

Histology-Devi

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Histology Test of Tilapia (*Oreochromis niloticus*) on Adsorbent Performance Results in Liquid Treatment of Industry Pulp and Paper Waste

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Abstract

Pulp and paper industry fly ash is categorized as B3 waste, namely hazardous and toxic waste. Liquid waste produced by the pulp and paper industry contains pollutants that are toxic and can cause pathological and histopathological changes in important tissues such as the gills of fish that live in the waters around the disposal. The purpose of this study was to analyze the histology of tilapia on the performance results of fly ash adsorbents in the management of liquid waste in the pulp and paper industry. The research method has 6 treatments, namely the ratio of fly ash to pulp and paper liquid waste (20 g: 1 L) namely P0: 100 % well water control, P1: lowest liquid waste 5 % + 9.5 % well water, P2: 5 % liquid waste fly ash adsorbent + 95 % well water, P3: 6.25 % liquid waste fly ash adsorbent + 93.75 % well water, P4: 7.5 % liquid waste fly ash adsorbent + 92.5 L of well water, P5: 8.75 % fly ash adsorbent + 91.25 % well water, P6: 10 % fly ash adsorbent + 9 % well water. Water quality measurements such as temperature, NH₃, pH and DO were carried out. Observation of gill histology used microtechnical method which includes deparaffinization and histological staining (HE). Analysis was carried out on changes in cell morphology in gill tissue. The results showed that the morphology of gill cells in liquid waste pulp and paper 5 % found gill damage in the form of congestion, secondary lamella fusion, and secondary filament rupture and goblet proliferation occurred in the treatment of wastewater treatment while Fly ash adsorbent 5 % damaged gills in the form of congestion and fusion of secondary lamellae. From the results of the Fly ash functions can reduce damage to body organs in fish, especially on the gills so that it can reduce the number of deaths in fish compared to treatment without the use of fly ash.

Keywords: Fly ash, pulp, histopathology, *Oreochromis niloticus*

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1. Introduction

The high demand for paper makes the pulp and paper industry in Indonesia growing. However, like two sides of a coin that cannot be separated, the positive impacts of industrial development are also followed by negative impacts on the environment and human health due to the generation of waste. Waste is a by product of the production process that cannot be used in the form of solid, liquid, gas, dust, vibration and other damage that can cause pollution if not managed properly [1]. The increasing growth of the paper industry has an impact on increasing environmental problems caused by B3 pollution (Hazardous and Toxic Materials). Several cases of environmental pollution caused by industrially produced B3 have become

hot topics in the mass media. Such as pollution of the OKI paper factory in South Sumatra which has an impact on the emergence of skin diseases that attack the surrounding population [2]. The source of solid B3 in the pulp and paper industry comes from the chemical recovery process that requires stabilization before stockpiling. Other sources of waste are paper machinery, boiler blow down and paper maturation processes that produce toxic residues. Fly ash is ash from combustion in boilers and is in the form of fine particles, slightly gray in color, has a low carbon content and is phenolic [3]. After the residue is treated, a toxic sludge concentrate is produced. B3 for the environment is not very good for the health of the general public and living things in the environment. B3 produced by industries is very detrimental to the surrounding environment, if it is not processed properly, especially solid B3 which is commonly

found around our environment [4]. In general, environmental pollution caused by the pulp and paper industry includes: 1) killing fish, shellfish and other aquatic invertebrates, 2) entering carcinogenic chemicals and substances that interfere with hormone activity into the environment, 3) consuming millions of liters of fresh water, 4) causing environmental pollution. The risk of exposure to the community by the discharge of hazardous chemicals from industrial waste that pollutes the environment [5]. Tilapia (*Oreochromis niloticus*) is one of the fish species that is very likely to be contaminated with paper mill wastewater. Several ecobiological characteristics of this fish such as its wide distribution in the aquatic environment, available at various stadia throughout the season and easily acclimatized to laboratory conditions make tilapia very suitable to be used as test animals in [6] toxicity study. Previous research has shown that the concentration of acute toxicity of paper mill effluent is on Tilapia [7]. This study aims to determine the effectiveness of Fly ash adsorbent in wastewater and to describe the histological changes that occur in the gills of Tilapia (*Oreochromis niloticus*) due to exposure to pulp and paper wastewater.

2. Materials and Methods

The method used is the experimental method, the ratio of fly ash with pulp and paper liquid waste (20 g; 1 L) is the best running result at the time of the adsorbent test, namely.

Kn: 100% well water control,

P1: Lowest liquid waste 5 % + 9.5 % well water,

P2: 5 % adsorbent fly ash + 9.5 % well water,

P3: 6.25 % adsorbent fly ash + 93.75 % well water,

P4: 7.5 % adsorbent fly ash + 92.5 L of well water,

P5: 8.75 % fly ash adsorbent + 91.25 % well water,

P6: 10 % fly ash adsorbent + 90 % well water.

The aquarium with a size of (30 x 20 x 25) cm³ is then filled with 10 L of water and installed with aeration. Each aquarium was filled with 10 test fish. The maintenance period begins with the acclimatization process of the fish to the new environment for seven days. The test fish were given artificial feed in the form of floating pellets by ad satiation at 07.00, 12.00 and 16.00 WIB. During the study, the water was not replaced (static). Observations on mortality and measured water quality parameters including temperature, pH, dissolved oxygen (DO) and ammonia were carried out at the beginning and end of the study.

The technique of making preparations for histology of tilapia gill tissue by taking three fish from each treatment after the fish showed clinical symptoms, namely the

test fish swam to the surface, slowed movement and opened overculum. Then the gill organs were cut into thin slices of thickness. 0.5 cm. Fish gills after being cut were fixed in 10 % formalin solution for 24 hours, after that, dehydration is carried out, namely: Dehydration begins by inserting the sample into a series of ascending alcohol bottles starting from 70 %, 80 %, 90 % and absolute alcohol for 1 hour each. The sample was put into xylol-alcohol (1:1) for 1 hour and put into pure xylol 1 and xylol 2 for 1 hour each. Paraffin infiltration, where the sample was put into a mixture of xylol paraffin (1:1) for 1 hour. Then the sample was put into pure paraffin 1 and pure paraffin 2 for 1 hour each. The entire infiltration process was carried out in an oven at a temperature of 60 °C. The samples were planted in paraffin blocks and allowed to freeze, then attached to the holder/wood block. Prior to cutting, paraffin blocks are placed on ice pads so that they freeze quickly and solidify and do not break during cutting. Samples were cut using a 5-6 m thick microtome. To make the sample expand/not shrink, the paraffin tape containing the sample is placed in a water bath with a temperature of 45 °C and after it has expanded, it is taken and attached to a glass object that has been smeared with new entellan. The samples were then dried in an oven at 45 °C for at least 24 hours. Staining the sample with hematoxylin eosin, with the following procedure (sample was put into a solution of xylol 1 and xylol 2 for 2 minutes each and then rehydrated in descending alcohol series, namely from absolute alcohol, 90 %, 80 %, 70 %, 35 % each for 2 minutes and washed with sufficient running water. Then the sample was immersed in a solution of Hematoxylin for 5 minutes, then washed with running water until clean. The sample was soaked with Eosin solution for 2 minutes, then the sample was washed with running water until the excess Eosin solution was washed off. Then the sample was immersed in ascending series alcohol, i.e. from 35 %, 70 %, 80 %, 90 % alcohol and absolute alcohol for 20 seconds each and then inserted into xylol 1 and xylol 2 then closed (mounting). Mounting is done by Cover the sample with a cover slip glued with New Entellan. The sample was dripped with new entellan covered with a cover slip carefully so as not to form bubbles, then dried in an oven at 45 °C. The dried sample was observed under an Olympus CX21 microscope, when the tissue was clearly visible at a low magnification of 100x then photographed using a digital camera.

3. Results and Discussion

During the observation Tilapia histology test the water quality parameters were measured. The results of water quality testing are temperature, pH, NH₃, and DO. The data on the results of water quality measurements are presented in Table 1.

Table 1. Water quality during Histology Test

Treatment	Parameter			
	Temperature(°C)	pH	NH ₃ (mg/L)	DO (mg/L)
Control	28	7,02	0,01	5,12
P1: Lowest liquid waste 5 % + 9.5 % well water,	31	8,12	2,52	2,48
P2: 5 % adsorbent fly ash + 9.5 % well water		6,89	0,5	5,45
P3: 6.25 % adsorbent fly ash + 93.75 % well water,	29	7,12	0,8	5,35
P4: 7.5 % adsorbent fly ash + 92.5 L of well water,	28	7,36	1,2	5,25
P5: 8.75 % fly ash adsorbent + 91.25 % well water,	29	7,38	1,3	5,35
P6: 10 % fly ash adsorbent + 90 % well water.	28	7,42	1,5	5,4

Based on Table 1, the results of water quality testing in wastewater during the study were control at 0 %, namely temperature 28 °C, pH 7.02 NH₃ 0,018 mg/L, and DO 5.5 mg/L. In the 5 % treatment, the results obtained water quality, namely, temperature 29 °C, pH 8.12 NH₃ 2.52 mg/L, and DO 2.48 mg/L. The results of water quality in the Fly ash adsorbent treatment obtained the following results, 5 % treatment, temperature 29 °C, pH 6.89 NH₃ 0.5 mg/L and DO 5.45 mg/L. Treatment was 6.25 %, temperature 29 °C, pH 7.12 NH₃ 0.8 mg/L and DO 5.35 mg/L. Treatment of 7.5 % resulted in temperature 28 °C, pH 7.36 NH₃ 1.2 mg/L, and DO 5.25 mg/L. Treatment of 8.75 % showed that the temperature was 29 °C, pH 7.38 NH₃ 1.3 mg/L, and DO 5.35 mg/L. Treatment of 10 % obtained water quality results, namely temperature 28 °C, pH 7.42 NH₃ 1.5 mg/L, and DO 5.4 mg/L.

The results of the water quality temperature in the wastewater treatment ranged from 28 to 29 °C while the fly ash adsorbent treatment reached 29-31 °C. The difference in temperature between the wastewater treatment and fly ash adsorbent is thought to be due to water temperature conditions. Each treatment had different physiological conditions of fish in the presence of liquid waste and also fly ash adsorbent. Higher temperatures occur in the treatment of liquid waste, presumably due to the entry of liquid waste content into the fish's body, resulting in an increase in temperature which causes the fish to die. According to [8] temperature has a universal influence in regulating natural processes in waters, because it affects biotic and abiotic components.

Pulp and paper mill liquid waste has a pH level of 7-9, the use of Fly ash adsorbent in liquid waste shows a decrease in the pH level of liquid waste in the toxicity test, which is 5-7, this is presumably because Fly ash adsorbent can reduce the alkaline pH level of wastewater to neutral. In accordance with the results of [9], stated that the decrease in the pH value was caused by the large number of H⁺ ions fighting over the adsorption site on the surface of the adsorbent with cations in methylene blue. Meanwhile, according to [5] stated that Fly ash contains minerals such as CaO which functions as an alkaline compound to form

an alkaline atmosphere so that it can increase the pH of the water. According to the [10] concerning Liquid Waste Quality Standards for Activities that are already operating, the maximum pH value of pulp and paper wastewater is 6-9 in this study the pH value obtained in the treatment of liquid waste and Fly ash adsorbents still within the maximum threshold of the quality standard. According to [6], the pH value of waters can fluctuate because it is influenced by photosynthetic activity, respiration of aquatic organisms, temperature and the presence of ions in these waters. The lowest oxygen content occurs at 5 % liquid waste concentration, which is 2.48 mg/L, which is 1.5 mg/L. While the highest oxygen content is at a concentration of 5 % Fly ash, which is 5.45 mg/L. According to [11], oxygen in water should not be less than 3 mg/L. Waters that are exposed to pollutants, the supply of oxygen from the air is very slow so that there is little oxygen in the water.

The value of NH₃ in wastewater treatment with a concentration of 5 % was 2.52 mg/L and in the Fly ash adsorbent treatment with a concentration of 10 % it was obtained 1.5 mg/L. Based on Table 1, the use of Fly ash adsorbent can reduce the ammonia value of wastewater. This is also supported by the results of research conducted by [9] which states that the use of Fly ash adsorbents can reduce ammonia levels in rubber liquid waste by 90-97 % this is because Fly ash is a porous solid consisting mostly of free carbon elements and each is covalently bonded. Fly ash surface is non-polar, in addition to composition and polarity, pore structure is also an important factor to consider. The pore structure is related to the surface area, the smaller the pores of the fly ash resulting in a larger surface area so that the adsorption ability increases.

The results of DO water quality in this study were obtained varied in liquid waste, the lowest DO value was obtained in 10% liquid waste treatment, namely 1.31 mg/L and in all wastewater treatments the DO value obtained was below the environmental quality standard according to [12], namely 3 mg/L. The low DO value is thought to be due to the presence of contaminants contained in pulp and paper liquid waste and the existence of oxygen competition between fish that experience changes in

adjusting the body to the existing waste so that the oxygen contained in the aquarium is decreasing. According to [13] the difference in DO values produced in waters can be caused by the influence of temperature and pressure values above the waters. According to [14], the higher the temperature, the lower the oxygen content of the water. According to the results of research by [3] states that the batik industry wastewater can reduce oxygen levels in the water, causing a lack of oxygen for gift tilapia seeds which can interfere with physiological processes in the body, and if it continues continuously it can cause the death of the fish seeds.

Histology of Tilapia gills

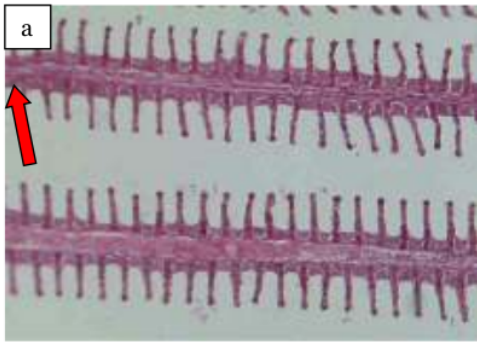
Gills are respiratory organs in fish that are directly related to water, so if the water is contaminated with hazardous materials it can cause damage to the gills [15]. Histological test of tilapia gills was carried out on live tilapia and treated with 5 % liquid waste and Fly ash adsorbent treatment (Table 2). Testing of tilapia gill samples was carried out at the Palembang Fish Quarantine Center Laboratory Station 1.

Table 2. Gill Histology Test Results Tilapia

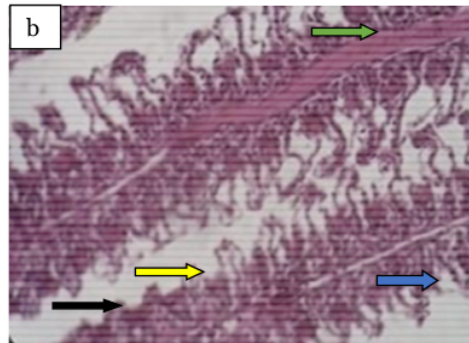
Treat- ment		Test results
Control	I	Normal Secondary Lamela
	II	Normal Secondary Lamela
	III	Normal Secondary Lamela
P1	I	Congestion, Secondary Filament Rupture, Secondary Lamellar Fusion and Goblet Proliferation
	II	Congestion, Secondary Filament Rupture, Secondary Lamellar Fusion and Goblet Proliferation
	III	Congestion, Secondary Lamellar Fusion and Goblet Proliferation
P2	I	Congestion
	II	Congestion, Secondary Lamellar Fusion
	III	Secondary Lamellar Fusion
P3	I	Congestion, Secondary Lamella Fusion
	II	Congestion, Secondary Lamella Fusion
	III	Congestion, Secondary Lamella Fusion
P4	I	Congestion, Secondary Filament Rupture
	II	Congestion, Secondary Filament Rupture
	III	Congestion, Secondary Lamella Function, Secondary Filament Rupture
P5	I	Congestion, Secondary Lamellar Fusion, Secondary Filament Rupture
	II	Congestion, Secondary Filament Rupture
	III	Congestion, Secondary Filament Rupture
P5	I	Goblet Cell Congestion and Proliferation
	II	Congestion, Secondary Lamella Fusion Goblet Cell Proliferation
	III	Congestion, Secondary Lamella Fusion and Goblet Cell Proliferation

Based on table 2 the results of histological tests on the gills, namely in control fish, the results of normal secondary gill lamellae were obtained, for the treatment of wastewater with a concentration of 5 % the results were congestion, secondary lamellae fusion and goblet proliferation. The treatment of Fly ash adsorbent obtained is that 5 % treatment occurs congestion, secondary lamella fusion, 6.25 % treatment occurs congestion, secondary la-

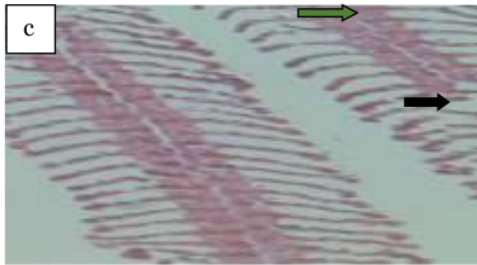
mella fusion, 7.5 % treatment occurs congestion, secondary filament rupture, 8.75 % treatment occurs congestion, filament rupture secondary and at 10 % treatment there was congestion, fusion of secondary lamellae and goblet cell proliferation. For a histological picture of the gills under a microscope for each treatment, it can be seen in the image below as follows:



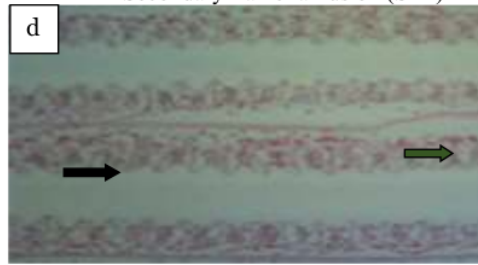
Histology of Tilapia Gill Control Treatment (0 %) with HE Staining with Cross Section. HE Staining, 100x Magnification, 5 µm Thickness:
 → Normal Secondary Lamellar (NSL)



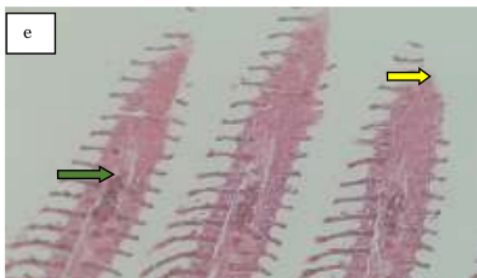
Histology of Tilapia Gills Treatment of 5 % Liquid Waste with HE Staining with Cross Section. HE Staining, 100x Magnification, 5 µm. Thickness:
 → Secondary Filament Rupture (SFR)
 → Concession (C)
 → Goblet Cell Proliferation (GCP)
 → Secondary Lamella Fusion (SLF)



Histology of Tilapia Gills treated with 5 % Adsorben Fly ash, with HE staining with Cross Section. HE Staining, 100x Magnification, 5 µm. Thickness:
 → Concession (C)
 → Secondary Lamella Fusion (SLF)



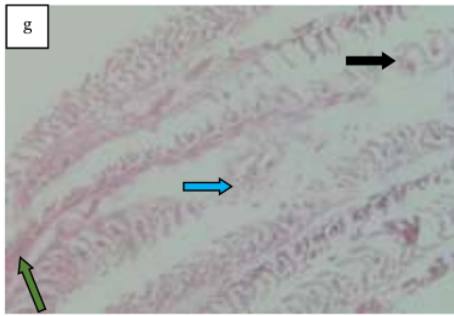
Histology of Tilapia Gills treated with 6.5 % Adsorben Fly ash, with HE staining with Cross Section. HE Staining, 100x Magnification, 5 µm. Thickness:
 → Concession (C)
 → Secondary Lamella Fusion (SLF)



Histology of Tilapia Gills Treated with 7.5 % Adsorbent with HE Staining with Cross Section. HE Staining, 100x Magnification, 5 µm Thickness:
 → Secondary Filament Rupture (SFR)
 → Concession (C)



Histology of Tilapia Gills 8.75 % Adsorbent Treatment with HE Staining with Cross Section. HE Staining, 100x Magnification, 5 µm Thickness:
 → Secondary Filament Rupture (SFR)
 → Concession (C)



Histology of Tilapia Gills Treated with 10 % Flyash Adsorbent with HE Staining with Cross Section. HE Staining, 100x Magnification, 5 μ m Thickness:




-  Concession (C)
-  Goblet Cell Proliferation (GCP)
-  Secondary Lamella Fusion (SLF)

Figure 2. Gill organs of Tilapia (*Oreochromis niloticus*) in Treatment A: Control, B: P1: Liquid Waste, P2: Fly ash adsorbent 5 %, P3: Fly ash adsorbent 6.25 %, P4: Fly ash adsorbent 7.5 %, P5: 8.75 % fly ash adsorbent and P6: 10 % fly ash adsorbent

Observation of gill tissue histology

Gills are the main respiratory organs that work by surface diffusion mechanisms of respiratory gases (oxygen and carbon dioxide) [16]. From the results of this study indicate that the observation of the gill tissue under a microscope each treatment showed differences. Normal gills can be seen in treatment A (control), in this treatment the fish are not contaminated by the compounds present in the waste so that the fish can breathe normally. Gill histology testing was carried out after the fish were exposed to liquid waste and Fly ash adsorbent for 96 hours in the LC50 test. Gills are respiratory organs in fish that are directly related to water, so if the water is contaminated with hazardous materials it can cause damage to the gills [5]. Histological testing in this study was carried out directly on the gills of fish exposed to liquid waste and Fly ash adsorbents because the gills are organs that are in direct contact with wastewater.

Observation under a microscope shows that the gill organs exposed to organophosphate group waste experience congestion (a soft structure, fresh red in color, has a large surface and is the main place for the respiration process), secondary filamentous rupture (a condition where the tissue swells or enlarges due to increased cell size), Secondary Lamella Fusion (bleeding) and Goble Proliferation (a condition in which the tissue swells due to the increasing number of cells).

Gill damage in the form of congestion occurred in all treatments, both in the treatment of liquid waste and Fly ash adsorbents. Congestion that occurs in the gills in the form of damage to the blood vessels in the gills which can cause blood vessels to burst. According to [16] con-

gestion is an increase in the volume of blood in the blood vessels, resulting in swelling of the blood capillaries. According to [17] stated that congestion at the most severe level will cause blood vessels to burst or exit the cardiovascular circulation (arteries, veins and capillaries), which in turn will cause cell death or necrosis caused by trauma, biological agents (viruses, bacteria, fungi and parasites), chemical agents or the occurrence of interference with the supply of blood in a particular area. Gill damage in the form of secondary lamella fusion in this study can be seen in Figure 2 there is a buildup of mucus in the gill lamella due to hyperplasia of the gills, resulting in accumulation of one lamella with another. According to [18], fusion is a condition where the efficiency of gas diffusion is reduced due to hyperplasia of the fish gill epithelial tissue and fused secondary lamellae. Lamella fusion is a severe level of damage because lamella fusion is an advanced stage of damage from hyperplasia. Fusion of secondary lamellae causes the lamellae to function incompletely because the lacunae contain red blood cells covered by pathological secondary lamellae epithelial cells [19]. According to [20] Lamella fusion occurs due to hyperplasia of lamellae cells that continuously fill the spaces between secondary lamellae with new cells, causing attachment between secondary lamellae. Cell proliferation is the process of developing new cells after necrosis of the gills, the proliferation process can be disrupted due to unfavorable environmental conditions and cause pathological damage to the gills. Excessive proliferation causes cell division, especially in cells that are able to divide rapidly, becoming uncontrolled, resulting in hyperplasia which will also cause secondary lamella fusion [21]. The number of cell deaths that have limited proliferative properties in gill lamellae can worsen

respiratory function in gills [18]. [12] Stated that there are several levels of damage to the gills associated with toxicity. Grade I, there is edema of the lamellae and detachment of epithelial cells from the underlying tissue. Grade II, there is hyperplasia of the proximal basal lamella secondary. Grade III, hyperplasia causes the fusion of the two secondary lamellae. Grade IV, almost all secondary lamellae are hyperplastic. Grade V, loss of secondary lamellae structure and filamentous damage. By observing the histological damage to the gills of tilapia on fly ash adsorbents, it can be concluded that the level of damage to the gills includes first and third degree damage, while in pulp and paper liquid waste the damage is first, second, third and fourth. The higher the concentration of pollutants, the damage to the gill organs will increase. The gill damage that occurs is thought to be due to the presence of heavy metals in the form of lead and cd in the pulp and paper liquid waste, according to [22], stating that congestion can occur due to an increase in the amount of blood and vasodilation of blood vessels caused by the reaction. Inflammation after changes in cell biochemical structure by Pb (lead).

4. Conclusion

The gill damage that occurs is thought to be due to the presence of contaminants in the form of pb and cd present in the 5 % pulp and paper liquid waste found gill damage in the form of congestion, secondary lamella fusion, secondary filament rupture and goblet proliferation occurred in the treatment of wastewater treatment while Fly ash adsorbent 5 % gill damage in the form of congestion and fusion of secondary lamellae. From the results of the Fly ash functions can reduce damage to body organs in fish, especially on the gills so that it can reduce the number of deaths in fish compared to treatment without the use of fly ash.

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