Histopathological Study of Kidney and Meat of Bungo Fish (Glossogobius sp) contaminated by Lead Metal (Pb) in Lake Tempe, Wajo Regency

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Histopathological Study of Kidney and Meat of Bungo Fish (Glossogobius sp) contaminated by Lead Metal (Pb) in Lake Tempe, Wajo Regency

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Abstract. Bungo fish (*Glossogobius sp*) is one of the freshwater aquatic fish that is in great demand by the community and is endemic in Lake Tempe and is in the Wallacea region. The purpose of this study was to determine the histopathological study of the kidneys and meat in bungos exposed to heavy lead metals (Pb). The samples used were nine bungo fish with nine kidney and nine meat samples each. Measurement of heavy metal content was carried out with Atomic Absorption Spectrophotometry and obtained the lowest metal concentration of 6.52 ppm, moderate 42.02 and the highest 435.30 ppm in the kidney and in meat, the lowest 0.07 ppm, medium 1.56 ppm, and the highest 4.73 ppm. Organ preparations were analyzed using histotechnique methods and they were analized with descriptive qualitative. Based on observations obtained by damage or histopathology that occurs in the kidneys which shows inflammation of the glomerulus, hemorrhage, formation of connective tissue, vacuoles, necrosis, changes in the number of melanomacrophages and also damage to the tubules, whereas damage to the meat does not occur. 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

Lake Tempe is one of the lakes that has considerable potential in South Sulawesi Province [1]. Lake Tempe is a tectonic lake in Indonesia located in the western part of Wajo Regency, South Sulawesi, covering several districts, namely Tempe, Belawa, Tanah Sitolo, Maniangpajo, and Sabbangparu [2]. The latitude of Lake Tempe is at coordinates 119 ° 53 '-120 ° 04' East Longitude and 4 ° 03 '-4 ° 09' South Latitude [1]. Lake Tempe is separated from Lake Buaya and Lake Sidenreng in the dry season while in the third rainy season the lake merges and forms an area of water covering an area of 35,000 ha [3]. Lake Tempe water sources are the Bila and Sidenreng watersheds in the north and the Batu-Batu watershed in the west [4].

Freshwater fish diversity in Indonesia is the second highest after Brazil, with as many as 1300 species. The diversity of fish in Indonesia is facing the threat from various human activities that can cause a decrease in the diversity of these fish. Indonesian fish that are endangered are 87 species, and 66 species of them are freshwater fish. Most (68%) of these endangered freshwater fish are endemic fish. In Sulawesi, 62 freshwater fish species have been recorded and among them there are endemic species. The decline in freshwater fish stocks is largely due to habitat destruction / loss (35%), introduction of exotic species (30%), and over-exploitation of species (4%). The rest (31%), due to pollution, competition for water use and global warming [5].

The potential of Lake Tempe resources that have been managed and utilized long ago by the community is the potential of fisheries. Lake Tempe is known for its freshwater fishery production and the fish products are marketed to outside Wajo Regency. The potential of this fishery has provided benefits to the community and government [6]. In 1948-1969 Lake Tempe fish production reached 55,000 tons per year. At that time Lake Tempe was dubbed the "fish bowl" of Indonesia [7].

The types of fish in the waters of Lake Tempe are cork (Channastriata), betok (Anabastestudineus), conjoined sepat (Trichogaster pectoralis), aquaculture (Helostoma temmincki), swamp sepat (Trichogaster trichopterus), catfish (Clarias batrachus), mas (Cyprinus carpio carpio)), tawes (Barbonymus gonionotus), nilem (Osteochilus vittatus), mujair (Oreochromis mossambicus), , bunaka (Bunaka gyrinoides), bungo (Glossogobius sp), masapi (Monarchusbutus alborus) and masapi (Anguillla albusorbus). mullet (Mugil cephalus) [8]. Several types of fish found in Lake Tempe are now introduced fish, including ponds, conjoined sepat, Tawes, carp, nilem, and catfish [9].

Bungo is one of the original fish of Lake Tempe [5]. However, there has not been much attention from researchers and local governments to conserve the fish. Bungos or belosoh fish are now increasingly pushed due to intensive fishing and habitat degradation [10]. Small amounts of heavy metals are needed for aquatic bodies, but in amounts that exceed the requirements for the normal life of aquatic bodies, heavy metals can be highly toxic poisons to aquatic organisms [7]. Heavy metals are toxic metals that are dangerous when entering the body beyond the threshold [11]. Heavy metal is an industrial waste that threatens aquatic organisms. Metal compounds can enter very easily and quickly into the body and can accumulate in the body tissues of aquatic organisms. It can also be toxic to humans if they consume contaminated fish. The process of accumulation of metals in tissues occurs after absorption of metals from water or through contaminated feed. The metal is absorbed by the blood, binding to blood proteins, which are then distributed to all body tissues [12]. Heavy metal pollution has negative effects on the life of living things such as disrupting chemical reactions, inhibiting the absorption of essential nutrients [11].

Water quality degradation that occurs due to pollution can trigger structural and functional damage to various fish organs. One organ that is sensitive to pollution is the kidney [13]. To find out the level of pollution that occurs can be determined by analyzing the content of heavy metals that have accumulated in the aquatic biota in these waters, including bungo fish. Bungo fish is one type of fish that is consumed by many people. Therefore, it is necessary to conduct a study to determine the presence of lead heavy metal (Pb) in bungo fish.

2.Materials and Methods

This research took place from May-July which included fish sampling activities in Tempe Lake, Tempe District, Wajo District. Samples were taken at a location in the middle of Lake Tempe, which is in the reservoir area or known as Beam Balanda. Measurement of metal content was carried out using the Atomic Absorption Spectrophotometer (AAS) in kidney and meat contaminated with lead metal (Pb)

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conducted at the Animal Food Chemistry Laboratory, Department of Nutrition and Animal Feed, Faculty of Animal Husbandry. Histopathological analysis of kidney and flesh of Pung fish contaminated by Pb heavy metals was carried out at the Pathology Laboratory of Educational Animal Clinics, Hasanuddin University. The number of samples used as many as nine fish consisting of 18 samples, namely nine kidney samples and nine meat samples.

Tissue samples were fixed with 10% formalin solution for 3 days. Organs that have been tissue fixed are inserted into the tissue cassette. Then proceed by inserting a tissue cassette into the tissue processor. The tissue in the tissue cassette is then dehydrated by putting the tissue into a multilevel alcohol solution, namely 70% alcohol, 80%, 90%, 95%, 100%. Alcohol 70% to 80% each for 1 day. Then 90% and 95% 12 hours respectively. Then 100% (1) and 100% (2) each 1 hour. Then clearing into xylol I and xylol II, each for 15 minutes. Then the infiltring stage, which is a tissue cassette is put into liquid paraffin I and II with a temperature of 56 ° C each for 1 hour. The next stage is embedding, which is printing tissue in liquid paraffin by means of the specimen placed on top of the mold and then filled with paraffin. The position of the specimen to be cut must face down, attached to the mold. Then put a pink cassette on top of the print and add paraffin.

Cutting is done with a microtome with a thickness of 5 mμ. Tissue pieces are then placed on a slide that has been given aquades and numbering. Then the slides were placed in an incubator at 40^0C for 1 day before staining. The tissue is soaked in xylol I solution for 30 minutes then soaked in xylol II solution for 30 minutes, then put in 100% I, 100% II, 95%, 80% and 70% alcohol respectively for 1 minute respectively . Furthermore, it is soaked in distilled water for 15 minutes so that the coloring of haematoxylineosin can stick properly. Then proceed to put the preparation into the eosin coloring solution for 10 minutes. The next step is the preparation put into 70% alcohol solution, 80%, 90%, 95%, 100%, 100%, xylol I, and xylol II sequentially for 1 minute each except xylol I and II respectively 30 minute. After that the preparation is dried and given 1-2 drops of adhesive, then carefully covered with a cover glass until no air bubbles are formed, then stored for several minutes until the adhesive dries and is ready to be observed under a microscope. Observations were made under a microscope, using a 10x subjective lens magnification and 4x, 10x and 40x objective lenses. Observation and image capture are done using optical lenses. Histological preparations for kidney and meat were then observed.

3.Results and Discussion

3.1 Timbel (Pb) heavy metal content in kidneys and Bungo fish meat

Timbel is a dangerous material if consumed in the body of living creatures exceeds the threshold because it can damage or decrease the function of the central nervous system, damage the composition of blood, kidneys, lungs, other vital organs and will be dangerous if consumed by humans in large quantities [14] . Based on laboratory tests on flesh of Bungo fish (Glossogobius sp), lead levels (Pb) in the kidneys and meat are shown in table 1.

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Table 1. Results of observations of the average content of heavy metals (Pb) in samples of kidney and flesh of bungo fish using the Atomic Absorption Spectrophotometry method

Sample	Meat (ppm)	Kidney (ppm)
1	0,61	42,02
2	0,07	9,54
3	0,79	39,52
4	3,72	6,52
5	1,36	435,30
6	2,86	10,02
7	4,58	60,14
8	1,56	162,72
9	4,73	145,59
Average	2,25 ± 1,77	101,26 ± 137,86

Based on the table above it can be seen that meat has a heavy metal content of Pb, from the AAS test results with a total of 18 samples including meat and kidney then averaged as seen in Table 1 showing the difference in lead levels (Pb) in each meat and kidney . In Bungo fish that was contaminated with lead (Pb) was 0.07-4.73 ppm with an average of 2.25 ± 1.76, whereas in the kidney of Bungo fish that was contaminated with lead (Pb) was 6.52-435.30 ppm and 101.26 ± 137.86 mean in the kidney. From the values obtained it can be seen that the concentration of Pb obtained in meat and kidneys is very high and exceeds the specified quality standard threshold. In Table 1 it can be seen that the lead content (Pb) detected in the flesh and kidney of Bungo fish (Glossogobius sp), exceeds the allowable limit according to SNI 7387: 2009 which is 1.0 mg / kg in meat and 0.25 mg / kg in the kidneys. This happens because tempe lakes are contaminated by various things, such as pepticides, fertilizers, excessive sedimentation, waste due to human activities, liquid waste, radioactive waste, waste heat and others [15].

3.2. Histopathology of Bungo fish

3.2.1. Kidney

The results of metal testing using the AAS method in this study showed various lead metal concentrations on kidney organs and bungo fish, causing various tissue damage to kidney and meat organs. Kidney damage in bungo fish can be seen in table 2.

Table 2. Results of observations of damage that occurs in the kidney of bungo fish with different Pb metal concentrations.

No.	Form of tissue damage	Pb concentration (lowest) 6,52 ppm	Pb concentration (medium) 42,02 ppm	Pb concentration (the highest) 435,30 ppm
1.	Glomerulus inflamation	-	++	-
2.	Haemorrhague	+	+++	-
3.	Connective tissue	+	+++	++++
4.	Vacuola	-	+++	-
5.	Necrosis	+	+++	++++
6.	Melanomacrophage	++	+++	+++
7.	Tubulus	-	+++	++++

Note: (-) no damage (+) minor damage (++) moderate damage (+++) severe damage (++++) very severe damage

Based on observations with the lowest level of contamination in the kidneys which is 6.52 ppm shows conditions with various types of damage. The structure of the kidney organ is seen in the form of necrosis damage. Cells that experience necrosis can be identified by their core form which shrinks (picnotics), enlarges, blurs or disappears (karyolisis) [16]. In glomerulus (Figure 1) which has undergone hypertrophy (swelling) causing a reduction in the filtrate cavity, cell lysis, cell necrosis and scar tissue (connective tissue). Hypertropy is tissue damage which is characterized by an increase in organ size due to increased cell size so that cells are separated from one another. Glomerular hypertrophy occurs due to the blockage of toxic compounds, although the concentration is low but being contaminated for a long time in a fish's body will cause severe damage [17]. Renal tubules are in the form of thin, short layers consisting of one thin layer of epithelial cells with long cilia [18]. At this level of contamination the kidney tubules still look normal but melanomacforags are starting to exceed the normal amount (Figure 1). Melanomakrofag is a collection of macrophages that contain hemosiderin, lipofuschin and seroids as well as the melanin pigment that is found in most lymphoid tissue teleost caused by inflammation. Melanomacrophages are normal, and their numbers will increase in pathological conditions [19].

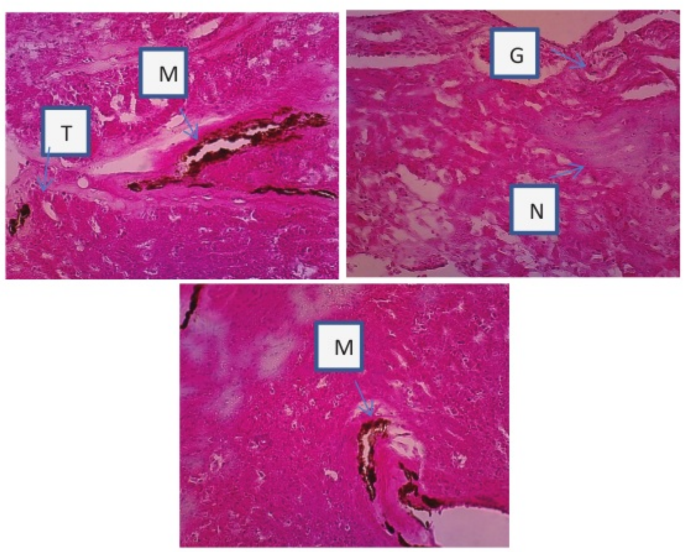


Figure 1. Histopathological of the kidney of bungo fish (Glossogobius sp) with low metal exposure (6.52) ppm in Lake Tempe. (M) melanomacrophages, (N) necrosis, (T) tubules, (G) glomerulus, HE, 40x.

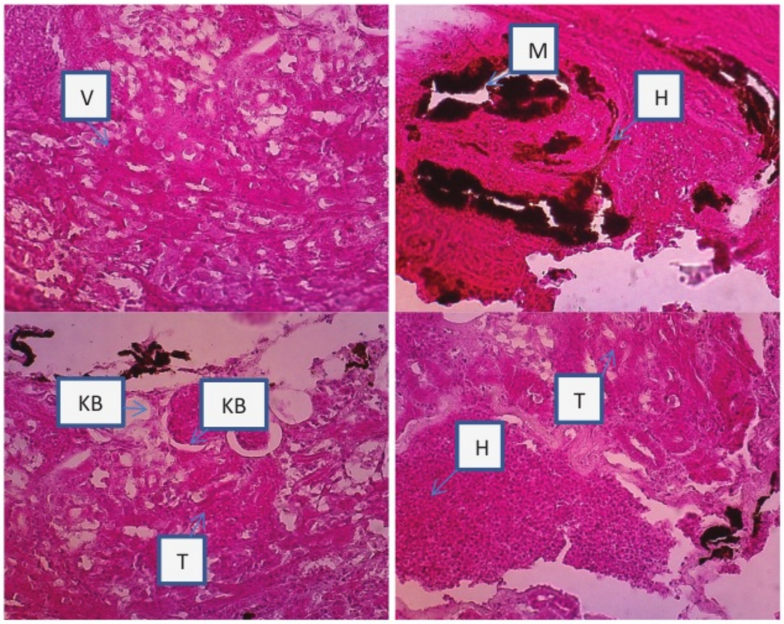


Figure 2. Histopathology of the kydney of bungo fish (Glossogobius sp) with 42.02 ppm metal exposure in tempe lake. (V) vacuoles, (H) Hemorrhages, (T) Tubules, (KB) Bowman capsules, (M) Melanomacrophages, HE, 40x.

In figure 2 with 42.02 ppm exposure which shows moderate exposure shows the formation of a dense connective tissue, where this tissue is formed as a reaction to inflammation (due to toxic entry from the blood) as well as defense and regeneration of the tissue [20]. Small vacuoles can unite to form larger vacuoles so that the cell nucleus is pushed to the edge (Figure 2). Melanomacropfage can be interpreted as a solid round cell that has a varying amount of pigment, found in healthy fish but the number increases in cases of chronic stress. In (Figure 2) visible macrophage images of more than 50% which means excess. The kidney tubules are narrowed. The kidneys function in the glomerulus, which forms ultrafilter from plasma. The ultrafilter will enter the Bowmen capsule and head to the lumen of the tubule. Filtering through various segments of the tubules resulting in changes in the volume and composition of the filtration fluid as a result of the process of reabsorption and secretion along the tubules [20].

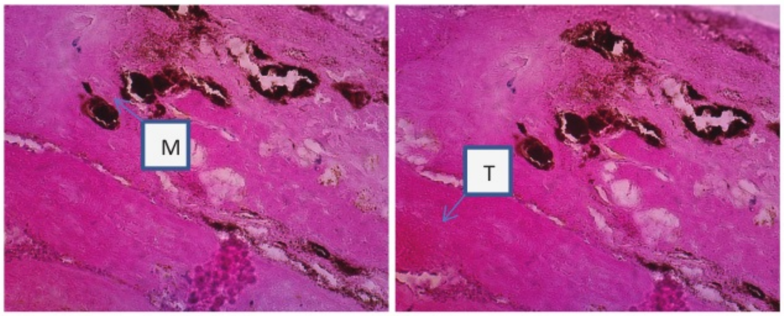


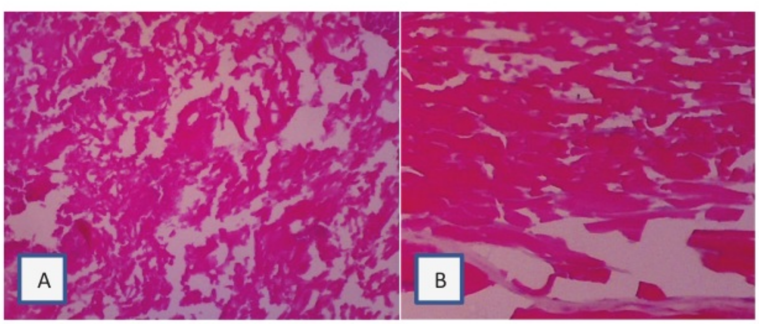
Figure 3. Histopathological of the kidney of bungo fish (Glossogobius sp) with metal exposure of 435.30 ppm in lake tempe. (M) Melanomacrophages. (T) Renal tubules.

In (Figure 3) the concentration of lead heavy metal exposure (Pb) 435.30 ppm with the category of high severity shows damage due to exposure to metals that are acute. It can be seen in the tissue that almost every part has suffered fatal damage, where the shape of the tubules and glomerulus are already unclear due to necrosis. Necrosis describes a condition in which decreased tissue activity is characterized by the loss of several parts of the cell one by one from one tissue so that in a short time will experience death [17].

The entry of lead heavy metal (Pb) into the kidney of Bungo fish (Glossogobius sp) on the observation showed that the damage varies according to the high levels of Pb contamination in the form of necrosis, swelling of the glomerulus, changes in the tubules, and changes in melanomacrophages. The highest metal accumulation is usually in detoxification (liver) and excretion (kidney) [21]. In addition, the kidney as an organ that filters all material and blood before it is released into the body so that if environmental conditions contain a lot of toxic substances it will cause kidney damage. Based on the Pb metal content that has exceeded the maximum limit set, this fish is not safe for consumption by humans because if consumed the metal can accumulate in the human body which can affect and disturb human health, even causing death [22].

3.2.2. Meat analysis

In observations made on Bungo fish (Glossogobius sp) there are 3 categories: low, medium and high. The picture of low contamination can be seen in (Figure 4)



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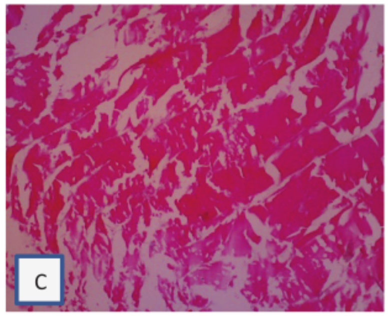

Figure 4. Histopathology of bungo fish (Glossogobius sp) (A) low Pb contamination rate (0.07 ppm) (B) moderate Pb contamination level (1.56 ppm), (C) high Pb contamination rate (4.73 ppm).

Based on observations made on Bungo fish (Glossogobius sp) with a low level of contamination (0.07 ppm) does not show any pathological changes, because the low contamination of the flesh of the Bungo fish has been below the threshold in accordance with SNI 7387: 2009 standards which is 1.0 mg / kg in meat. So that in (Figure 4A) no damage was seen. In (Figure 4B) is a moderate level of contamination (1.56 ppm) and (4.73 ppm) is a high level of contamination. Where the level of contamination is moderate and high already exceeds the predetermined threshold. The metal used also exceeds the specified threshold but does not show changes or damage to the meat tissue [23]. The Pb metal content in the flesh (muscle) organ is lower when compared to the kidney organ but it is not much different from the content in the gill organ [22].

4. Conclusion
The conclusion of this research is the kidney organs and flesh of Bungo fish (Glossogobius sp) in Lake Tempe were found to contain heavy metals. The results of measurements of metal content in Lake Tempe show that exposed fish show different results. In the meat that is 0.07-4.73 ppm with an average of 2.253333 ± 1.76614, while in the kidney is 6.52-435.30 ppm and 101.2633 ± 137.8633 mean on the kidney. From the results of measurements of metals in the lake containing lead (Pb) on average approached the maximum limit. Damage or histopathology that occurs in the kidney organ that is inflammation of the glomerulus, hemorrhage, formation of connective tissue, vacuoles, necrosis, changes in the number of melanomacrophages and also damage to the tubules. 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.

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