Histopathological view of silver barb (*Barbonymus gonionotus*) kidney that contaminated by heavy metal Cadmium (Cd) in Tempe Lake, Wajo District

AY Ihzamahendra¹, DK Sari² and AW Jamaluddin³

¹Veterinary Study Program, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia
²Division of Veterinary Pathology, Veterinary Medicine Study Program, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia
³Division of Veterinary Pharmacy, Veterinary Medicine Study Program, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia

Email: dwiks73@yahoo.com

Abstract. Start Silver barb (*Barbonymus gonionotus*) is widely consumed by the community and is the dominant type of fish caught throughout the year in Lake Tempe. The purpose of this study was to determine the histopathological view of the kidneys in silver barb contaminated with heavy metal cadmium (Cd). Samples were used is 15 silver barb with each of 5 kidney samples at each station. Measurement of heavy metal content was carried out with an Atomic Absorption Spectrophotometer (AAS) and obtained cadmium (Cd) concentrations in the kidney organs of contaminated fish which were 0.29 - 1.94 µg / g with an average of 0.77 ± 0.12 µg / g. Kidney organ preparations were fixed using 10% neutral buffered formalin (NBF), dehydration using graded alcohol, embedding using paraffin, cutting with a thickness of 5 µm and stained with haematoxilin eosin and then observed. Analysis of the data used is descriptive qualitative. Based on observations obtained by damage or histopathology that occurs in the kidneys namely fat degeneration, connective tissue formation, necrosis, hemorrhage and an increase in the number of melanomacrophages. The level of damage to the tissue depends on the concentration of the metal contaminated in the fish's organs. Damages that occur are thought to be caused by exposure to heavy metals dissolved in the waters of the fish ecosystem which have passed the threshold.

1. Introduction
Tempe Lake is located in Wajo Regency, Sidrap Regency and Soppeng Regency, where the water comes from river water. Resources in Tempe Lake are utilized in the fisheries, agriculture, and water transportation services sectors [1]. Silver barb (*Barbonymus gonionotus*) is a type of introduced fish and is the dominant fish in the waters of Lake Tempe throughout 2016 with a percentage of the number of fish caught reaching 54.5% and production reaching 1815.4 tonnes [2].

The waters of Lake Tempe have been polluted, one of which is heavy metals. The status of Lake Tempe water quality is heavily polluted because several water quality parameters are already outside the acceptable threshold according to class II water quality standards, one of which is cadmium (Cd) [3]. Cadmium (Cd) is useless to the body and can interfere with health if it accumulates in the body.
[4]. Contamination causes structural and functional damage to various fish organs. The kidneys are one of the organs that are sensitive to pollution [5].

2. Methodology
The data analysis used was descriptive qualitative data analysis. This method will explain the histopathological view of the kidney of tawes fish (Barbonymus gonionotus) contaminated with heavy metal cadmium (Cd). The relationship between cadmium content (Cd) with body weight and body length of Tawes fish was determined using regression analysis.

2.1. Sampling
Fish samples were taken at Lake Tempe, Tempe District, Wajo Regency. Fish samples were taken from three different stations, namely Station 1 located at coordinates 4°09'49" South Latitude and 119°56'51" East Longitude, where this location is around the Panincong River which is the place or point of entry of water from Soppeng Regency to the lake and also a place tawes fishing. Station 2 is located at coordinates 4°06'13" South Latitude and 119°58'49" East Longitude, where this location is in the middle of a lake and is often used by fishermen as a fishing area. Station 3 is located at coordinates 4°07'43" South Latitude and 120°01'12" East Longitude, where this location is around of the Gerak Dam, which is the exit route for river water from the lake. The samples used were 5 fish samples from each station, so the total sample was 15 fish. After that, a necropsy was performed to separate the fish kidneys. The three stations in Lake Tempe that were selected may have been contaminated with heavy metal cadmium (Cd) due to the large activity of citizens in these locations and naturally polluted.

2.2. Heavy metal measurement and making of histological preparations
Making samples of tawes fish kidneys and analysis of Atomic Absorption Spectrophotometer (AAS) on kidney organs contaminated with heavy metal cadmium (Cd) was carried out at the Makassar Health Laboratory Center. Histopathological analysis of tawes fish kidney contaminated with heavy metal cadmium (Cd) was carried out at the Laboratory of Animal Clinical Pathology of Education, Hasanuddin University. The stages of making histopathological preparations are fixation, trimming, processing and embedding, blocking, and staining. After the histopathological preparations were completed, microscopic observations were made to see the histopathological changes that occurred in the fish kidney organs due to heavy metals.

3. Results and discussion

3.1. The content of heavy metal cadmium (Cd) in the kidney of Tawes fish
Based on the test by the Makassar Health Laboratory Center for the kidney organ of tawes fish (Barbonymus gonionotus), the levels of heavy metal cadmium (Cd) contamination are shown in table 1.

Table 1. The observations on the average content of heavy metal cadmium (Cd) in the tawes fish kidney samples using the Atomic Absorption Spectrophotometer method.

| Sample Code | Body Weight (g) | Body Length (mm) | Cadmium Content (µg.g⁻¹) |
|-------------|----------------|------------------|-------------------------|
|             |                |                  |                         |
| G.Cd.SP.1   | 113.4          | 200              | 1.56                    |
| G.Cd.SP.2   | 99.22          | 185              | 0.62                    |
| G.Cd.SP.3   | 76.54          | 185              | 1.01                    |
| G.Cd.SP.4   | 93.55          | 185              | 0.35                    |
| G.Cd.SP.5   | 85.05          | 190              | 0.38                    |
| **Range**   | **76.54 – 113.4** | **185 – 200** | **0.35 – 1.56**        |
|          | Average ± SE  | Station 2       | Station 3       | Combined Station |
|----------|--------------|-----------------|-----------------|------------------|
|          | 93.55 ± 6.28 | 189.00 ± 2.92   | 0.78 ± 0.23     |                  |
| Station 2| 107.73       | 190             | 0.96            |                  |
|          | 62.37        | 165             | 0.58            |                  |
| G.Cd.PB.3| 59.54        | 160             | 0.53            |                  |
| G.Cd.PB.4| 76.54        | 175             | 0.84            |                  |
| G.Cd.PB.5| 70.88        | 175             | 1.94            |                  |
| Range    | 59.54 – 107.73| 160 – 190       | 0.53 – 1.94     |                  |
| Average ± SE | 75.41 ± 8.63 | 173.00 ± 5.15   | 0.97 ± 0.26     |                  |
| Station 3| 90.72        | 180             | 0.45            |                  |
|          | 85.05        | 182             | 0.29            |                  |
| G.Cd.BG.3| 73.71        | 178             | 0.72            |                  |
| G.Cd.BG.4| 65.21        | 172             | 0.85            |                  |
| G.Cd.BG.5| 70.88        | 172             | 0.52            |                  |
| Range    | 65.21 – 90.72| 172 – 182       | 0.29 – 0.85     |                  |
| Average ± SE | 77.11 ± 4.69 | 177.00 ± 1.95   | 0.57 ± 0.10     |                  |
| Combined Station | Range | 59.54 – 113.40 | 160 – 200       | 0.29 – 1.94      |
|          | 82.03 ± 4.21 | 17.97 ± 0.26    | 0.77 ± 0.12     |

Based on the table, it can be seen that the tawes fish kidney contains heavy metal Cd from the AAS test results. The concentration of cadmium (Cd) in contaminated fish kidney organs was 0.29 - 1.94 µg / g with an average of 0.77 ± 0.12 µg / g. From the value obtained, it can be seen that the concentration of Cd contained in the kidney organ exceeds the quality standard threshold allowed for fish and its processed products according to SNI 7387: 2009, namely 0.1 mg / kg.

The highest concentration of heavy metals is at station 2, namely 0.53 - 1.94 µg / g with an average of 0.97 ± 0.26 µg / g, this is because the location of station 2 is an area that is often used by fishermen to catch fish so that pollution originates of the large number of motorized boat traffic. This location is also the closest to several industries where their production waste has undergone processing, but the levels are still above the threshold so that the industry has the potential to be the highest contributor to heavy metal Cd. In contrast, the lowest Cd heavy metal content is found at station 3, namely 0.29 - 0.85 µg / g with an average of 0.57 ± 0.10 µg / g, this is because the location of station 3 is not in an area close to industry and rarely it is used by fishermen as a fishing area so that cadmium (Cd) contamination only comes from community activities along the river, which causes the content of heavy metal Cd at station 3 to be the lowest among other stations.

At station 1, the content of Cd heavy metal was higher than station 3, namely 0.35 - 1.56 µg / g with an average of 0.78 ± 0.23 µg / g, this is because other than the place or route of water entry from Soppeng Regency to the lake, this location also has a floating house which is an area for tourism and a trading facility so that the floating house waste and motorized boat activities cause an increase in the heavy metal content of Cd at the station.

Information about the relationship between cadmium content in the kidneys and body size (length and weight) of tawes fish was subjected to regression analysis. The closeness of this relationship can be seen in Figure 1 (with fish body length) and Figure 2 (with fish body weight).
Figure 1. Graph of the relationship between body length and cadmium content in the tawes fish kidneys combined from all stations in Lake Tempe.

The simple linear regression test results between the body length of the fish and the accumulated cadmium content in the tawes fish kidneys as seen in the graph show a negative relationship because the correlation coefficient (r) is 0.2161. This suggests that the relationship between body length measurement and the concentration of cadmium in the kidneys has a very weak correlation.

Figure 2. Graph of the relationship between body weight and cadmium content in the combined tawes kidneys of all stations in Lake Tempe.

The simple linear regression test results between the cadmium content in the kidneys and the body weight of tawes fish as seen in the graph also show negative results because the correlation coefficient (r) is 0.1253. This shows that the relationship between fish body weight and the concentration of cadmium in the kidneys has a very weak correlation. If the correlation coefficient (r) has a value between 0–0.25, there is a very weak closeness relationship [6].

The existence of a negative relationship between the heavy metal content of cadmium and body size (length and weight) of fish in this study was probably caused by differences in metabolic activity between small and large fish. Fish with a large size have a smaller concentration of heavy metals. This is because the heavy metals that enter the fish's body will have a dilution process through the growth process, so that the increase in heavy metals in the fish's body will decrease along with the increase in individual body size of the fish [7].

Based on the test by the Makassar Health Laboratory Center for the quality of Lake Tempe water at three stations, the levels of heavy metal cadmium (Cd) contamination are shown in table 2.
Table 2. Tempe Lake water test results on cadmium (Cd) content.

| Sample Code | Cadmium Content (mg/L) |
|-------------|------------------------|
| Station 1   | 0.0006                 |
| Station 2   | 0.0011                 |
| Station 3   | 0.0006                 |

Range: 0.0006 – 0.0011  
Average ± SE: 0.0008 ± 0.0002

Based on table 2, it can be seen that Lake Tempe water contains heavy metal Cd from the AAS test results. Lake Tempe water contaminated with cadmium (Cd) is 0.0006 – 0.0011 mg / L with an average of 0.0008 ± 0.0003 mg / L. From the value obtained, it can be seen that the concentration of Cd in Lake Tempe water has not exceeded the water quality standards set by PPRI No. 82 of 2001, which is 0.01 mg / L.

Although the levels of heavy metals in river flows are relatively small but they are easily absorbed and accumulated biologically by aquatic plants or animals and will be involved in the food web system [8]. Heavy metals can accumulate in the body of the fish through several ways including inhalation (respiration), the food channel (biomagnification) and through the skin (diffusion). The highest metal accumulation is usually found in the liver and kidneys [4].

Pollution in Lake Tempe occurs because the main source of water comes from several rivers and those rivers are place for various community activities, both as a means of transportation and as a place for bathing, washing and other activities so that the sources pollution of Lake Tempe include domestic, agricultural, and household industrial waste. These pollutant sources cause the metal content in the waters of Lake Tempe to be classified as high [3].

3.2. Kidney histopathology of tawes fish (Barbonymus gonionotus)
3.2.1. Kidney histopathology of tawes fish (Barbonymus gonionotus) at Station 1. The kidneys of tawes fish (Barbonymus gonionotus) at Station 1 in Lake Tempe suffered various histopathological changes depending on the concentration of cadmium (Cd) contamination that occurred. The histopathological changes that occurred in the kidneys with a concentration of Cd 0.35 µg / g were fat degeneration, hemorrhage and connective tissue. Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 1 with exposure to cadmium are shown in figure 3.
Figure 3. Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 1 with exposure to cadmium. a. 0.35 µg / g, b. 0.62 µg / g, c. 1.56 µg / g. CT (blue): Connective tissue, FD (green): Fat degeneration, G (gray): Glomerulus, H (yellow): Hemorrhage, HC (red): Hematopoietic cells, MM (black): Melanomacrophage, N (white): Necrosis, T (dark blue): Tubule (HE, 400x).

The kidneys with a concentration of Cd 0.62 µg / g had an increase in histopathological changes, namely necrosis, hemorrhage and fat degeneration. In contrast, at a concentration of Cd 1.56 µg / g, the most severe histopathological changes occurred, namely necrosis, hemorrhage, fat degeneration, connective tissue and there were many melanomacrophages and in this condition the kidney structure was difficult to identify compared to the exposure of 0.35 µg / g and 0.62 µg / g.

3.2.2. Kidney histopathology of tawes fish (Barbonymus gonionotus) at Station 2. The kidney of tawes fish (Barbonymus gonionotus) at Station 2 in Lake Tempe suffered various tissue damage depending on the concentration of cadmium (Cd) contamination that occurred. Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 2 with exposure to cadmium are shown in figure 4.

Figure 4. Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 2 with exposure to cadmium. a. 0.53 µg / g, b. 0.96 µg / g, c. 1.94 µg / g. BC (orange): Bowman's capsule, CT (blue): Connective tissue, FD (green): Fat degeneration, G (gray): Glomerulus, H (yellow): Hemorrhage, HC (red): Hematopoietic cells, MM (black): Melanomacrophage, N (white): Necrosis (HE, 400x).

Tissue damage that occurs in the kidneys with a concentration of Cd 0.53 µg / g is fat degeneration, hemorrhage, necrosis and melanomacrophages. The kidneys with a concentration of Cd 0.96 µg / g had increased tissue damage, namely necrosis, fat degeneration, melanomacrophages and a lot of...
hemorrhage. In contrast, the Cd content of 1.94 µg / g experienced the most severe tissue damage, namely there were many necrosis cells, hemorrhages, fatty degeneration, connective tissue and melanomacrophages. The glomerular and tubular structures in the kidneys with concentrations of Cd 0.53 µg / g and 0.96 µg / g are still clearly visible, while at a concentration of 1.94 µg / g it is difficult to identify.

3.2.3. Kidney histopathology of tawes fish (Barbonymus gonionotus) at Station 3. The kidneys of tawes fish (Barbonymus gonionotus) at Station 3 in Lake Tempe suffered various histopathological changes depending on the concentration of cadmium (Cd) contamination that occurred. Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 3 with exposure to cadmium are shown in figure 5.

![Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 3 with exposure to cadmium.](image)

**Figure 5.** Histopathology of the kidney of tawes fish (Barbonymus gonionotus) at Station 3 with exposure to cadmium a. 0.29 µg / g, b. 0.52 µg / g, c. 0.85 µg / g. CT (blue): Connective tissue, FD (green): Fat degeneration, H (yellow): Hemorrhage, MM (black): Melanomacrophage, N (white): Necrosis (HE, 400x).

Histopathological changes that occurred in the kidneys with a concentration of Cd 0.29 µg / g were fat degeneration, hemorrhage, and necrosis. The kidneys with a concentration of Cd 0.52 µg / g had an increase in histopathological changes, namely fat degeneration, necrosis and a lot of hemorrhage. In contrast, the Cd content of 0.85 µg / g experienced the most severe histopathological changes, namely there were many necrotic cells, hemorrhage, fatty degeneration, connective tissue and melanomacrophages.

From the histopathology of fish kidneys, it can be seen that the higher the concentration of cadmium that enters the fish's body and the longer the exposure, the cell damage increases because the kidneys are organs that will be exposed to the main contaminants in the water and organs that accumulate cadmium in the fish's body [9].

Kidney cells that experience necrosis can be characterized by a smaller and dark nucleus (pycnosis), lysis (karyolysis), and rupture (cariorexis) [9]. The longer the exposure to toxic substances
in the kidneys can increase the number of necrotic cells in the kidneys [10]. Cell degeneration is characterized by the cell nucleus being pushed to the edge, shrinking, and having a darker color and a vacuole filled with fat. The presence of fat vacuoles in cells is due to disruption of fat metabolism in cells [11]. Chronic loss of renal function results in the progressive formation of scar tissue (connective tissue) throughout the kidney. This tissue is formed as a reaction to inflammation (due to toxic input from the blood) as well as a defense from the tissue [12].

Hemorrhage causes disruption of blood supply to epithelial cells and is characterized by bleeding, namely finding small and large red spots that spread [13]. Hemorrhage can be caused by trauma, rupture of blood vessels or increased damage due to infection with bacteria, viruses or toxic materials [14]. Melanomacrophages are a collection of macrophages that are found in the lymphoid tissue of most teleosts and can be characterized as solid round cells that have varying amounts of pigment, found in healthy fish but the number increases in pathological conditions and cases of chronic stress [15].

Cadmium contamination (Cd) in tawes fish (Barbonymus gonionotus) has exceeded the maximum limit set and there have been various tissue damage to the kidney organs based on observations of histopathological changes. If the tawes fish which accumulates Cd metal is then consumed by humans, the metal that accumulates in the fish will also accumulate in the human body and in the short term will cause acute poisoning which is characterized by nausea, stomach pain and lung problems. If cadmium is consumed for a long time, it will cause chronic poisoning, marked by kidney damage and nervous system damage, and even death.

4. Conclusion
The kidney organ of tawes fish (Barbonymus gonionotus) in Lake Tempe has a concentration of heavy metal cadmium (Cd) that exceeds the maximum threshold. Histopathology that occurs in the kidney organs, namely fatty degeneration, connective tissue formation, necrosis, hemorrhage and an increase in the number of melanomacrophages. Based on the linear regression test, there is a very weak relationship between body size (length and weight) of fish and the concentration of cadmium contained in the tawes fish kidney.

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