

Histopathological changes in gill of Nile tilapia (*Oreochromis niloticus*) after palm oil mill effluent exposure

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Abstract. Histopathological studies have been widely used as a tool for detection and assessment the negative effects of pollutants on fish. This present study aimed to determine and analyze the histopathological changes of gills in Nile tilapia after exposed to Palm Oil Mill Effluent (POME). Toxicological/chronic concentration in each treatment was based on LC₅₀-96 hours value of POME on Nile tilapia obtained from the previous study which was 15.65 mg/L. The fish were divided into five treatment with 10 fish were used in each treatment. Treatment were design as follows: Treatment control (0% POME: 0 mg/L), Treatment A (10% of LC₅₀-96 hour value: 1.565 mg/L), Treatment B (15% of LC₅₀-96 hour value: 2.347 mg/L), and Treatment C (20% of LC₅₀-96 hours: 3.130 mg/L). The exposure period lasts for 45 days. The result shows that POME exposure has a negative impact caused histopathological changes in the gill of Nile tilapia. The gill lesion due to POME exposure observed in this study were erythrocyte infiltration, hyperplasia, epithelial lifting and lamellar fusion.

1. Introduction

Palm oil mill effluent (POME) has been considered as pollutant especially for the aquatic environment [1]. POME contains a high concentration of chemical oxygen demand (COD), biochemical oxygen demand (BOD), oil and grease which have the potential impacts on aquatic ecosystems [2, 3]. However, study to underlying the negative impacts of POME on aquatic biota is still scarce. Several previous studies revealed that contamination of POME has cause negative effect in phytoplankton diversity and disturbance reproductive performance of fish [4, 5].

Fish gills are vulnerable organs and considered to be initial indicators to assess fish health exposed to the pollutant [6]. The negative effects of various pollutants on gill performance have been widely reported previously. Mishra and Mohanti's research has indicated that cadmium exposure to *Channa punctatus* caused gill histopathology changes such as epithelial lifting and hyperplasia which resulted in decreased levels of oxygen consumption and disruption of the osmoregulation process [7]. In addition, exposure to various types of heavy metals has been also reported reducing the performance of the enzyme Na, K-ATPase on the gill of tilapia (*Oreochromis niloticus*) [8].

Histopathological studies have been widely used for detection and assessment the negative effects of pollutants on fish [9, 10]. Histologically, the changes that occur in fish organs due to pollutants exposure tend to be easy to identify and can be served quickly [11]. Histopathology changes in gill due to different pollutants exposure have been reported previously by many authors including sugarcane vinasse, endosulfan, diesel oil and mercury [12, 13, 14, 15]. However, the histopathological impact of POME



on fish gills is largely unknown. Therefore, the aims of the present study were to determine and analyze the histopathological changes of gills in Nile tilapia after exposed to POME.

2. Materials and Methods

2.1 Fish and chemical exposure

Nile tilapia with a weight range from 9-10 g and a total length range from 7-9 cm were collected from Fish Hatchery Center Batee Iliak, Bireuen, Indonesia and transferred in the oxygenated container to the Aquaculture Laboratory, Almuslim University using land transportation. Before the toxicological test, fish were acclimatized to laboratory conditions (DO, 5.2 mgO₂/L; temperature, 28.5 °C dan pH, 7.1) for one week and fed twice a day. All procedures of fish test handling in this study refer to international standards of animal welfare [16]. A total of 30 liters of raw POME were obtained from the Syaikat Sejahtera palm oil mill factory (Bireuen, Indonesia) and diluted into the required concentration for toxicological test. Fish exposure media were 50x30x40 cm glass aquaria containing 25 liters of water. The water was taken from urban tap water and aerated for 24 hours before being used.

Toxicological/chronic concentration in each treatment was based on LC₅₀-96 hours value of POME on Nile tilapia obtained from the previous study which was 15.65 mg/L [17]. The fish were divided into five treatment with 10 fish were used in each treatment. Treatment were design as follows: Treatment control (0% POME: 0 mg/L), Treatment A (10% of LC₅₀-96 hour value: 1.565 mg/L), Treatment B (15% of LC₅₀-96 hour value: 2.347 mg/L), and Treatment C (20% of LC₅₀-96 hours: 3.130 mg/L). The exposure period lasts for 45 days. Fish were fed twice a day with commercial food and water was completely changed every 15 days.

2.2 Histological examination

After the exposure period was over, the test fish were removed from the tank and sacrificed with anesthetic overdosed using clove oil. Fish gills were dissected by using surgical instruments and preserved with Buffered Neutral Formalin (BNF) for 24 h. The method for making the histological section of gill was based on Humason's method [18]. Dehydration process was done by soaking the tissues in graded alcohols from 80%, 90%, 95% to absolute alcohol. Gill tissues were sectioning using a microtome to obtain a section of 5 µm in size.

The sectioning tissue was floated in warm water (40°C), then placed in a glass object. Object glass was heated on the hotplate for 10-15 minutes until all the water evaporated. The stained process was done by immersing the slides with Hematoxylin for seven minutes followed by counter stained with eosin for three minutes. Micrographs were generated using a microscope equipped with a digital camera. Histological appearance of gills in each treatment was reported in qualitative and semiquantitative evaluation. Qualitatively, the observations were carried out on morphological abnormalities that occurred, while semiquantitatively was carried out through scaling/scoring methods referring to the intensity of alteration: 1 (none), (2) mild, (3) moderate, and (4) severe [19].

3. Results and Discussion

Gills are multifunctional organs that play an important role to perform respiration, osmoregulation, acid-base balance and nitrogenous waste excretion [20]. Gill alteration induced by pollutants can result in hypoxia, respiratory failure and disrupt the osmoregulation function which impacted lower growth and death. In normal fish, gill consists of several primary lamellae where one primary lamella is composed of several secondary lamellae. The gill epithelium consist of two or three layers located on the basal membrane, the secondary lamellae show normal arrangement with large spaces between them [15]. During the exposure period, there were no histopathology changes observed in the treatment control (Table 1). The gill structure of Nile tilapia in the treatment control is exhibited in Figure 1A.

Table 1. Frequency of gill lesion of Nile tilapia after POME exposure for 45 days

Lession	Treatment			
	Control	A (1.565 mg/L)	B (2.347 mg/L)	C (3.130 mg/L)
Erythrocyte infiltration	-	+	+	++
Hyperplasia	-	-	++	+++
Epithelial lifting	-	+	++	++
Lamellar fusion	-	-	+	+++

None (-), mild (+), moderate (++), and severe (+++)

Most common gill lesion due to POME exposure observed in this study were erythrocyte infiltration, hyperplasia, epithelial lifting and lamellar fusion (Figures 1B, 1C and 1D). Similar finding with this result was documented by many author in several other related studies. According to Malat [21] most common gill alteration that occurs due to various pollutants exposure are hyperplasia, hypertrophy, rupture of gill tissues, lamellar fusion, hypersecretion, a proliferation of mucous cells, alteration in chloride cells, and vascularization. However, the prevalence of different type of gill lesion observed in this study was still lower when compared with the fish exposed to chromium and diesel oil [7, 14].

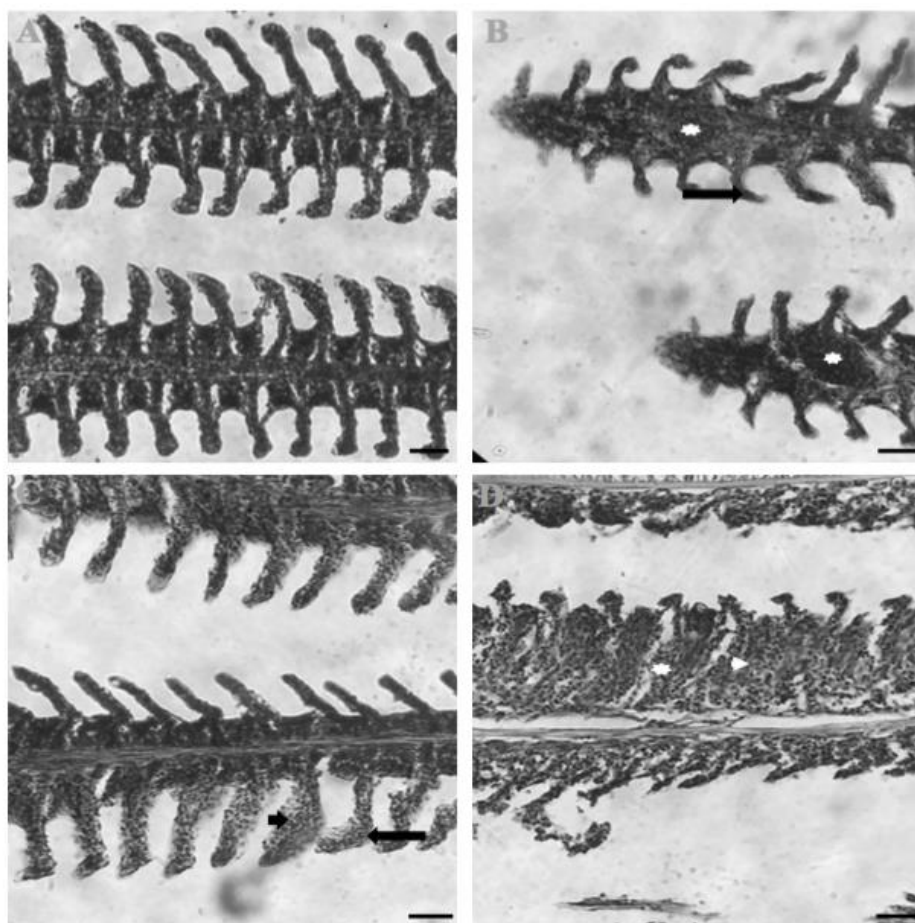


Figure 1. Histological alterations observed in gill tissue. (A) Control (B), Erythrocyte infiltration (star) and epithelial lifting (long black arrow) (C), Hyperplasia (short black arrow) and epithelial lifting (long black arrow) (D) Lamellar fusion (white arrow). Bar: 50 μm, magnification: x 100

In general, the gills of exposed fish with higher doses of POME exhibited the severe histological alteration. The dominant gill lesion observed in the highest dose treatment was hyperplasia and lamellar fusion. Hyperplasia occurred as a response to the pollutant in order to reduce the contaminant absorption into the bloodstream. The occurrence of secondary lamellar fusion results in impaired function of the secondary lamellar in respiration process. As a result, fish encounter hypoxia, stimulates the organism to bind red blood cells and stimulates hematocrit and hemoglobin to improve the mechanism of oxygen adaptation [22].

Erythrocyte infiltration was characterized by accumulation of very dense red blood cells in blood vessels induced by disruption of capillary blood flow [23]. On other hand, a similar finding was also observed in Great sturgeon (*Huso huso*) gills exposed to diesel oil [14]. Epithelial lifting is a gill response to pollutant infiltration through increased epithelial mucosal cell production when severe edema occurs [22]. The mucous contains sugar and protein which serves to inhibit pollutants entry the fish's body. However, excessive mucous secretion can inhibit gill function.

4. Conclusion

The result confirms that POME exposure has a negative impact caused histopathological changes in the gill of Nile tilapia. The gill lesion due to POME exposure observed in this study were erythrocyte infiltration, hyperplasia, epithelial lifting and lamellar fusion. Further research is needed to assess the impact of POME exposure on the performance of fish's respiratory physiology.

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