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Analysis of Liver Histology Several Fish in the Air Kotok River in the Gold Mining Area, Lebong Regency

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Abstract

Fish have habitats that are easily exposed to contamination from the environment, such as heavy metal waste, which can cause changes in the structure and function of body tissues. One type of waste that enters the Lebong Regency River is gold mining processing waste that uses mercury and cyanide. Heavy metals can enter organisms, and the accumulation and biomagnification of mercury can occur in the bodies of aquatic biota such as fish. There has been no research related to fish histology in the Lebong gold mining area, so the aim of this research was to determine the histological condition of the liver organs of several fish in the Air Kotok river in the gold mining area of Lebong Regency. The research using the paraffin method with hematoxylin-eosin staining. The data were analyzed descriptively and qualitatively based on the histology of the fish liver. The results of the research found four fish species: Hemibagrus nemurus, Rasbora sp., Trichogaster trichopterus, and Macrognathus circumcinctus in the Air Kotok river. The data of the liver histology shows damage to the fish liver structure, such as vacuolization, congestion, pyknosis, dilated sinusoids, melanomacrophages, and leukocyte infiltration. The damage to the hepatocytes in these fish can be used as a bio-indicator of exposure to harmful substances in the Air Kotok river, one of which is heavy metal activity from gold mining, which can be a threat to the health and survival of the fish

Keywords: *Bengkulu, gold mining waste, heavy metal, hepatocyte, histology*

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INTRODUCTION

Aquatic environments such as rivers have an important role in the life of aquatic biota. The aquatic environment includes various parameters that play an important role in maintaining balance and homeostasis, both in terms of physical factor parameters such as temperature, pH, light, and other physical factors, and the most important is the chemical composition of the water and the biological content of the water. According to (Atifah & Harahap, 2019) low river water quality can make the aquatic environment unsuitable as a habitat, which can have an effect on species productivity and variety by reducing their ability to survive and reproduce.

The direct release of heavy metals like mercury into rivers by gold mining activities frequently poses a substantial danger of river contamination. One of the remaining gold mining enterprises in Bengkulu, specifically in Lebong Regency. Generations of gold miners have worked in the same way, generally in their own village. Mercury is commonly used in gold processing drums to bind gold (Trimiska et al., 2016). The research from Nordan et al., (2020) discovered that a village in Lebong Tambang, Lebong Regency, amalgamated gold ore by utilizing mercury metal (Hg) to separate it from sand and rock. Microorganisms will transform heavy metals like mercury in water into methyl mercury components (CH_3-Hg), which can damage aquatic life. Mercury can biotransform into phenylmercury chemical compounds as well as methylmercury as a result of bacterial degradation (Arifin & Goang, 2018). Fish, shrimp, shellfish, and other aquatic organisms that are consumed can accumulate heavy metals in their bodies, which can then enter humans' bodies (Said, 2018).

Fish can acquire heavy metals in two different ways, directly through permeable membranes or indirectly through food and water consumption through the digestive system (Das et al., 2017). Additionally, according to (Ezraneti, 2016), heavy metals like mercury can harm fish organs like the digestive and respiratory systems as well as through skin penetration. According to (Nordan et al., 2020), fish samples from former mining ponds revealed mercury levels of 155.7 ppb, which was beyond the allowable limit for human consumption.

Fish can undergo a variety of morphological, physiological, and biochemical changes as a result of heavy metal exposure. Heavy metal contamination is expected to cause structural and functional damage to a variety of fish organs. The liver is an organ that is frequently used as an indicator of chemical exposure. The liver plays a significant role in the detoxification of xenobiotics, which is why it is frequently cited as a sign of water contamination (Zeitoun & Mehana, 2014). Because heavy metals accumulate in the liver to the point that they are regularly found in significant concentrations, the liver is frequently used for biomonitoring fish health, particularly exposure to heavy metals.

The liver will endure degeneration or severe damage and may become inactive if xenobiotic pressure continues to enter and surpass its physiological capacities. For the accurate identification of fish disorders, the effect of heavy metal bioaccumulation through ultrastructural studies is crucial (Sudrajat et al., 2019). According to Tayel et al., (2014), waste exposure in the aquatic environment can effect in pathological alterations in fish organs. Indicators of the effects of numerous anthropogenic contaminants on organisms, as well as the general health of all aquatic ecosystems, can be found in histopathological alterations. The harmful effects of pollution can be detected in fish tissue before alterations in performance and behavior are observed in fish (Mahboob et al., 2020).

The histological methods employing fish as a bio-indicator of water quality have not yet been extensively studied, notably in Bengkulu Province. The aim of the research is to investigate the histology of the livers of numerous fish species in the Air Kotok River in the Lebong Regency's gold mining zone.

METHOD

This research was conducted from June to November 2022. The sampling location was in the Air Kotok River, North Lebong District, Lebong Regency, Bengkulu Province. Sampling location 1 at coordinates S 03°07'05.2” E 102°12'06.7”, sampling location 2 at coordinates S 03°107'18.22” E 102°12'05.25”, and sampling location 3 at coordinates S 03°187'13.3” E 102°12'09.11”. Sampling locations can be seen in Figure 1.

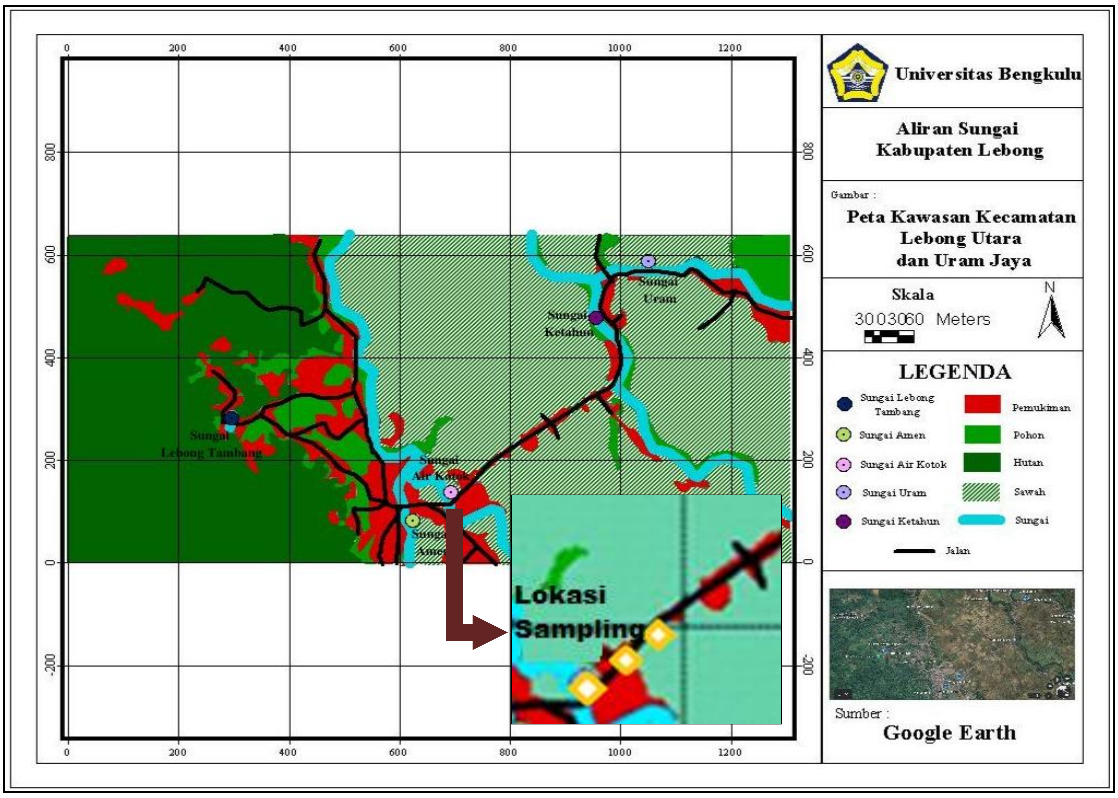


Figure 1. Map of Sampling Location

The water samples were analyzed in the Biology Basic Science Laboratory, and the histology research was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Bengkulu. Mercury analysis using the AAS method and cyanide analysis using spectrophotometry were sent to the Chemical Analysis Laboratory at Politeknik AKA Bogor.

Tools and materials

The tools used in this research were a fishing net with 10 holes, a dissecting kit, flacon bottles, pH meter, DO meter, thermometer, tube, plastic bottle, stopwatch, pH meter, camera, water sample container, microtome, brushes, ovens, glass slides, glass decks, petri dishes, beakers, and Bunsen lamps. The materials used in this research were 10% NBF, distilled water, 70% alcohol, 80% alcohol, 90% alcohol, absolute alcohol, 0.9% NaCl, paraffin, toluol, xylol, hematoxylin, eosin stain, and canada balsam.

Environmental Parameter

Sampling of water in the Air Kotok River, North Lebong District, Lebong Regency, Bengkulu Province. Analysis was carried out on parameters such as: water temperature, pH, water salinity, TDS, TSS, conductivity, and Dissolved oxygen using the Indonesian National Standard (SNI) for water quality analysis. Data on class 2 river water quality parameters compared to the Environmental Quality Standards according to PP RI No. 82/2021.

Histological Preparations

Fish were caught at three sampling points using a fishing net. Fish caught in nets are used as samples, then identification and measurement are carried out, and then they are euthanized and surgically removed for organs. Fish livers were washed with 0.9% NaCl and then fixed with a 10% NBF fixative. Fish liver organs are then processed using the paraffin method. Samples were cut using a microtome with a thickness of about 5 µm. Staining of preparations using hematoxylin-eosin (H&E). Preparations were observed in five fields of view. The histopathological data were examined descriptively and qualitatively.

RESULTS AND DISCUSSION

The Air Kotok river still meets the proper standards for temperature, pH, and dissolved oxygen, according to data from environmental parameter measurements (Table 1). This is seen in the water's pH, which is normally normal given that freshwater fish can survive in a pH range of 6 to 9. Due to the heavy rainfall that occurred during the sampling period, the water temperature tends to be low. According to PP RI No. 82/2021, the normal temperature ranges between 26°C and 30 °C.

The metabolic rate of aquatic species will increase with a rise in water temperature, which can enhance the accumulation and toxicity of heavy metals (Jakfar et al., 2014). Because biological processes double with every 10°C increase in temperature, the amount of heavy metals that are ingested and excreted by fish can be affected by temperature changes. Biological processes are influenced by both the level of temperature and the amount of heavy metals that are absorbed and excreted. Similarly, since freshwaters have a minimum dissolved oxygen level of 4 ppm, amounts of dissolved oxygen that are within the usual range can support fish life.

According to class 2 water quality regulations, the levels of Total Suspended solids (TSS), Total Dissolved solids (TDS), and river conductivity still fall within safe ranges. In accordance with PP RI No. 82/2021, the maximum TSS level is 50 mg/L, the maximum TDS level is 1000 mg/L, and the maximum conductivity range is 200 to 1000 S/cm. Based on the findings of water sample tests, both TSS, TDS, and conductivity are still below the maximum limits. According to the water quality threshold value PP RI No. 82/2021, the Air Kotok river still meets the normal criteria based on the parameters of the waters, which include pH, temperature, salinity, DO, TDS, TSS, and conductivity.

Other water parameters, the water in the Air Kotok River contained mercury and cyanide, but the levels were still below the maximum limit of 0.001 mg/L. This shows that the village still employs mercury in gold processing near the Air Kotok River. Aside from that, cyanide usage was identified, but it was still below the quality limit of 0.1 mg/L. This shows that gold mining or processing activities in the Lebong Regency area still use mercury and cyanide. Although the amounts of cyanide and mercury were below the threshold for class 2 river quality requirements based on laboratory analysis, the timing of water sampling during the rainy season and after many days of heavy rainfall could be a factor in low levels of mercury and cyanide. This is also consistent with the findings of (Rezki & Anita, 2017), who discovered that seasonal factors such as the rainy season might trigger a decrease in mercury levels in water.

Table 1. Result of physical-chemical testing on the Air Kotok River

Parameter	**Air kotok**	**Quality Standards Class 2**
pH (unit)	6,34±0,02	6-9
Temperature (°C)	25,3±0,05	26-30
Salinity (ppt)	1±0,00	1
DO (mg/L)	5,97±0,1	>4
TSS (mg/L)	4±0,2	50
TDS (mg/L)	57,84±0,4	1000
Conductivity (mg/L)	100,9±0,6	200-1000
Cyanide (mg/L)	0,005	0,02
Mercury (mg/L)	<0,001	0,002

The sepat fish (*Trichogaster trichopterus*), sili fish (*Macrognathus Circumcinctus*), wader fish (*Rasbora* sp.), and baung fish (*Hemibagrus nemurus*) are among the various fish species that can be found in the Air Kotok river. In comparison to other species, the sili fish in the Air Kotok River are particularly abundant. Figure 2 shows a picture of some of the fish that were found in the Air Kotok River in the Lebong Regency.

a. *Trichogaster trichopterus*

b. *Macrognathus circumcinctus*

c. *Rasbora* sp

d. *Hemibagrus nemurus*

Figure 2. Types of Fish species in the Air Kotok River in the Lebong Regency

The liver is one of the organs most intimately associated with the detoxification and biotransformation processes due to its role, location, and blood supply. The liver is frequently used as an indicator of water pollution because it is one of the organs most affected by chemicals in water (Younis et al., 2015). After absorbing heavy metals into the fish's body, metal ions go through the circulation to the liver, one of the primary locations for heavy metal accumulation (Onita et al., 2021).

The histological structure of the liver of *T. trichopterus* is shown in Figure 3. The liver exhibits extensive damage, primarily due to vacuolization, which results in numerous large cells that resemble vacuoles due to loss of function as a result of altered cell structure. Hepatocyte cell nuclei exhibit substantial pyknosis, leading the nucleus to become contracted and smaller than normal. Binuclear cells were also found in the fish's histological structure, indicating that there had been severe cell damage and that the cells needed to engage in a cell-regeneration process to replace the tissues or cells. The liver of *T. trichopterus* fish also exhibits significant congestion (blood retention), which is indicated by the presence of erythrocytes or blood in various tissues throughout the organ. Congestion, which is characterized by an increase in the volume of blood in the veins, making the sinusoids seem dilated, is frequently regarded as the first clinical indication of tissue injury (Andayani et al., 2018).

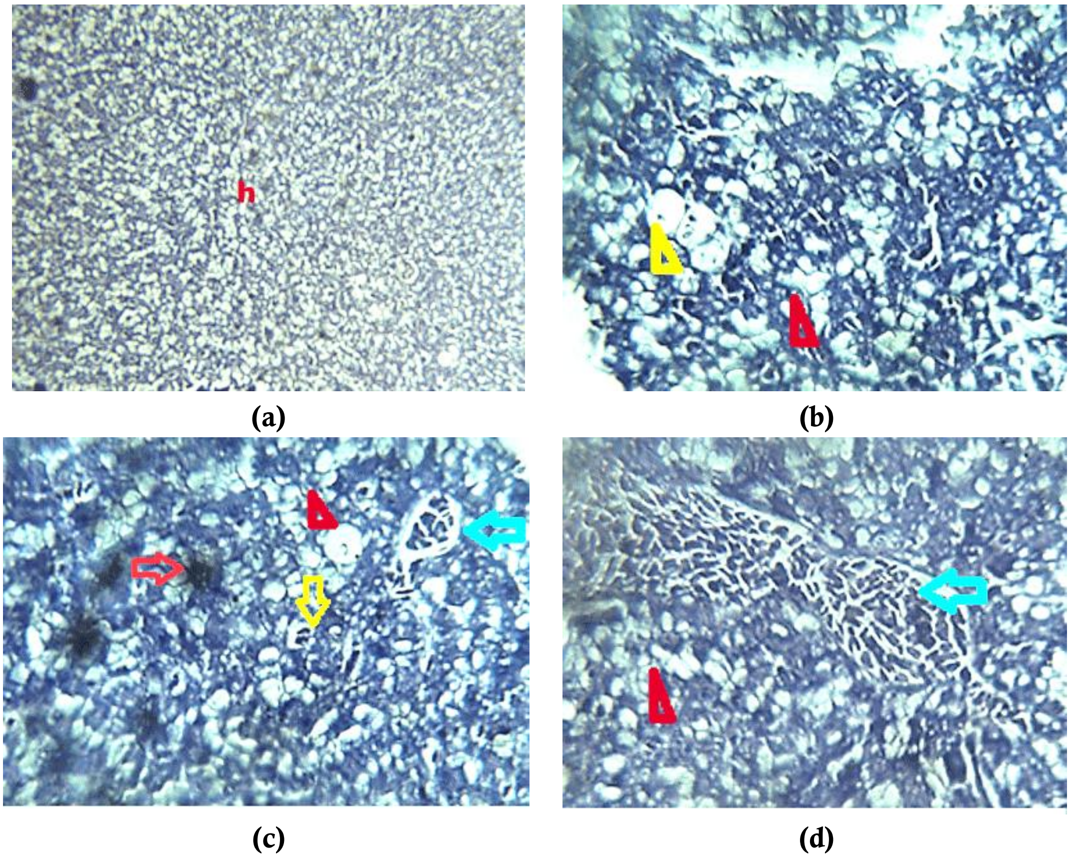


Figure 3. Photomicrograph of *T. trichopterus* liver structure, magnification: 100x (a) and 400x (b,c,d). h: hepatocyte, ◣red: vacuolization, ◣yellow: pyknosis, ⇨red: melanomacrophage, ⇦ blue: congestion, ⇩yellow: binuclear

The histological structure of the liver in *M. circumcinctus* fish (Figure 4) describes a structure that has not experienced significant alterations in the structure of the liver tissue. Melanomacrophages were discovered in the liver tissue of this fish. Important liver abnormalities like degenerative and necrotic processes are frequently linked to an increased aggregate density of melanomacrophages. According to several earlier studies, the existence of melanomacrophages in tissues is caused by the creation of hazardous chemical activity, vitamin shortages, and a rise in heavy metal concentrations in water (Poleksic et al., 2010). Although the role of melanomacrophages in fish liver is still unknown, numerous investigations have shown that they may be involved in endogenous recycling, detoxification, or destruction.

The histopathology of *M. circumcinctus* fish revealed mild sinusoidal dilatation in some areas but also in other areas. According to (Letsoin et al., 2017), blood clots in the venous system caused by harmful substances or the beginning of severe fatty degeneration can cause sinusoidal dilatation. Cells with pyknosis and necrosis were also discovered in fish histology; however, they were less numerous than in other fish species.

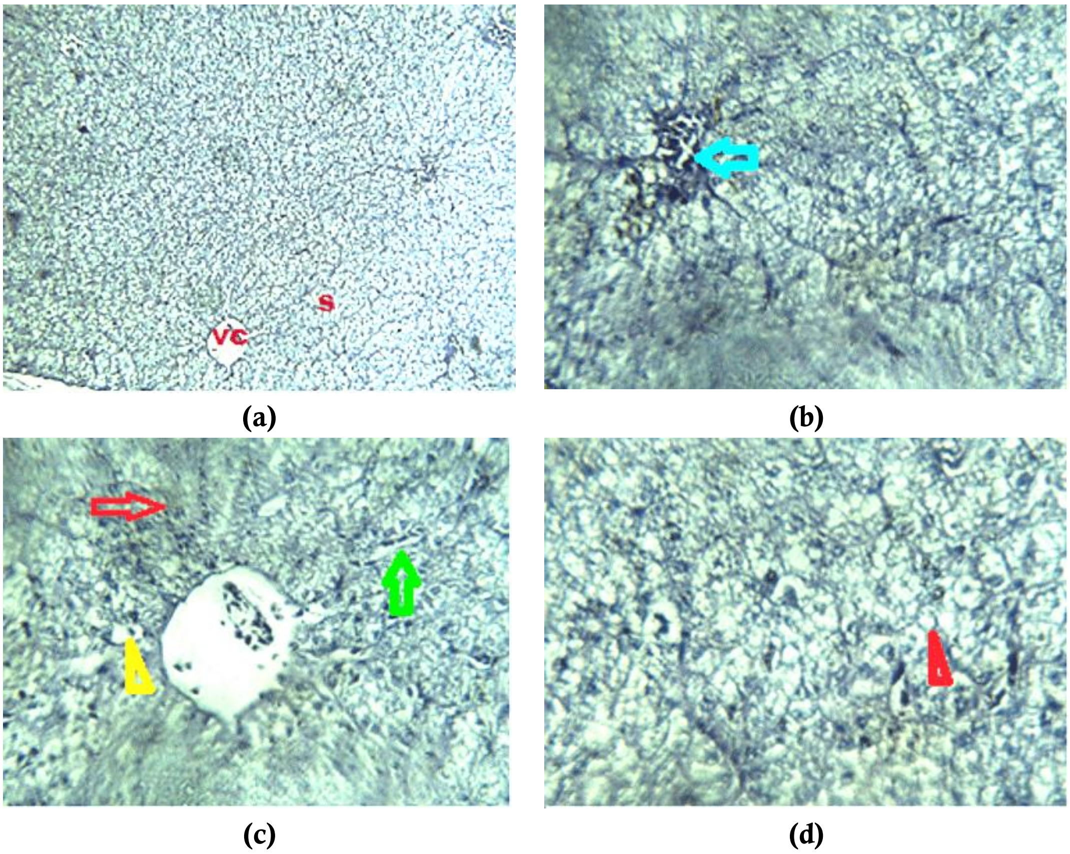
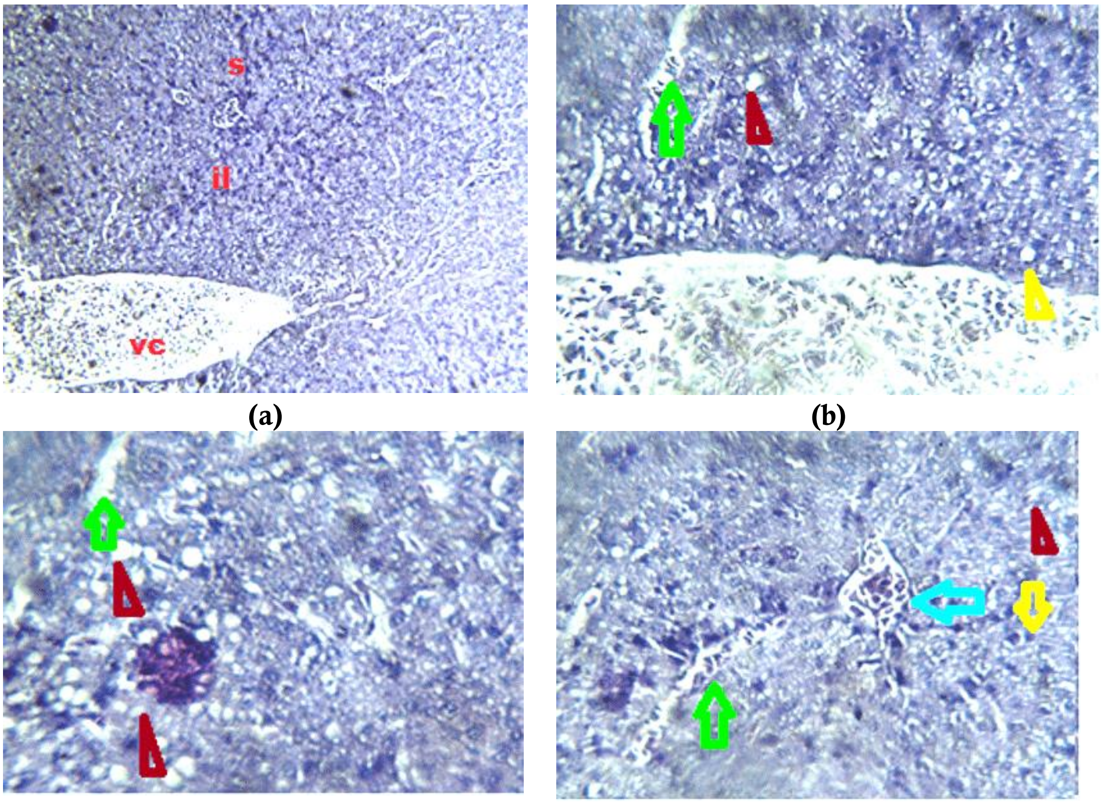


Figure 4. Photomicrograph of *M. circumcinctus* Liver Structure
Magnification: 100x (a) and 400x (b,c,d). vc=central vein, s=sinusoid, ◣red=vacuolization, ◣yellow=pyknosis, ⇨red=melanomacrophage, ⇦ blue=congestion, ⇧green=sinusoidal dilatation

The histological photomicrograph of *Rasbora* sp. liver shows a range of alterations in the tissue structure, most notably of vacuolization, which was characterized by large cells that had lost their function as a result of the altered cell structure. Mustafa (2020) state that vacuolization in the hepatic cytoplasm may be caused by the accumulation of glycogen and lipids, which indicate metabolic issues caused by hazardous substance exposure. The massive volume of erythrocytes in this hepatic sinusoids fish showed that severe congestion or blood retention in the central vein.

Leukocyte infiltration was also discovered in the hepatic histology of *Rasbora* sp. Accordingly, it is possible that the infiltration of leukocyte cells is a sign of a defense mechanism in response to pathological alterations brought on by heavy metals (Santoso et al., 2021). The presence of cellular infiltration in fish can be interpreted as a sign of an inflammatory reaction caused by the presence of toxins.



(d)

Figure 5. Photomicrograph of *Rasbora* sp Liver Structure,
Magnification: 100x (a) and 400x (b,c,d). vc=central vein, s=sinusoid, il=leukocyte infiltration, ◣red=vacuolization, ◣yellow=pyknosis, ⇦ blue=congestion, ⇧green=sinusoidal dilatation

The liver of *H. nemurus* has significant histological damage, with approximately 80% of the hepatocytes altered, many of which are vacuolated (Figures 6b and 6d). Congestion, necrosis, and pyknosis were also found in the liver tissue of *H. nemurus*. Hepatic cell death that also results in the rupture of the plasma membrane is known as necrosis. The first indicators of necrosis are hepatic inflammatory reactions such as hepatocyte edema and tissue death (Oktafa et al., 2017). Necrosis, which is likewise irreversible, is characterized by hypertrophic signs. The function and metabolism of the liver will change as the liver's structure changes.

According to research by (Younis et al., 2015), cadmium induction caused changes in the liver histology in the form of vacuolization, nuclear degeneration, congestion, and leukocyte cell infiltration in nile tilapia. The increase in cadmium levels was accompanied by an increase in vacuolization. Oxygen deficiency can also injure liver cells, causing nuclear degeneration and necrosis. Santoso et al., (2021) collected *Periophthalmodon schlosseri* fish samples from lead (Pb)-polluted waterways in 2021, congestion, vacuolization, pyknosis, and fatty degeneration were all signs of structural injury to the liver in these fish.

The histology of fish livers in rivers contaminated with mercury, cadmium, and lead was investigated by (Atifah & Harahap, 2019), who discovered vacuolization and necrosis in numerous hepatocytes. Hepatic alterations occur in fish exposed to high levels

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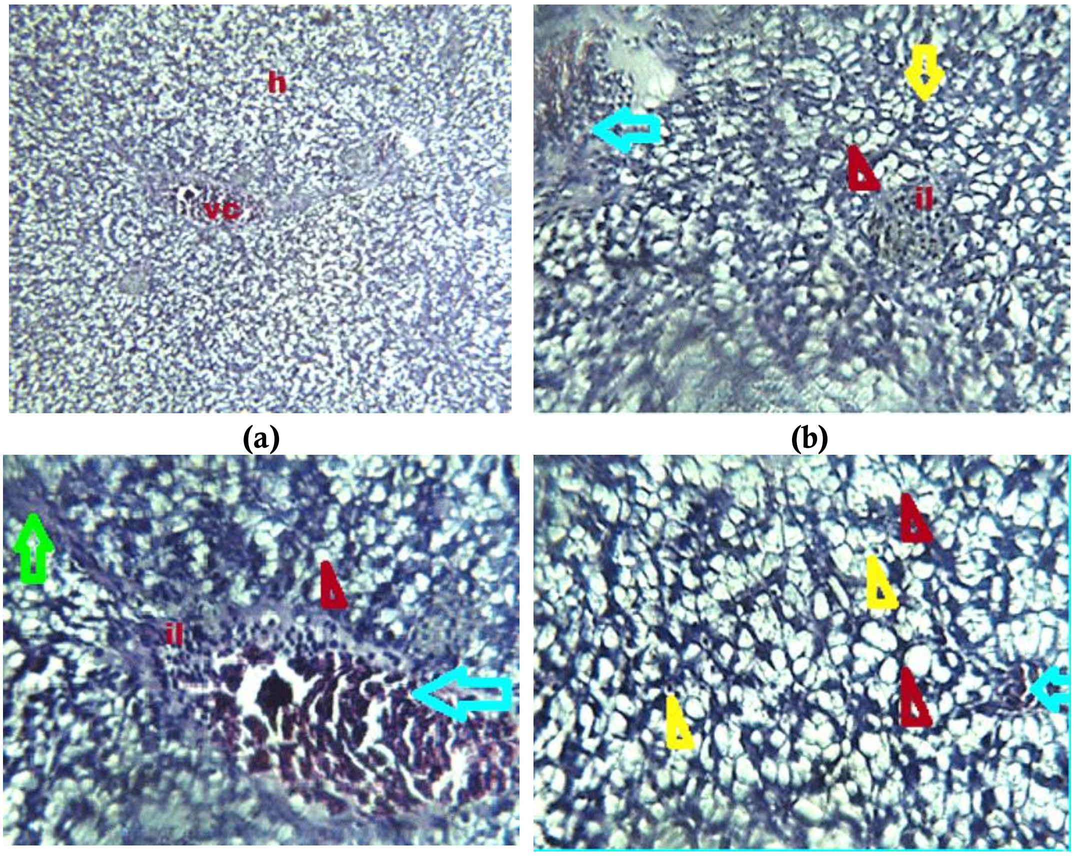
of inorganic Hg as a result of the loss of lipids stored in hepatocytes, causing cells to respond rapidly, and this state is regarded as a possible biomarker (Jasim et al., 2016). According to the histological structure of the fish, the Air Kotok River in the Lebong Regency's fish habitat environment contains harmful substances. The histological structure of fish liver organs can also change as a result of other toxins that enter the water, such as household waste.



Gambar 6. Photomicrograph of *H. nemurus* Liver Structure,
Magnification: 100x (a) and 400x (b,c,d). vc=cenral vein, s=sinusoid, il=leukocyte infiltration, ◣red=vacuolization, ◣yellow=pyknosis, ⇦ blue=congestion, ⇧green=sinusoid dilatation

Enzymatic and molecular disruptions in cells contribute to changes in the histological makeup of the liver caused by exposure to heavy metals found in water. The histological alteration detected in the livers of *H. nemurus*, *Rasbora* sp., *T. trichopterus*, and *M. circumcinctus* is one of the impacts of waste such as heavy metals from gold mining. Although these signs cannot be used as biomarkers, there is a general impairment of the histology of the hepatocyte structure in these fish due to waste. Toxins, chemicals, and other potentially dangerous materials must be avoided in the water or river.

The quantity of materials that enter the waters, such as anthropogenic household trash, mining, and other activities, can also have an impact on the physicochemical characteristics of the water. Pathology in fish organs or tissues is an indication that the presence of toxic compounds in river waters has caused damage to the fish liver structure. The toxic material has the potential to endanger fish populations in the rivers of the

Lebong gold mining zone. This fish liver histology photomicrograph data, as an environmental biomarker, can help in environmental monitoring and management.

CONCLUSION

Analyzing the histological structure of the liver of the fish *Hemibagrus nemurus*, *Rasbora* sp., *Trichogaster trichopterus*, and *Macrognathus circumcinctus* in the Air Kotok river in the gold mining area of Lebong Regency, Bengkulu Province, it was shown that there was damage to the fish liver structure, which included vacuolization, congestion, pyknosis, dilated sinusoids, melanomacrophages, and leukocyte cell infiltration. Histology alteration in fish indicates that the river water habitat of the fish is polluted by waste, one of which is heavy metals from gold mining activities that use mercury and cyanide, which can threaten the health and sustainability of fish life.

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