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NILE TILAPIA (*OREOCHROMIS NILOTICUS*) HEALTH ASSESSMENT AS THE INDICATOR OF Pb CONTAMINATION IN SELOREJO RESERVOIR, INDONESIA

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ABSTRACT

Selorejo reservoir is polluted by heavy metals, whose levels exceed quality standards of Hg (0.03 mg/l), Pb (0.0015 mg/l), Cd (0.0018 mg/l) and Phenol (0.009 mg/l) (Perum Jasa Tirta, 2019) and it is feared that such condition will undermine Nile Tilapia's health. This study aimed to conduct an analysis of Tilapia's health status as the indicator of the environmental assessment of Selorejo Reservoir. Additionally, the histology of kidneys, livers, gills and histology of Tilapia were used to measure the impact of environmental contamination. This research was conducted from March to April 2021 using survey method. 36 fish samples were taken randomly for each sampling. Water quality parameters such as temperature, pH, and dissolved oxygen were in accordance with the water quality standards, while the results of histological analysis on the kidneys, liver and gills showed that 50-75% of those organs suffered from mild to moderate damage such as necrotic, congestion, cell degeneration and hypertrophy. The results of hematological and micronucleus analysis showed that Tilapia fish from Selorejo Reservoir were unhealthy as a result of exposure to heavy metal Pb of 0.03-0.08 mg/l. Histological and hematological analyses in this research showed that Tilapia from Selorejo Reservoir were unhealthy, so there is a need for better environmental management and programs for monitoring hazardous materials in the ecosystem.

KEY WORDS: Selorejo reservoir, Tilapia, Hematology, Histology, Water pollution, SEM

INTRODUCTION

Selorejo Reservoir is a reservoir used for tourism and traditional fishing using nets. The fish found in Selorejo Reservoir generally are local fish from rivers that enter the reservoir such as red rasbora, Tilapia and black Tilapia. The Malang Regency Fishery Department periodically supplies the reservoir with several species of fish fries (one of which is Tilapia). Fishing activities by the local communities living around Selorejo Reservoir prove that tilapia fish is high in demand.

Selorejo Reservoir was polluted in the aftermath of the eruption of Mount Kelud which brought eruptive materials into bodies of water such as rivers flowing into dams, including the Selorejo Reservoir. The eruptive materials in the form of volcanic ash contain Lead (Pb) and Mercury (Hg). Data from water quality report of Selorejo Reservoir from Perum Jasa Tirta 1 in 2019 showed heavy metal levels of Hg (0.03 mg/l), Pb (0.0015 mg/l), Cd of (0.0018 mg/l) and Phenol of (0.009 mg/l). In 2020, the levels of heavy metals showed an increase due to human activities and industrial waste that was brought by the rivers into the reservoir.

The entry of heavy metal pollutants into the waters can potentially reduce water quality and fish well-being, especially in the form of hematological conditions and damage to the histological structure of the fish organs. Heavy metals can affect red blood cells, decrease hemoglobin synthesis and inhibit heme synthesis which causes symptoms of anemia. Gills, kidneys and livers are most susceptible to pollutants, since these organs functions as filters, and most of biochemical metabolisms are conducted by in the liver (Sari, *et al.*, 2017). Furthermore, Alturiqi and Albedair, (2012) found the accumulation of heavy metals lead in the fish meat as much as .01–10.49 and 10.02–15.43 µg/g in meat and meat products, respectively. However, the levels of lead were higher than the standard permissible levels, 0.4 mg/kg (EC, 2001) and0.5 mg/kg (FAO, 1983). Assumption of collateral impact of heavy metals transfer to human could be greater since Tilapia is one of the most consumable fish commodities in Selorejo Reservoir area.

The purpose of this study was to conduct analysis of Tilapia health status as the indicator of environment assessment of Selorejo Reservoir. In addition, histological analyses of the kidney, livers, gills and hetalogy of Tilapia were used to measure the impact of environmental contamination.

MATERIALS AND METHOD

Procedures of Fish Sampling

This research used non-experimental random sampling. Tilapia samples were obtained from fishermen in Selorejo Reservoir. The average size of fish sample was 14.8 ± 20.9 cm, taken from Selorejo reservoir with three replicates every two weeks in February - March 2021, and the total number of samples was 108 fish. Organs and blood preservation steps were conducted in Selorejo Reservoir. Fish organs were preserved with 4% formalin solution, and fish blood was preserved in a coolbox filled with ice gel.

Tilapia Histology

Histological analysis of Tilapia was carried out through 11 stages. The first stage was tissue preparation by performing surgery to remove the fish's kidneys. The second stage was fixation or preservation by immersing the tissue in Boynce's fluid for 24 hours. The third was the washing stage, conducted by immersing the sample into 70% alcohol for 2 x 15 minutes. The fourth stage was dehydration stage, conducted by gradually immersing the organs into 70%, 75%, 80%, 85% and 96% alcohol solutions for 1x5 minutes each. The fifth stage was clearing, conducted by immersing the organs into Xylen fluid twice for 15 minutes. The next stage was the immersion stage, done by compacting the organs using paraffin. The seventh stage was casting, by pouring a little liquid paraffin to the edge so it wouldn't leak. The next stage was the tissue cutting stage. The ninth stage was tissue staining by inserting a glass slide containing the tissue into Xylene Solution I for 10 minutes. Next was the gluing or mounting stage and the last stage was labeling the preparation and observing under a microscope.

Water Quality Measurement

Water physical parameters were temperature and chemical parameters including pH and dissolved oxygen (DO). Water temperature and dissolved oxygen were measured using DO Meter (Lutron PDO-519), and water pH was measured using pH paper. Water quality measurements were carried out in three replicates and the instruments were calibrated prior to usage.

Data analysis

For data analysis, this study used descriptive analysis method to describe the relationship between the dependent variable (fish histology) and the independent variable (the heavy metal lead in water that exceeded the quality standard level).

RESULTS AND DISCUSSION

Tilapia Organs Histology

The percentage of damaged fish kidneys, livers and gills was around 50%≤P<75; this indicates that the organ structure was in a moderately damaged condition. Histological changes commonly experienced by Tilapia in Selorejo Reservoir were cell degeneration, hypertrophy, congestion and necrosis.

Kidney

Microscopic observations showed changes in the tissue structure. The damages were in the form of necrosis, degeneration, and congestion. Tissue damage in the kidneys of Tilapia (*Oreochromis niloticus*) are presented in Table 1. Necrosis damage at the first station was recorded at 70.1% and the second station was 30.4%. Necrosis is a damage that occurs due to premature death of tissue cells which is characterized by shrinking, destruction, and loss of the cell nucleus (Wagiman, *et al.*, 2014). The results of histological observations of tilapia kidney tissue showed necrotic damage which was indicated by symptoms of loss of tissue structure which can be seen in (Fig. 1.A).

Table 1. Tissue damage in the kidneys of Tilapia (*Oreochromis niloticus*)

Location	Damage			Average (%)	Kidney Damage Status
	Necrosis	Cell degeneration	Congestion		
Poin 1	70.1	60.2	64.4	64.9	Medium
Poin 2	30.4	34.4	45.9	36.9	Mild

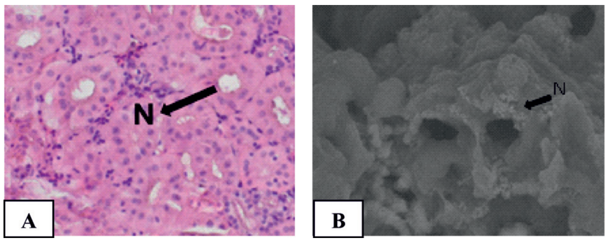

Fig. 1. Tilapia's (*Oreochromis niloticus*) necrotic kidney tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

Damage in the form of cell degeneration was recorded at 60.2% at the first station, and 34.4% at the second station. According to Maftuch *et al.* (2018), degeneration is characterized by swelling of the tubular epithelium which can then lead to necrosis. Damage to cell degeneration in the kidneys of Tilapia shown by the presence of empty spaces marked by swelling of the tubular epithelium is shown in (Fig. 2.A).

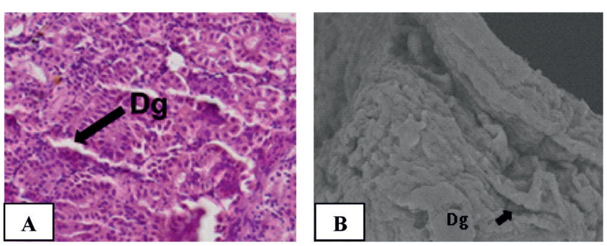

Fig. 2. Cell degeneration of Tilapia's (*Oreochromis niloticus*) kidney tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

The last damage is congestion, which was recorded at 64.4% at the first station and 45.9% at the second station. Congestion or blockage of Congestion damage in the kidneys of Tilapia is shown in (Fig. 3.A).

Kidney histological studies using electron microscopy (SEM) showed necrotizing damage characterized by cell damage and rupture (Fig. 1.B). Fish sample treatment showed damage in the form of cell degeneration which was marked by swelling of the tubular epithelial cells (Fig. 2.B). Damage in

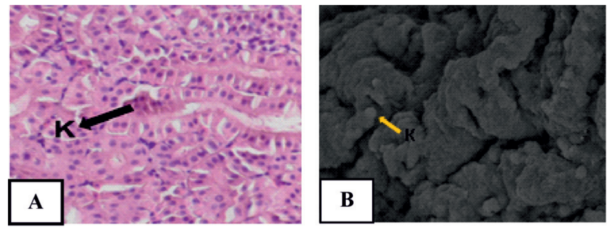

Fig. 3. Tilapia's (*Oreochromis niloticus*) congested kidney tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

the form of congestion is characterized by swelling of the kidneys due to obstruction of blood vessels (Fig. 3.B).

Liver

Miscroscopic observation of the revealed changes in the structure of liver tissue damage in the form of in necrosis, hypertropy and congestion. Tissue damage in the livers of Tilapia (*Oreochromis niloticus*) are shown in in Table 2. Damage in the form of necrosis was recorded at 76.9% at the first station and 35.8% at the second station. The results of histological observations of tilapia liver tissue showed necrotic damage indicated by the gradual loss of several cell parts (Fig. 4.A).

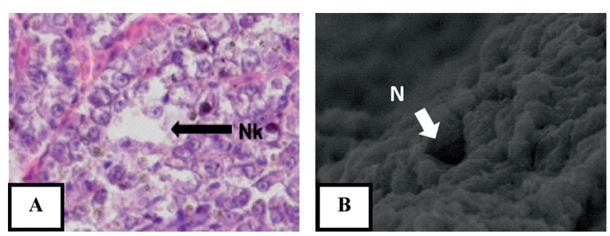

Fig. 4. Necrosis of Tilapia's (*Oreochromis niloticus*) liver tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

Hypertrophic damage to the liver of Tilapia was recorded at 61.5% at the first station and 32.1% at the second stationHypertrophy is a tissue damage which is characterized by an increase in organ size due to an increase in cell size so that cells separate from one another (Fig. 5.A).

Table 2. Tissue damage in the livers of Tilapia (*Oreochromis niloticus)*

Location	Damage			Average (%)	Liver Damage Status
	Necrosis	Hipertrophy	Congestion		
Poin 1	76.9	61.5	63.9	67.4	Medium
Poin 2	35.8	32.1	45.0	37.6	Mild

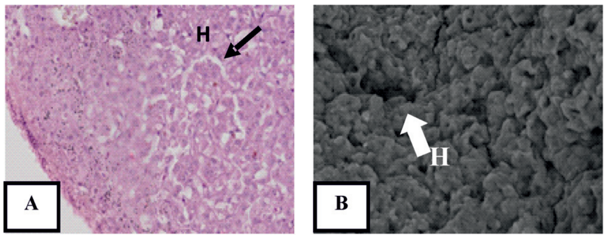

Fig. 5. Tilapia's (*Oreochromis niloticus*) hypertrophic liver tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

The next damage is congestion, which was recorded ar 63.9% at the first station and 45.0% at the second station. Histological observations on congested organs characterized by an increase in blood in the blood vessels is shown in Fig. 6.A.

Histological studies of the liver using electron microscopy (SEM), showed hypertrophic damage, congestion, and necrosis. Necrotic damage is characterized by cell death and pyknosis, namely shrinkage of the cell nucleus, characterized by crushed and fragmented nuclei (Fig. 4.B). Hypertrophic damage is characterized by cell damage due to swelling so that cells disintegrate (Figure 5.B). Damage in the form of congestion is characterized by swelling of the vessels due to increased blood (Fig. 6.B).

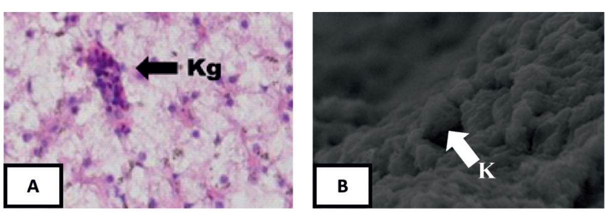

Fig. 6. Tilapia's (*Oreochromis niloticus*) congested liver tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

Gills

Results of microscopic observations showed changes in the structure of the gill tissue. Tissue damage in the gillss of Tilapia (*Oreochromis niloticus*) are shown in Table 3. The damage experienced were secondary lamellae, hyperplasia, and necrosis. Damage to the secondary lamellae was recorded at 74.6% at the first station and 31.4% at the second station.

Secondary lamellae are damages characterized by swelling of cells caused by the entry of lead into the gills or excessive accumulation of fluid in body tissues which is marked by the basement membrane starting to stretch off, the lacunae cells narrowing, causing the gills to experience deficiency of function and difficulty in the respiratory and and disturbed metabolic processes. Histological observation of the liver of Tilapia (*Oreochromis niloticus*) secondary lamella damage is shown in Fig. 7.A.

The next damage is hyperplasia, whose percentage reached 60.8% at the first station and 31.9% at the second station. Hyperplasia is the formation of excessive tissue due to an increase in the number of cells. Lamellar hyperplasia results in

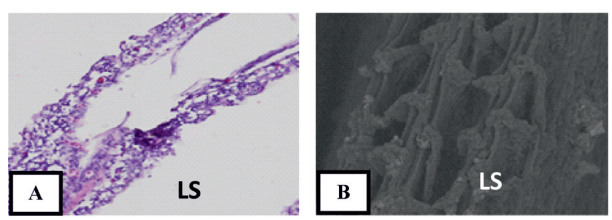

Fig. 7. Tilapia's (*Oreochromis niloticus*) gill tissue secondary lamella, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

Table 3. Tissue damage in the gills of Tilapia (*Oreochromis niloticus*)

Location	Damage			Average (%)	Gill Damage Status
	Secondary lamella	Hyperplasia	Necrosis		
Poin 1	74.6	60.8	60.9	65.4	Medium
Poin 2	31.4	31.9	40.1	34.4	Mild

thickening of the epithelial tissue at the end of the filament or thickening of the epithelial tissue located near the base of the lamella (Siregar, *et al.,* 2019). Histological observation of the gills of Tilapia (*Oreochromis niloticus*) showed the presence of hyperplasia, which is presented in Fig. 8.

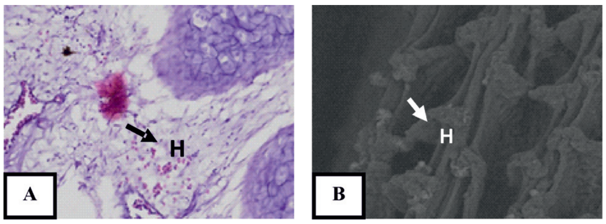

Fig. 8. Tilapia's (*Oreochromis niloticus*) gill tissue hyperplasia, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

The last kind of damage to the gills is a necrosis. At the first station it was found that 60.9% percent of the gills were damaged, while the damaged gills at the second station amounted to 40.1%. Necrosis is the death of a cell that occurs due to toxic compounds that enter the body. These compounds undermine the cells' ability to carry out metabolic processes so that energy is not formed for survival. Histological observation of the gills of Tilapia (*Oreochromis niloticus*) found necrosis, which is shown in Fig. 9.A.

Histological studies of gill organs using electron microscopy (SEM) showed damages in the form of secondary lamellae damage, hyperplasia, and necrosis. The first signs of pathology include edema of the epithelial cells in the gills. This is due to the epithelial layers of the secondary lamellae being lifted up in a continuous sheet of the pillar cell system, thereby, increasing the diffusion distance between cells (Fig. 7.B). Epithelial hypertrophy and hyperplasia and chloride cells with partial or complete fusion of the lamellae also occurred in this study (Fig. 8.B) as well as necrosis (Fig. 9.B) or cell loss in the gills leading to cell's death.

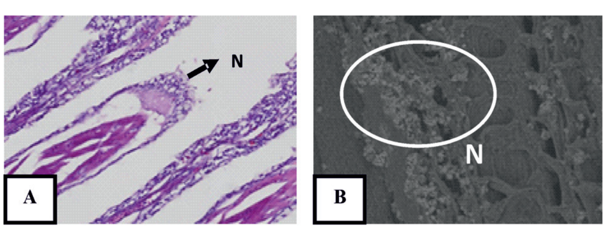

Fig. 9. Tilapia's (*Oreochromis niloticus*) necrotic gill tissue, through histological microscopic test (**A**), through SEM test (**B**) (Research documentation, 2021)

Both Tilapia research locations showed mild to moderate damage of kidney and liver; these conditions occur because the heavy metals that accumulated in the fish organs also exceeded the quality standard, especially heavy metal Pb at the first station because this location got input from the Konto River which is heavily polluted.

This study indicates that Tilapia has been exposed to water pollution which is thought to be the result of industrial waste, household waste or pesticides residues brought by rivers entering the reservoir which eventually accumulated at the reservoir. Claims on water pollution in both locations is supported by quantitative data from the histological condition of the kidneys, liver and gills in the form of the number and percentage of tissue damage. The results of this study can confirm that histopathological changes are good indicators of water quality.

Hematology

Hematological analyses conducted in this study were erythrocytes, leukocytes, hemoglobin, hematocrit and micronuclei. The average number of erythrocytes (cells/mm^3) of Tilapia (*Oreochromis niloticus*) at both stations showed the highest results at the second station, (1,220,556 cells/mm^3), and the lowest average of erythrocytes in fish was recorded at the first station (984,444 cells/mm^3) (Table 4).

The average leukocyte at both stations showed the highest yield at the first station (155.703 cells/mm^3) and the lowest average leukocyte count was recorded at the second station (118,044 cells/mm^3).

Hemoglobin levels (g%) at both stations showed the highest results at the second station, (7 g%) and the lowest at the first station in the second week (5 g%).

The average hematocrit value (g%) obtained at the second station was 18%, while the lowest average hematocrit value obtained at the first station was 14%. The fish samples at the first station had a hematocrit value of <15% which means it was not normal. Based on the statement of Royan *et al.* (2014), normal hematocrit in Tilapia ranges from 15 - 35.8% and a value <15% indicates a deficiency of erythrocytes.

The average number of micronuclei at both stations showed the highest results at the first station (33 cells/1000) and the lowest number of micronuclei was recorded at the second station (19 cells/1000). Ibrahim *et al*. (2019) state that the number of micronuclei indicating a healthy fish is

Table 4. Hematology of Tilapia (*Oreochromis niloticus*)

Location	Hematology				
	Erythrocytes (sel/mm³)	Leukocytes (sel/mm³)	Hemoglobin (g%)	Hematocrit (%)	Micronuclei (sel/1000)
Poin 1	984.444	155.703	5	14	33
Poin 2	1.220.556	118.044	7	18	19

<30 cells/1000.

Water Quality Analysis

In this study, temperature, pH, dissolved oxygen (DO), phenol and heavy metals (Pb, Cd, Hg) were measured. The temperature value in the Selorejo Reservoir ranges from 27.2 – 28.1°C. The temperature value in the Selorejo Reservoir is still classified as a decent temperature for a living medium for Tilapia.

The pH value in the reservoir is constant, namely at a value of 7. The pH value in the Selorejo Reservoir is still classified as a decent pH for living medium for Tilapia. A good pH range for the growth of Tilapia is 6.5 - 8.5. Water conditions that are very acidic or alkaline will endanger the survival of organisms because it will cause metabolic and respiratory disorders.

The value of dissolved oxygen in the Selorejo Reservoir ranges from 7.5 to 8.2 mg/L. The value of dissolved oxygen in the Selorejo Reservoir is still classified as decent oxygen for a living medium for Tilapia. The optimal dissolved oxygen level for the growth of Tilapia is more than 5 mg/L (Gusrina, 2008).

Phenol values in Selorejo Reservoir ranged from 0.0019 to 0.0057 mg/l and were quite high as a natural living medium and exceeded the water quality standard criteria for level III, namely 0.001. According to Hudori and Yulianto (2011), phenolic compounds in waters that have a value of more than 0.002 mg/l can cause poisoning effects on fish and biota that become their food.

Based on Government Regulationn Number 82 of 2001, Pb quality standard in water is 0.03 mg/l. The Pb value in the Selorejo reservoir is high, ranging from 0.03-0.08 mg/l. Exposure to Pb that exceeds the quality standard will cause adverse effects on the blood which include the ability of Pb to inhibit calcium and interact with hemoglobin protein. Pb can potentially bind biological molecules, causing some physiological function disorders (Yedjou *et al*., 2010).

The Cd value in the Selorejo reservoir exceeds the quality standard (0.01 mg/l), which is in the range of 0.027-0.028 mg/l. Agricultural activities by farmers around the Selorejo Reservoir still rely on pesticides. Cd toxicity can potentially cause damage to red blood cells through oxidative stress (ROS) mechanisms. This oxidative stress causes single-stranded DNA damage and interferes with the synthesis of nucleic acids and proteins.

The Hg value in the Selorejo reservoir also exceeds the quality standard (0.002 mg/l), which is in the range of 0.0027-0.0032 mg/l. Hg can enter aquatic systems from natural and anthropogenic sources, including cigarette industry or domestic waste, use of pesticides and inorganic fertilizers around reservoirs. Based on research by Leaner *et al*. (2007), fish exposed to heavy metal Hg with high concentrations will cause changes in histology (liver, kidney, gills) and hematology (red blood cells, white blood cells or plasma, hemoglobin, hematocrit).

Pollution Status

Pollution status in Selorejo Reservoir is determined by the pollution index method with water parameters whose value exceeds the quality standard based on government regulations (PP) no. 82 of 2001, the results of the analysis of the pollution index values from each location in the study is shown in Table 5.

According to Walukow (2010), the dominant factors affecting water quality include settlements, agriculture, industry, erosions and natural factors such as soil content around water bodies. Although the results of the analysis of water quality at each station are evenly distributed for each class, at several stations a score is obtained which indicates the condition of the worst water quality. Based on the pollution index, the highest score was recorded at the first station (heavy metal Pb). This is possible because the location at the first station is close to the input from the Konto River which carries Pb from activities around the river, while the second station is far from the input from the river.

Table 5. Value of Pollution Index in Selorejo Reservoir

Location	Heavy metals	Pollution Index	Quality Standart	Category
Poin 1	Fenol	3,96	1.0 < IP ≤ 5,0	Lightly polluted
	Pb	10,61	IP >10	heavily polluted
	Cd	2,62	1.0 < IP ≤ 5,0	Lightly polluted
	Hg	1,13	1.0 < IP ≤ 5,1	Lightly polluted
Poin 2	Fenol	1,32	1.0 < IP ≤ 5,2	Lightly polluted
	Pb	7,78	5.0 < IP ≤ 10	Moderately polluted
	Cd	1,91	1.0 < IP ≤ 5,2	Lightly polluted
	Hg	0,95	0 ≤ IP ≤ 1,0	According to quality standards

The cause a high index of heavy metal pollutant in water are generally domestic urban wastes, urban stormwater, residential liquid waste (sewage), mining activities, industrial waste (industrial wastes), agricultural wastes (agricultural wastes) and aquaculture waste.

CONCLUSION

The well-being of Tilapia in Selorejo Reservoir based on this study was categorized as unhealthy, judging from the results of histology and hematology as well as water quality obtained during the study (temperature, pH and DO) which were still in the normal range˙while the values of phenol, Cd, Hg and Pb tend to be high with pollution status from mild to heavily polluted status.

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REFERENCES

Alturiqi, A. S. and Albedair, L. A. 2012. Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. *The Egyptian Journal of Aquatic Research.* 38(1): 45-49.

Chaudhary, M., Mishra, S. and Kumar, A. 2017. Estimation of water pollution and probability of health risk due to imbalanced nutrients in River Ganga, India. *International Journal of River Basin Management.* 15(1): 53-60.

da Cruz, A. L., Prado, T. M., da Silva Maciel, L. A. dan Couto, R. D. 2015. Environmental effects on the gills and blood of Oreochromis niloticus exposed to rivers of Bahia, Brazil. *Ecotoxicology and Environmental Safety.* 111: 23-31.

EC. 2001. Commission Regulation No. 466/2001 of 8 March 2001, Official journal of European communities 1.77/1.

FAO. 1983. Compilation of legal limits for hazardous substance in fish and fishery products (Food and agricultural organization). FAO fishery circular No. 464, Rome. 5-100.

Fitriawan, F., Sutarno, S. and Sunarto, S. 2011. Microanatomy alteration of gills and kidneys in freshwater mussel (Anodonta woodiana) due to cadmium exposure. *Nusantara Bioscience.* 3(1): 28-35.

Gomez Isaza, D. F., Cramp, R. L. and Franklin, C. E. 2020. Simultaneous exposure to nitrate and low pH reduces the blood oxygen-carrying capacity and functional performance of a freshwater fish. *Conservation physiology.* 8(1): 1-15.

Gusrina. 2008. Budidaya Ikan Untuk SMK. Direktorat Pembinaan Sekolah Menengah Kejuruan, Jakarta. 499

Hamed, S. B., Guardiola, F., Cuesta, A., Martínez, S., Martínez-Sánchez, M. J., Pérez-Sirvent, C. and Esteban, M. Á. 2017. Head kidney, liver and skin histopathology and gene expression in gilthead seabream (*Sparus aurata* L.) exposed to highly polluted marine sediments from Portman Bay (Spain). *Chemosphere.* 174: 563-571.

Hardi, E. H., Harris, E. and Lusiastuti, A. M. 2012. Karakteristik dan patogenisitas streptococcus agalactiae tipe-hemolitik dan non-hemolitik pada Ikan Nila. *Jurnal Veteriner.* 12(2): 167-178.

Hudori, H. and Yulianto, A. 2011. Penurunan fenol melalui proses adsorptive micellar flocculation. *Jurnal Sains & Teknologi Lingkungan.* 3(1): 66-72.

Ibrahim, A. T. A., AbouelFadl, K. Y. and Osman, A. G. 2019. Ultraviolet A-induced hematotoxic and genotoxic potential in Nile tilapia Oreochromis niloticus. *Photochemical & Photobiological Sciences.* 18(6): 1495-1502.

Khairuman, A. and Amri, K. 2005. Budi Daya Ikan Nila Secara Intensif. AgroMedia, Jakarta.

Leaner, V. D., Donninger, H. and Birrer, M. J. 2007. Transcription factors as targets for cancer therapy: AP-1 a potential therapeutic target. *Current Cancer Therapy Reviews.* 3(1): 1-6.

Lesmana, D. S. 2005. Kualitas Air untuk Ikan Hias Air Tawar. Penebar Swadaya, Jakarta.

Maftuch, M., Sanoesi, E., Farichin, I., Saputra, B. A., Ramdhani, L., Hidayati, S., Fitriyah, N. and Prihanto, A. A. 2018. Histopathology of gill, muscle, intestine, kidney, and liver on Myxobolus sp.-infected Koi carp (Cyprinus carpio). *Journal of parasitic diseases.* 42(1): 137-143.

Osman, A. G., Reheem, A. E. B. M. A. E., Moustafa, M. A., Mahmoud, U. M., Abuel-Fadl, K. Y. and Kloas, W. 2011. In situ evaluation of the genotoxic potential of the river Nile: I. Micronucleus and nuclear lesion tests of erythrocytes of Oreochromis niloticus (Linnaeus, 1758) and Clarias gariepinus (Burchell, 1822). *Toxicological & Environmental Chemistry.* 93(5): 1002-1017.

Ramesh, M., Anitha, S., Poopal, R. K. and Shobana, C. 2018. Evaluation of acute and sublethal effects of chloroquine (C18H26CIN3) on certain enzymological and histopathological biomarker responses of a freshwater fish Cyprinus carpio. *Toxicology reports.* 5: 18-27.

Royan, F., Rejeki, S. dan Haditomo, A. H. C. 2014. Pengaruh salinitas yang berbeda terhadap profil darah Ikan Nila (Oreochromis niloticus). *Journal of aquaculture management and technology.* 3(2): 109-117.

Sari, W., Okavia, I. W., Ceriana, R., dan Sunarti, S. 2016. Struktur mikroskopis hati Ikan Seurukan (Osteochilus vittatus) dari Sungai Krueng Sabee Kabupaten Aceh Jaya yang tercemar limbah penggilingan bijih emas. BIOTIK: *Jurnal Ilmiah Biologi Teknologi dan Kependidikan.* 4(1): 33-40.

Sayekti, R. W., Yuliani, E., Bisri, M., Juwono, P. T., Prasetyorini, L., Sonia, F. dan Putri, A. P. 2015. Studi evaluasi kualitas dan status trofik air Waduk Selorejo akibat erupsi Gunung Kelud untuk budidaya perikanan. *Jurnal Teknik Pengairan: Journal of Water Resources Engineering.* 6(1): 133-145.

Siregar, A. S., Alfiyah, S., Prayogo, N. A. and Sulistyo, I. 2018. Effects of Leads Pb on Gill Microanatomy Structure of Hard-Lipped Barb Fish Osteochilus hasselti. *Omni-Akuatika.* 14(2): 116-122.

Sukarni, S., Maftuch, M. and Nursyam, H. 2012. Kajian penggunaan ciprofloxacin terhadap histologi insang dan hati Ikan Botia (Botia macracanthus, bleeker) yang diinfeksi bakteri Aeromonas hydrophila. *The Journal of Experimental Life Science.* 2(1): 6-12.

Suprapto, H., Arief, M., Ermawati, L., Hakim, H. Z. and Hidayati, N. 2019. Toxicity and severe stress of lead (Pb) to hematology responses of Java Barb (Barbonymus gonionotus). *International Journal of Fisheries and Aquatic Studies.* 7(6): 26-30.

Wagiman, W., Yusfiati, Y. and Elvyra, R. 2014. Struktur ginjal Ikan Selais (Ompok hypopthalmus Bleeker, 1846) Di Perairan Sungai Siak Kota Pekanbaru. JOM FMIPA. 1(2): 1-9.

Walukow, A. F. 2010. Penentuan status mutu air dengan metode storet didanau sentani jayapura propinsi papua. *Berita Biologi.* 10(3): 277-281.

Wang, S. and Shi, X. 2001. Molecular mechanisms of metal toxicity and carcinogenesis. *Molecular and cellular biochemistry.* 222(1): 3-9.

Wolf, J. C., Baumgartner, W. A., Blazer, V. S., Camus, A. C., Engelhardt, J. A., Fournie, J. W., Frasca, S., Groman, D. B., Kent, M. L., Khoo, L. H., Law, J. M., Lombardini, E. D., Ruehl-Fehlert, C., Segner, H. E., Smith, S. A., Spitsbergen, J. M., Weber, K. and Wolfe, M. J. 2014. Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies: a guide for investigators, authors, reviewers, and readers. *Toxicologic Pathology.* 20: 1-29.

Yedjou, C. G., Milner, J. N., Howard, C. B. and Tchounwou, P. B. 2010. Basic apoptotic mechanisms of lead toxicity in human leukemia (HL-60) cells. *International journal of environmental research and public health.* 7(5): 2008-2017.