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Histopathological Analysis of Gills, Liver, and Kidneys of Nile Tilapia (*Oreochromis niloticus*) Affected by Feverish Lapindo Mud in Porong River, Sidoarjo-East Java

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Abstract

Direct discharge of feverish Lapindo mud resulted in abundant toxic substances in the Porong River. The impact extended beyond water quality, affecting fish in their habitat which was the Porong River. This study aimed to characterize the histopathology analysis of Nile tilapia affected by feverish mud and assessed the environmental conditions of the Porong River. Histopathological analysis revealed edema, congestion, hyperplasia, fusion, and necrosis in the gill lamellae. The kidneys exhibited degeneration, inflammation, edema, hemorrhage, and necrosis, while the liver showed hydropic degeneration, vacuolization, congestion, and necrosis. These results indicated that the toxic substances in the feverish mud affected the internal organ conditions of Nile tilapia.

INTRODUCTION

The Porong River is a floodway connected to the Brantas River, originating from Mojokerto City (Lengkong Baru Dam) and flowing into the Java Strait. It forms the boundary between the Sidoarjo Regency and the Pasuruan Regency. According to the master plan of water supply systems in Sidoarjo Regency for 2018-2037, the Porong River is used as a mixed channel, serving as both an irrigation and a drainage channel. However, since the disaster of the feverish mud eruption in the Porong Subdistrict, Sidoarjo, in 2006, the Porong River has acquired an additional function as a discharge channel for the feverish mud, resulting in an increased concentration of toxic substances polluting the river.

The presence of heavy metals in the Sidoarjo feverish mud leads to pollution that directly affects the river environment. The accumulation of these heavy metals also has repercussions on the biota that inhabit the river, influencing the ecosystem and its inhabitants (Akbar and Rahayu, 2023). This environmental contamination requires careful attention and management to mitigate its adverse effects on both the river ecosystem and the organisms that depend on

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it for their habitat. Fish are primary aquatic organisms that are capable of accumulating specific metals in large amounts, exceeding their concentrations in the aquatic ecosystem (Sukowati *et al.*, 2018). High accumulation of toxic substances, especially heavy metals, leads to abnormalities in blood cells and causes damage to gills and muscles (Shah *et al.*, 2020).

Histopathological changes have been widely used as biomarkers to evaluate the health of fish exposed to toxic pollutants (Thabet *et al.*, 2023). Histopathological analysis is extensively utilized to detect physiological changes and diagnose the health status of fish exposed to pollutants either chronically or acutely (Kaur *et al.*, 2018). Previous research has demonstrated histopathological abnormalities in the gills, kidney, liver, and muscle tissues of tilapia in waters contaminated with heavy metals. (Mahboob *et al.*, 2016; Yacoub *et al.*, 2021).

The study aimed to characterize the histopathological alterations in the gills, liver, and kidneys of Nile tilapia as potential biomarkers for assessing water quality in the Porong River, Sidoarjo. This study contributes to the field of aquatic toxicology and environmental monitoring by providing valuable insights into the impact of water pollution on fish health and developing tools for effective water quality assessment.

METHODOLOGY

Ethical Approval

No animals were harmed or improperly treated during this research, which was approved through due diligence. All procedures followed standards, ensuring no environmental or laboratory damage, in accordance with the principles of ethical approval and animal welfare.

Place and Time

This research was conducted in January- February 2024. Tilapia fish were collected from the Porong River in Sidoarjo, East Java.

Figure 1. Location of the research areas in Porong River, Sidoarjo.

Research Materials

The equipment and materials used in this research included plastic bags, cooler box, fishnet, surgical instruments, trinocular microscope (Olympus BX43), wax dispenser, microtome, a water bath, 10% Neutral Buffered Formalin (NBF), absolute ethanol (Merck), xylene (Merck), paraffin (Leica), Mayer's hematoxylin, eosin, slide glasses, and cover glasses.

Research Design

Sixteen samples of Nile tilapia (*Oreochromis niloticus*) were randomly collected from three locations (upstream, discharge point, and downstream) within a 100-meter radius from specific points along the feverish mud discharge channel into the Porong River.

Work Procedure

Sample Collection

The samples were packed in plastic bags, placed in a cooled cooler box, and transported to the laboratory for necropsy and histopathology examination.

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Histopathological Examination

Organ samples (gills, liver, and kidneys) were cut to a size of 0.5 x 0.5 cm and fixed using a 10% Neutral Buffer Formalin (NBF) solution for 24 hours. The samples underwent histopathological preparation processes (dehydration, clearing, impregnation), followed by embedding and cutting to a thickness of 5 µm. Tissue sections were stained with hematoxylin and eosin (HE) and observed under a microscope at 400x magnification.

Data Analysis

The data of this research were analyzed descriptively and presented as images. Data collection was conducted to identify the types of tissue damage in the gills, liver, and kidneys.

RESULTS AND DISCUSSIONS

Water pollution is caused, in part, by human activities that produce waste. The toxic substances present in this waste are hazardous to both human life and the aquatic environment. Heavy metals such as cadmium (Cd), nickel (Ni), mercury (Hg), copper (Cu), chromium (Cr), and lead (Pb) (Koul *et al*., 2022), natural components found in water, are present in low compositions. However, human activities indirectly increase the concentration of toxic substances affecting aquatic biota. In the aquatic ecosystem, fish are considered important indicators of environmental pollution because they are at the top of the aquatic food chain and are directly affected by toxic substances, including suspended matter found in their habitat (Authman *et al.*, 2015). Histopathology of gills can be used as a biomarker for water quality and environmental pollution levels (Wood, 2017).

The pathological changes observed in the gill tissues of Nile tilapia in the Porong River include edema, congestion, hyperplasia, fusion, and necrosis (Figure 2). Edema in the gill lamellae is caused by the infiltration of toxic substances, resulting in swelling of the irritated gill cells. The edema mechanism, as explained by Prabaningsih *et al*. (2020), occurs due to the entry of toxic substances into the gill lamellae, causing lesions in the more permeable endothelial walls and allowing fluid to easily flow into the interstitial tissue. The outflow of fluid from the interstitial tissue disrupts the normal reabsorption mechanism in the tissue, leading to edema, as the lymphatic system cannot reabsorb the fluid. Edema is also one of the physiological adaptations of fish when experiencing environmental disturbances (Aliza *et al.*, 2021).

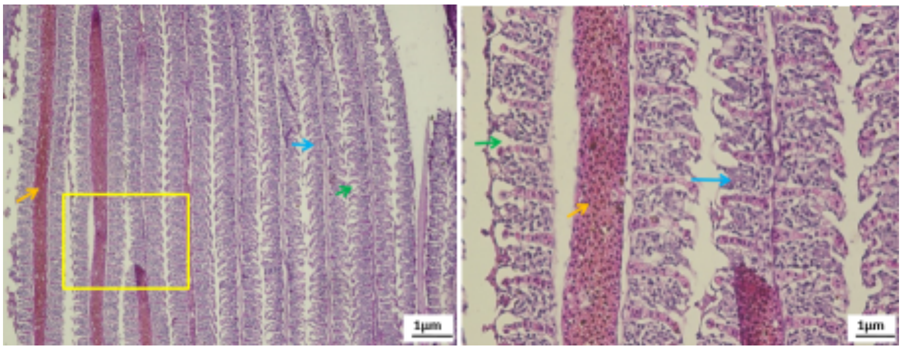

Figure 2. Tilapia gills: edema (green arrow), congestion (yellow arrow), hyperplasia (blue arrow), and necrosis (black circle).

The gills also have congestion due to disturbances in the circulatory system, suspected to be caused by heavy metals altering the biochemical structure of the cells, resulting in inflammation. If this process continues, it can hinder the oxygen carried by the blood flow, disrupting the gills' function as a respiratory organ. Abalaka (2015) explains that this condition can lead to death if the exposure occurs for

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a long time since the gills experience asphyxia, a condition where oxygen levels in the body decrease. Aliza *et al.* (2021) stated that congestion is triggered by the damaged structure of gill cells, leading to increased blood flow in the lamellae. Furthermore, the presence of heavy metals affects the permeability of the gill cell membrane, causing resistance in the ion exchange system, ultimately disrupting the transport of fluids in and out of the cells.

Fusion in the lamellae due to hyperplasia is quite severe, where the secondary lamellae will appear fused. Hyperplasia can trigger fusion in the secondary lamellae. This study reports severe damage to the gills and finds some necrosis in certain gills, indicating a high concentration of toxic substances in the water. If the exposure to toxic substances containing high levels of lead, such as this feverish mud, continues, it can cause necrosis in the fish gills. Damage to the gills, such as necrosis, fusion in the lamellae, and epithelial cell proliferation due to the exposure to heavy metals, has also been reported by Aldoghachi *et al.* (2016). Furthermore, it is explained that the gills are the main gateway for toxic substances, including lead, iron, and zinc (Vasanthi *et al.*, 2013), making it possible for significant damage to occur in these organs.

In addition to osmotic balance, the kidneys also play a role in eliminating contaminants from the body. The changes observed in the kidneys of Nile tilapia are suspected to be a result of exposure to toxic substances, leading to damage such as degeneration, inflammation, edema, hemorrhage, and necrosis. Edema in Nile tilapia is due to disturbances in blood circulation, resulting in an increase in fluid volume accompanied by the infiltration of fluid into the intercellular and extravascular spaces within tissues. Pathological changes in the kidney organs indicate contamination in the aquatic environment, making them useful as environmental bioindicators (Ortiz *et al.*, 2003).

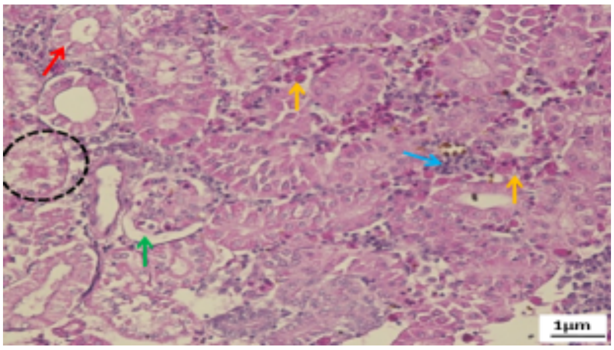

Figure 3. Kidney abnormalities: degeneration (red), hemorrhage (yellow), inflammation (blue), edema (green), and necrosis (black circle).

Hydropic degeneration (Figure 3) occurring in the kidneys of Nile tilapia may be a result of acute anoxia or exposure to toxic agents, both of which can disrupt the sodium pump's function. This condition allows water to enter cells through osmosis and accumulate in the cytoplasm, causing swelling. Histopathological studies of Nile tilapia (*O. niloticus*) living in polluted waters have been conducted by Shahid *et al.* (2022), revealing various cellular, biochemical, and histological changes as responses to contamination in their habitat. According to Robert-Nicoud *et al.* (2001), all changes in the kidneys affect their function as excretory and osmoregulatory organs, disrupting physiological processes and potentially causing death.

The liver of fish plays a role in the detoxification process, and its function as a neutralizer of toxic substances makes it highly susceptible to the influence of chemicals in its environment, potentially due to exposure to toxic agents such as

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heavy metals. The liver is a major metabolic organ vulnerable to lesion formation when exposed to water pollutants (Hossain *et al.*, 2021; Popović *et al.*, 2023). Besides hydropic degeneration, other damage observed in the liver cells of Nile tilapia includes vacuolization and congestion. This damage was also found by Mustafa (2020), where the livers of *Luciobarbus xanthopterus* fish living in an environment polluted by heavy metals exhibited vacuolization, degeneration, and inflammation.

Vacuolization in the livers of Nile tilapia may indicate disturbances in metabolism due to exposure to chemicals and other organic substances. As mentioned above, in addition to heavy metals, the feverish mud from PT. Lapindo also contains organic pollutants. This is consistent with the study by Araújo *et al.* (2019), which reported that histopathological analysis of the liver of *Pimelodus maculatus* in the Paraíba do Sul river in Brazil, which was contaminated with organic waste, showed damage such as cytoplasmic vacuolization, inflammatory infiltrates, congestion, steatosis, cytoplasmic granules, and infiltration of adipocytes.

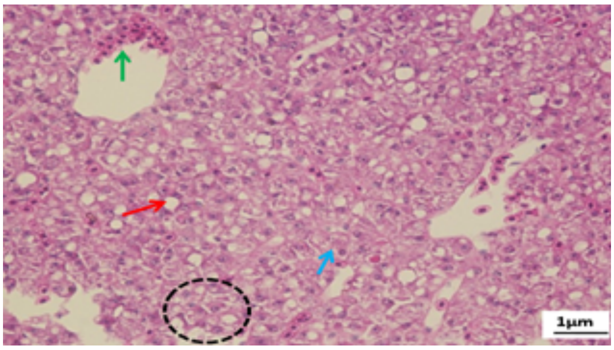

Figure 4. Liver abnormalities: degeneration (blue), congestion (green), vacuolization (red), and necrosis (black circle).

The liver tissue of Nile tilapia experienced necrosis, characterized by the loss of hepatocyte cells. Furthermore, congestion was observed in the liver, possibly caused by the high concentration of toxic substances in the Porong River (Figure 4). The accumulation of toxic substances also affects blood circulation in the liver, indicated by the accumulation of blood cells in the liver tissue, blocking venous vessels. One cause of congestion is disturbances in the liver's circulatory system, resulting from the inflammatory reaction that affects blood vessel permeability. The above results are in line with those of Savassi *et al.* (2020), who found pathological changes in the livers of fish living in heavy metal-contaminated waters, including congestion followed by the appearance of macrophages indicating phagocytosis and necrosis. Suhermanto *et al.* (2019) stated that the liver experiences histopathological changes that cause liver dysfunction so that the liver's main function of detoxifying toxins or foreign objects is disrupted, as indicated by marked degeneration of hepatocytes and congestion in the sinusoids. In addition to its vital role in metabolism, the liver also functions to filter blood and supply it to the entire body (Shahid *et al.*, 2022).

CONCLUSION

Fish are used as water quality biomarkers due to their sensitivity to pollution. Histopathological analysis can be used to describe tissues, especially organs related to the changes in environmental conditions, such as gills, liver, and kidneys. The histopathological changes found in the gills, liver, and kidneys of Nile tilapia in the Porong River are suspected to be caused by exposure to toxic substances in the water.

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CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

The contributions of each author are as follows: Meilya Suzan Triyastuti and Diah Ayu Satyari Utami collected and analyzed the data; Budi Rianto Wahidi and Achmad Suhermanto conceived and designed the experiments and contributed to the drafting of the manuscript; and Nisa Hakimah drafted and revised the manuscript.

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