Analysis of kidney microanatomy of Tilapia Fish 
(*Oreochromis mossambicus*) contaminated by lead metal (Pb) 
in Lake Tempe, Wajo Regency

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**Abstract.** Tilapia fish (*Oreochromis mossambicus*) is a freshwater fish that is consumed by many people. The purpose of this study was to determine the Analysis of Kidney Microanatomy of tilapia fish (*Oreochromis mossambicus*) contaminated by Lead Metal (Pb) in Lake Tempe, Wajo Regency. The samples used were twenty-one tilapia fish with seven kidney samples in each station. Measurement of heavy metal content was carried out with Atomic Absorption. Kidney preparations were fixed using 10% neutral buffered formalin (NBF), dehydration using multilevel alcohol, embedding using paraffin, cutting with a thickness of 4 µm stained using haematoxillin eosin then observed. Data analysis used is qualitative descriptive. Based on the results of observations obtained by damage or histopathology that occurs in the kidneys which shows the presence of necrosis, inflammatory cell infiltration, edema, fatty degeneration hemorrhage and melanomacrophages and the level of damage to the tissue depends on the concentration of metal contaminated in the fish organs. Damages that occur allegedly due to exposure of heavy metals that are dissolved in the waters of the fish ecosystem that has passed the threshold.

1. Introduction

Tempe Lake potential resources that have been managed and utilized for a long time by the community is fishery potential. This lake is known for its freshwater fishery production and the fish products are marketed outside the district Wajo. This fishery potential has provided benefits to the community and government [1]. In 1948-1969, Tempe Lake fish production reached 55,000 tons per year. At that time Tempe Lake was dubbed the “fish bowl” of Indonesia [2].

Types of fish in the waters of Tempe Lake are gabus (*Channa striata*), betok (*Anabas testudineus*), sepat siam (*Trichopodus pectoralis*), tambakan (*Helostoma temmincki*), sepat rawa (*Trichopodus trichopterus*), lele (*Clarias batrachus*), mas (*Cyprinus carpio*), tawes (*Barbounymus gonionotus*), nilem (*Osteochilus vittatus*), tilapia (*Oreochromis mossambicus*), nila (*Oreochromis niloticus*), bunaka (*Bunaka gyrinooides*), bungo (*Glossogobius sp*), masapi (*Anguilla marmorata*), belut (*Monopterus albus*), dan belanak (*Mugil cephalus*). The types of fish caught were tawes (*B. gonionotus*), and tilapia fish (*O. mossambicus*) [3].

Heavy metals in small amounts are needed by aquatic bodies. However, if in an amount that exceeds the need for normal aquatic bodies, heavy metals can be very dangerous toxins for aquatic
organisms [2][4]. It can also be toxic to humans if you eat contaminated fish [4][5]. According to the Ministry of Environment of the Republic of Indonesia, 2014, one of the heavy metals found in the waters of Tempe Lake is Timbel (Pb). Lead is a non-essential heavy metal that is very dangerous and can cause poisoning (toxicity) in living things [5].

The decline in water quality that occurs due to pollution can lead to structural and functional damage to various fish organs. One of the organs that is sensitive to contamination is the kidney. The kidneys perform important functions related to electrolytes and water balance and maintain a stable internal environment (osmoregulation). The kidney organ can be used as an indicator of water pollution [6].

2. Materials and methods
The kidneys of 21 tilapia fish suspected to have been contaminated with lead from Lake Tempe were tested for metal content and made histological preparations. The procedure for measuring heavy metals in the kidney is based on the method in the Makassar Health Laboratory Center, where 2 g of each sample is put into a digestion block tube then the sample is mixed in 0.5 ml distilled water. To avoid water splashing and to facilitate a quick reaction with acids, the sample which was added with water was digested with 10 ml of HNO₃ concentration which was carried out at a temperature of about 100 °C for approximately 2 hours. After cooling for about 15 minutes, as much as 0.5 ml of perchlorate (HClO₄) is added to the solution little by little then the solution is heated again in the digestion block for about 1 hour and added with 50 ml of distilled water, filtered using Whatman filter paper. no. 42. The filter results are ready for analysis. Standard lead minerals were prepared, measured 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 fish kidneys were then observed. The results of microscopic examination are recorded and then processed using a computer program that is available to provide definitive diagnostic answers.

3. Results and discussion

3.1. Content of lead heavy metal (Pb) in kidney of Tilapia Fish

Table 1. The results of the observation of the heavy metal content of lead (µg.g⁻¹) in the kidneys of Tilapia fish (Oreochromis mossambicus) using the Atomic Absorption Spectrophotometer method.

| No. | Sample code | Body Weight (G) | Body length (mm) | Lead levels (µg.g⁻¹) |
|-----|-------------|-----------------|------------------|---------------------|
|     |             |                 |                  | Station 1           |
| 1   | G. S1. 1 (Pb) | 232.47          | 230              | 0.50                |
| 2   | G. S1. 2 (Pb) | 192.78          | 203              | 0.10                |
| 3   | G. S1. 3 (Pb) | 116.23          | 177              | 0.36                |
| 4   | G. S1. 4 (Pb) | 121.9           | 190              | 0.12                |
| 5   | G. S1. 5 (Pb) | 226.8           | 222              | 0.16                |
| 6   | G. S1. 6 (Pb) | 172.93          | 216              | 0.40                |
| 7   | G. S1. 7 (Pb) | 119.07          | 170              | 0.40                |
|     | Range       | 116.23 – 232.47 | 170 – 230        | 0.10 – 0.50         |
|     | Average± SE | 168.88 ± 19.18  | 201.14 ± 8.69    | 0.29 ± 0.06         |
|     |             |                 |                  | Station 2           |
| 1   | G. S2. 1 (Pb) | 187.11          | 215              | 0.27                |
| 2   | G. S2. 2 (Pb) | 150.25          | 196              | 0.59                |
| No. | Sample code | Lead levels (µg/L) |
|-----|-------------|-------------------|
| 3.  | G. S2. 3 (Pb) | 147.42 ± 1.91 ± 0.25 |
| 4.  | G. S2. 4 (Pb) | 127.57 ± 1.70 ± 1.08 |
| 5.  | G. S2. 5 (Pb) | 136.08 ± 1.92 ± 0.12 |
| 6.  | G. S2. 6 (Pb) | 184.27 ± 2.10 ± 0.29 |
| 7.  | G. S2. 7 (Pb) | 110.56 ± 1.69 ± 0.41 |

**Range** 110.56 – 187.11 169 – 215 0.12 – 1.08

**Average ± SE** 149.04 ± 10.70 191.86 ± 6.70 0.43 ± 0.12

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| No. | Sample code | Lead levels (µg/L) |
|-----|-------------|-------------------|
| 1.  | G. S3. 1 (Pb) | 158.76 ± 2.01 ± 0.50 |
| 2.  | G. S3. 2 (Pb) | 127.57 ± 1.78 ± 0.30 |
| 3.  | G. S3. 3 (Pb) | 104.89 ± 2.00 ± 0.12 |
| 4.  | G. S3. 4 (Pb) | 94.4 ± 1.65 ± 0.46 |
| 5.  | G. S3. 5 (Pb) | 113.4 ± 1.81 ± 0.30 |
| 6.  | G. S3. 6 (Pb) | 94.4 ± 1.60 ± 0.33 |
| 7.  | G. S3. 7 (Pb) | 235.3 ± 2.34 ± 0.26 |

**Range** 94.4 – 235.3 160 – 234 0.12 – 0.50

**Average ± SE** 132.67 ± 19.09 188.43 ± 9.62 0.32 ± 0.05

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**Combined table**

**Range** 94.40 – 235.30 160 – 234 0.10 – 1.08

**Average ± SE** 150.20 ± 9.78 193.81 ± 1.42 0.35 ± 0.01

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Base on the table, it can be seen that the lead content in tilapia fish kidneys in Lake Tempe ranges from 0.10 to 1.08 µg.g⁻¹ with a mean of 0.35 ± (plus and minus) 0.01 µg.g⁻¹. Fish samples with the lowest lead content (0.10 µg.g⁻¹) were obtained at Station 1 and the highest (1.08 µg.g⁻¹) was found in fish samples at Station 2. The lead content obtained showed that the Pb concentration in the kidney organ of tilapia fish has exceeded the quality standard threshold that has been determined according to SNI 7387: 2009, namely 0.1 mg / kg for the kidney (fish and 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 = 0.00003 W² – 0.0106 W + 1.1331 | 0.0747 |
|         | Length    | 7 | Pb = 0.0003 L² – 0.1393 L + 13.996 | 0.6086 |
| 2       | Weight    | 7 | Pb = -0.00003 W² + 0.0036 W + 0.4742 | 0.1326 |
|         | Length    | 7 | Pb = 0.0003 L² – 0.1407 L + 14.747 | 0.3871 |
| 3       | Weight    | 7 | Pb = -0.00002 W² + 0.0063 W – 0.1244 | 0.0903 |
|         | Length    | 7 | Pb = 167.17 L⁻¹209 | 0.1153 |
| Combined| Weight    | 21| Pb = -0.000009 W² + 0.0023 W + 0.2198 | 0.0224 |
|         | Length    | 21| Pb = 0.0001 L² – 0.0522 L + 5.6807 | 0.1490 |

The table shows the relationship between lead content and body weight and between lead content and body length following polynomial regression, except at Station 3 for the relationship between lead content and body length. Based on the value of the coefficient of determination (R²), it is better to estimate the lead content in fish kidneys using the fish body length parameter.

**Table 3.** The lead content (µg/L) of Lake Tempe water.

| No. | Sample code | Lead levels (µg/L) |
|-----|-------------|-------------------|
| 1   | Station 1   | 0.008             |
| 2   | Station 2   | 0.016             |
| 3 | Station 3 | 0.011 |
|---|-----------|-------|
|   | Average   | 0.012 ± 0.004 |

These results indicate that the Pb concentration in Lake Tempe water does not exceed the water quality standard threshold. Based on Permenkes 416 / Menkes / Per / IX / 1990, the Pb threshold value for clean water is 0.05 and Permenkes 492 / Menkes / Per / IV / 2010, the Pb threshold value for drinking water is 0.01 mg/L.

3.2. Microanatomy kidneys of Tilapia Fish

3.2.1. Station 1

![Histopathology of kidney of tilapia (Oreochromis mossambicus) with lead concentration (Pb) at station 1. (A) Pb concentration 0.50 µg.g⁻¹, (B) Pb concentration 0.36 µg.g⁻¹, (C) Concentration Pb 0.10 µg.g⁻¹. Ed (green): edema, Ir (orange): Infiltration of inflammatory cells, N (white): Necrosis, Mm (black): Melanomagrophages, H (red): Hemorrhage, Fd (blue): Fat degeneration (400x).](image)

3.2.2. Station 2

![Histopathology of kidney of tilapia (Oreochromis mossambicus) with lead concentration (Pb) at station 2. (A) Pb concentration 0.50 µg.g⁻¹, (B) Pb concentration 0.36 µg.g⁻¹, (C) Concentration Pb 0.10 µg.g⁻¹. Ed (green): edema, Ir (orange): Infiltration of inflammatory cells, N (white): Necrosis, Mm (black): Melanomagrophages, H (red): Hemorrhage, Fd (blue): Fat degeneration (400x).](image)
Figure 2. Histopathology of kidney of tilapia fish (*Oreochromis mossambicus*) with lead (Pb) content at station 2. (A) Pb concentration 1.08 µg.g⁻¹, (B) Pb concentration 0.41 µg.g⁻¹, (C) Pb concentration 0.12 µg.g⁻¹. Ed (green): edema, Ir (orange): Infiltration of inflammatory cells, N (white): Necrosis, Mm (black): Melanomagrophages, H (red): Hemorrhage, Fd (blue): Fat degeneration (400x).

3.2.3. Station 3

Figure 3. Histopathology of the kidney of tilapia fish (*Oreochromis mossambicus*) with lead (Pb) content at station 3. (A) Pb concentration 0.50 µg.g⁻¹, (B) Pb concentration 0.33 µg.g⁻¹, (C) Pb concentration 0.12 µg.g⁻¹. Ed (green): edema, Ir (orange): Infiltration of inflammatory cells, N (white): Necrosis, Mm (black): Melanomagrophages, H (red): Hemorrhage, Fd (blue): Fat degeneration (400x).
Table 4. The results of observations of damage that occurred in the kidneys of tilapia (*Oreochromis mossambicus*) with different concentrations of Pb from each station.

| No  | Forms of tissue damage | Concentration of lead metals (Pb) | Station 1 | Station 2 | Station 3 |
|-----|------------------------|----------------------------------|-----------|-----------|-----------|
|     |                        | µg/g                              | 0.10      | 0.36      | 0.50      |
|     |                        | µg/g                              | 0.12      | 0.41      | 1.08      |
| 1   | Fatty degeneration     | -                                 | -         | +         | +         |
| 2   | Hemorrhage             | +                                 | +         | -         | +         |
| 3   | Edema                  | -                                 | ++        | -         | +         |
| 4   | Necrosis               |                                   | ++        | ++        | +++       |
| 5   | Melanomacrophages      |                                   | -         | ++        | -         |
| 6   | Inflammatory cell infiltration |              | ++      | ++       | +++       |

The level of fish kidney damage is stage I: it has not changed the normal function of the tissue too much, stage II: it is more severe and damages the normal function of the tissue, and stage III: is very severe and causes irreparable damage. Stage I, the corpuscles undergo glomerular capillary dilatation and glomerular enlargement, the tubules undergo nuclear and cellular hypertrophy, cytoplasmic vacuolization, cloudy swelling, tubular lumen dilation, tubular regeneration and melanomacrophage aggregates. Stage II bleeding occurs in the bowman capsule space, the bowman capsule space is reduced, hyaline and tubular degeneration and decreased tubular lumen function. Stage III necrosis occurs which is the final stage of cell damage [7].

4. Conclusion

Based on the results of observations obtained by damage or histopathology that occurs in the kidneys which shows the presence of necrosis, inflammatory cell infiltration, edema, fatty degeneration hemorrhage and melanomacrophages and the level of damage to the tissue depends on the concentration of metal contaminated in the fish organs. Damages that occur allegedly due to exposure of heavy metals that are dissolved in the waters of the fish ecosystem that has passed the threshold.

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