.7022 \) | 0.1742 |
| Combined| HIS       | 21| \( y = -0.1665x^2 - 0.1075x + 1.2839 \) | 0.0453 |

Table 3 shows the relationship between lead content and fish hepatosomatic index (HSI) following polynomial regression. Based on the value of the coefficient of determination (R²) obtained, the HSI value can be used to estimate the lead content in fish livers. Hepatosomatic Index (HSI) is defined as the ratio of liver weight to body weight. The HSI of fish was calculated using the formula (HSI: liver weight / body weight x 100%). This index value gives an indication of the status of energy reserves in animals [5].

3.2. Histopathology livers of tilapia fish

![Histopathology of tilapia (*Oreochromis mossambicus*) liver at Station 1 with exposure to lead (Pb) a). 0.16 µg / g, b). 0.92 µg / g, c). 1.08 µg / g. DL (blue): Fat degeneration, IR (red): Infiltration of inflammatory cells, N (black): Necrosis (HE, 400x).](image)
Figure 2. Histopathology of tilapia (*Oreochromis mossambicus*) liver at Station 2 with exposure to lead (Pb) a). 1.62 µg / g, b). 1.65 µg / g, c). 2.80 µg / g. H (Green): Hemorrhage, DL (blue): Fat degeneration, IR (red): Infiltration of inflammatory cells, N (black): Necrosis. (HE, 400x).

Figure 3. Histopathology of tilapia (*Oreochromis mossambicus*) liver at Station 3 with exposure to lead (Pb) a). 0.14 µg / g, b). 0.16 µg / g, c). 2.17 µg / g. H (green): Hemorrhage. DL (blue): Fat degeneration, IR (red): Infiltration of inflammatory cells, N (black): Necrosis. (HE, 400x).
Based on the results of histopathological observations of tilapia fish liver at station 3 the content of heavy metal lead (Pb) has varying degrees of histopathological changes depending on the level of contamination contained in the liver of tilapia (*Oreochromis mossambicus*) in Tempe Lake. Station 2 is the station with the highest lead contamination level (Pb) among the three stations in Lake Tempe. This caused the liver of tilapia (*Oreochromis mossambicus*) at station 2 to experience tissue damage compared to station 1 and station 3 in Tempe Lake. Infiltration of inflammatory cells at the acute level is characterized by an increase in the permeability of blood vessels, fluids, and cells that come out of the blood vessels and the presence of neutrophils in inflamed tissues [6]. Fat degeneration occurs due to the accumulation of fat (neutral fat) with damage to the cell nucleus and the shrinking of liver cell tissue [7]. This degeneration of fat is characterized by a histological appearance in the form of vacuoles. Supported by research Alifa and Djawad (2000) states that milkfish (*Chanos chanos forskall*) exposed to lead (Pb) causes the liver to degenerate in fat so that complex liver function is lost [8]. Hemorrhage is a moderate level of liver damage, this occurs when the congestion is very severe, the blood vessels will burst and the blood is in an improper place (bleeding) [9]. Hemorrhage is characterized by bleeding in the cells. Based on the results of the study Fahmi et al., (2019) stated that the liver of the naked pangkilan fish experienced hemorrhage caused by the presence of iron and nickel metals in Lake Matano [10]. If the hemorrhage is very severe, then necrosis (cell death) occurs. Necrosis is the death of liver cells. Cell death occurs with the rupture of the plasma membrane. This is because fat is stored in large quantities, resulting in the death of liver cells [11]. Necrosis begins with an inflammatory reaction of the liver in the form of swelling of the hepatocytes and tissue death. Supported by research results Andini et al., (2019) stated that the hepatopancreas of butini fish experienced necrosis caused by metal contamination in Matano lake [12]. The damage seen in the structure of the liver cells found in tilapia shows the effect of the toxicity, namely the heavy metal lead (Pb) which is constantly exposed to fish.

4. Conclusion
The liver of tilapia (*O. mossambicus*) in tempe lake was found to contain heavy metals with an average concentration of 0.83 ± 0.18. This concentration has set the maximum limit set. The results of histopathological analysis of liver damage, such as inflammatory cell infiltration, fat degeneration, hemorrhage and necroses. This is thought to be due to exposure to heavy metals. Based on the regression equation to estimate the content of heavy metal lead (Pb) in the liver of fish, it is better to use the parameter of fish body length.

References
[1] Nasution S H 2015 Biodiversitas dan distribusi ikan di Danau Tempe Pros. Semin. Nas. Ikan Ke 8 381–92
[2] Hidup K L 2011 Gerakan Penyelamatan Danau (Germadan) Danau Rawapening
[3] Ramadhan A, Triyanti R and Koeshendrajana S 2017 Karakteristik Dan Nilai Ekonomi Sumberdaya Perairan Komplek Danau Tempe, Sulawesi Selatan J. Sos. Ekon. Kelaut. dan Perikan. 3 89–102
[4] Darmono 2001 Lingkungan Hidup dan Pencemaran (Jakarta: UI Press Jakarta)
[5] Tresnati J, Umar M T and SulfiCRYana S 2018 Perubahan Hati Terkait Pertumbuhan Oosit Ikan Sebelah (Psettodes erumei) J. Pengelolaan Perair. 1 46–53
[10] Fahmi U, Andriani I, Salmah S, Hatta T H, Omar S B A and Sari D K 2019 Histopathology of liver and intestine of pangkilan bare fish (Oryzias matanensis) Polluted by nickel and iron in Lake Matano, South Sulawesi IOP Conf. Ser. Earth Environ. Sci. 370 12078

[11] Lu F C 1995 Toksikologi dasar Edisi kedua (Jakarta: Universitas Indonesia Press)

[12] Andini N S, Anshary H, Putra A and Sari D K 2019 Histopathological study of hepatopancreas and kidney of butini fish (Glossogobius matanensis) in Matano Lake, South Sulawesi, Indonesia, caused by metal contamination IOP Conf. Ser. Earth Environ. Sci. 343 12033