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THE IMPACT OF LEAD (PB) BIOACCUMULATION ON THE HISTOPATHOLOGICAL CONDITIONS OF THE LIVER, KIDNEY AND MUSCLE OF TILAPIA (*OREOCHROMIS NILOTICUS*) FROM THE FLOATING NET CAGE AT SAGULING RESERVOIR IN BANDUNG REGENCY WEST JAVA

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KeyWords
Bioaccumulation, Histopathology, Tilapia, Lead, Saguling Reservoir.

ABSTRACT

This research aimed to analyze the impact of Pb heavy metals on histopathological conditions of the liver, kidneys, and muscle of tilapia. The method used in this research was a survey method with the object used was Tilapia (Oreochromis niloticus) from Saguling Reservoir and the results of cultivation in the Center for Freshwater Aquaculture Fisheries (BBPBAT) Sukabumi. Data from observations were analyzed in a comparative descriptive manner. The results showed the levels of Pb heavy metals that accumulated in organs at each station were very diverse, and most of them had exceeded the quality standards based on National Standardization Agency of Indonesia 7387: 2009. Pb content in the liver ranged from 0.345 to 0.577 ppm, kidneys from 0.276 to 1.513 ppm and muscles from 0.063 to 0.331 ppm, while the concentration of Pb in water showed a value of <0.05 ppm at each station. Histopathological observations made, in general, test fish have experienced histopathological changes in liver, kidney and muscle tissue. Liver damage that occurs is with a diagnosis of hepatic necrosis, a kidney with a diagnosis of nephrosis and nephritis, while a muscle with a diagnosis of myopathy.

INTRODUCTION

The problem of environmental pollution, especially the problem of water pollution, receives the most attention from the government, because water is an important element for living things and life. In line with the increase of industrialization, the concentration of heavy metal elements in the waters has also increased, making it possible to achieve levels of toxic concentrations for the life of aquatic organisms.

Based on research conducted by the Department of Environmental Engineering, Bandung Institute of Technology (Ministry of Environment in 2003), Saguling Reservoir has been contaminated with heavy metals worse when compared to Cirata and Jatiluhur Reservoirs. Based on research by Nandang et al. (2008) Pb concentrations in tilapia from floating net cages in cirata with a range of 0.003-0.065 ppm. This is due to the Saguling Reservoir being the first place of deposition of the Citarum River. Almost all activities, such as large industries, small industries, agriculture, dump their waste into the Citarum River basin, which will ultimately lead to the Saguling Reservoir, thus allowing the reservoir to experience very large pollution. According to Regional Environmental Management Agency (2013), there is heavy metal lead pollution (Pb) which has exceeded the quality standard found in citarum river which empties into the Saguling Reservoir, by 0.33 ppm.

Polluted waters will experience a decrease in quality, which causes the carrying capacity of these waters to decline against aquatic organisms that live in them. The problem of water pollution has biological, physical or chemical effects. Biological effects can be seen from the mass death of fish or in the form of structural and functional abnormalities towards abnormal (Wikiandy et al. 2013).

Fish that live in bodies of water that have been polluted by industrial waste for a long time will experience structural or functional abnormalities, will also experience changes in histological conditions (Wikiandy et al. 2013). Gills are organs of respiration in fish that are directly related to water, so that if the water is contaminated with harmful substances it can cause damage to the gills. The highest accumulation of heavy metals (chromium) is usually found in the liver (detoxified) and kidney (excretion) (Suyanto et al. 2010).

The Environmental Protection Agency (EPA) recommends that tilapia (Oreochromis niloticus) is one animal that can be used as a test animal. Many of these fish live freely in the waters of Saguling Reservoir and some are cultivated in the floating net cages. The accumulation of heavy metals in the organs of tilapia will result in the concentration of heavy metals Pb will increase, and cause damage to the organs of tilapia and can eventually lead to death in fish. Anatomic damage in tilapia due to pollution can be reviewed with histopathological studies. Histopathological analysis can be used as a biological marker commonly called a biomarker. The use of histopathological biomarkers can be used in monitoring the environment by observing organs that have an important function in the body's metabolism so that it can be used as an initial diagnosis of health problems in an organism (Tugiyono et al. 2009).

Based on the description above, research is needed on the impact of Pb bioaccumulation on the histopathological conditions of the liver, kidneys and muscle of tilapia (Oreochromis niloticus) from floating net cage in Saguling Reservoir.

MATERIALS AND METHODS

Research Tools and Materials

There were 3 types of materials used. Materials used in the field included ice cubes, labels, fixative solutions (formalin buffer), tilapia with a size of ± 200 gr. Materials for heavy metal testing include deionized water, HCL 37%, HNO3 65%, NH4H2PO4, lead standard solution (Pb). Materials for the preparation of histopathological preparations included fixative fish organs, formalin buffer, alcohol, paraffin, aquades, xylol, eosin, hematoxylin, and entelan.

Research Method

The method used in this research was the survey method. Fish samples were taken from 3 (three) Saguling Reservoir water stations and comparative fish were taken from existing aquaculture ponds in Sukabumi. Station 1 is located in Maroko, station 2 in Ciminyak, and station 3 in Rabian (Figure 1).

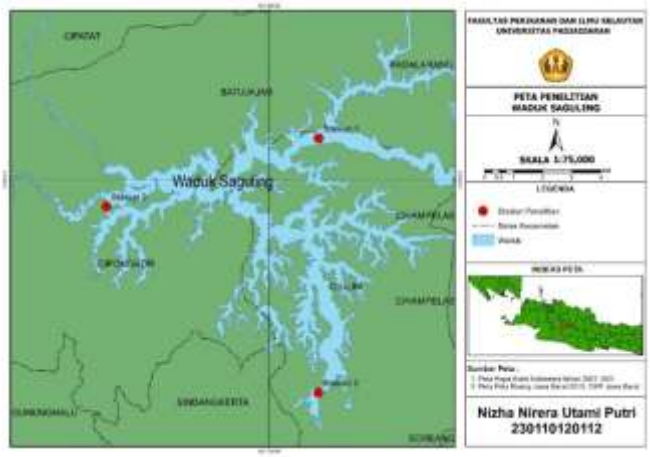

Figure 1. Map of Research Location

Data Analysis

The results of water quality observations and histopathological preparations of the liver, kidneys, and muscle of tilapia in Saguling Reservoir were analyzed in a comparative descriptive manner.

RESULTS AND DISCUSSION

Content of Pb Heavy Metal

Pb is a nonessential metal whose existence in the body of living things can be said to be unexpected. The presence of Pb in the body often replaces essential metals in enzyme activity and has the effect of inhibiting the action of enzymes (Palar 2004).

Content of Pb Heavy Metal in the Water

The results of laboratory analysis on water samples showed that the Pb concentration values at each observation station and Sukabumi BBPBAT aquaculture pond were the same, i.e. less than 0.05 ppm (Table 1). This can be caused by the heavy metals present in the waters absorbed into the body of aquatic biota, it was tilapia, and sedimentation in sediments. In accordance with the statement of Cloutier et al. (2003), heavy metals that are absorbed in water particles will be deposited on the surface of sediments and aquatic organisms will absorb it and transfer it through the food chain. High and low concentrations of heavy metals are caused by the amount of heavy metal waste input into the waters. The greater the waste that enters into a water, the greater the concentration of heavy metals in water. Heavy metals have properties that are easy to bind and settle at the bottom of the water and unite with sediments, therefore heavy metal content in sediments is higher than in sea water (Zainuri et al., 2011).

Table 1. Measurement Result of Lead (Pb) in the Water

Observation	Lead Concentration (ppm)				
	Station 1	Station 2	Station 3	BBPBAT Sukabumi	Quality Standard*
1	<0.05	<0.05	<0.05	(tt)	0.03
2	<0.05	<0.05	<0.05	(tt)	

*Information: Quality standard based on Government Regulation of the Republic of Indonesia No. 82 of 2001

Content of Pb Heavy Metal in Tilapia Organs

Pb concentration at station 1, showed in the kidney organ of 0.672 ppm, in the liver of 0.345 ppm, and in the muscle of 0.081 ppm. Station 2 showed the concentration of Pb in kidney organs at 1,513 ppm, in the liver at 0.415 ppm, and muscle organs at 0.331 ppm. Whereas at station 3, the Pb concentration in the kidney organ was 0.276 ppm, in the liver was 0.577 ppm, and in the muscle was 0.063 ppm (Table 2).

Table 2. Measurement Results of Lead (Pb) on Tilapia Organ

Organ	Konsentrasi Timbal (ppm)				
	Station 1	Station 2	Station 3	BBPBAT Sukabumi	Quality Standard*
Kidney	0.672	1.513	0.276	(tt)	0.3
Liver	0.345	0.415	0.577	(tt)	
Muscle	0.081	0.331	0.063	(tt)	

*Information: Quality standard is based on National Standardization Agency Of Indonesia 7387: 2009 concerning the maximum limit of heavy metal contamination in food

Based on the analysis of Pb in muscle organs, the Pb concentration value at station 1 was 0.081 ppm, station 2 was 0.331 ppm, and station 3 was 0.063 ppm. At the three research stations, it was found that only at station 2 had exceeded the quality standard set by National Standardization Agency Of Indonesia 7387: 2009. The results of the Pb metal analysis show that muscles were the organs with the lowest Pb heavy metal exposure compared to the liver and kidneys. As said by Ahmed *et al*. (2014) that the level of accumulation of heavy metals in fish body tissue from large to small was found in the liver, kidneys, gills, then muscles. Although the Pb concentration value is the smallest, but if left exposed for longer, it will be more dangerous and exceeds the established quality

standards. Given if the muscle is a part that is usually consumed by humans. If fish that are exposed to Pb metal are consumed continuously, it is also possible that the human body will also be exposed to Pb metal, and will accumulate in their bodies.

In contrast to the condition of fish taken in the Floating Net Cage in Saguling Reservoir that has exceeded the Pb metal quality standard set by National Standardization Agency Of Indonesia 7387: 2009, the condition of fish taken in the Sukabumi BBPBAT aquaculture pond showed that the value of Pb metal concentrations in liver, kidney, and muscles were below the standard quality standard of <0.05 ppm. This happens because, the area of the fish lives was controlled with good water quality. Thus, minimizing the contamination of fish cultivated at the BBPBAT. This showed that there was a difference from fish culture in controlled aquaculture ponds, with fish farming in Karuling Floating Net Cage, Saguling Reservoir, which was open water which was input from various sources, including industrial waste, fuel residue (pollution) and can also be from fish feed containing Pb, and Pb that was in the water itself.

Based on the results of research conducted (Table 2), it showed that tilapia cultivated in floating net cage from Saguling Reservoir, when viewed from the concentration of Pb in muscle, was still safe for consumption of floating net cage results at stations 1 and 3, while at station 2 was not safe for consumption. This referred to National Standardization Agency Of Indonesia 7387: 2009 concerning the maximum limit of heavy metal contamination in food and Indonesia National Agency of Drug and Food Control No. 5 of 2018 concerning the maximum limit of heavy metal contamination in processed food. The Pb limit in fish according to National Standardization Agency Of Indonesia is 0.3 ppm, whereas according to Indonesia National Agency of Drug and Food Control it is 0.2 ppm.

Water Quality Condition

Physical and chemical parameters of water are very important in the research of the distribution of contaminants that enter the waters because, because when the contaminants entered the waters, the interaction of contaminants with physical and chemical parameters will affect the fluctuations in the concentration of heavy metals dissolved in water. The physical and chemical parameters of the waters observed in this research included temperature, degree of acidity (pH) and dissolved oxygen (DO). In general, the water quality of Floating Net Cage in Saguling Reservoir and Sukabumi BBPBAT aquaculture pond still meet the threshold based on Government Regulation of the Republic of Indonesia No.82 of 2001 for grade III quality standards. Following are the results of observations of water quality of Floating Net Cage in Saguling Reservoir and Sukabumi BBPBAT aquaculture pond.

Table 3. Water Quality Measurement Results

Parameter	Quality Standard*	Station 1	Station 2	Station 3	BBPBAT Sukabumi
Temperature ◦C	Deviation 3	27.3	27.55	26.33	27
DO mg/L	> 3	3.6	4.1	4.5	6.5
pH	6 – 9	7.54	8.42	7.05	7

*Information: Quality standard based on Government Regulation of the Republic of Indonesia No. 82 of 2001

Histopathological Conditions

Tilapia organ analyzed histopathologically showed changes in various organ tissues. This can be used as a positive indication of pollution in waters by heavy metals and by other elements that cause tissue conditions in the organ to change.

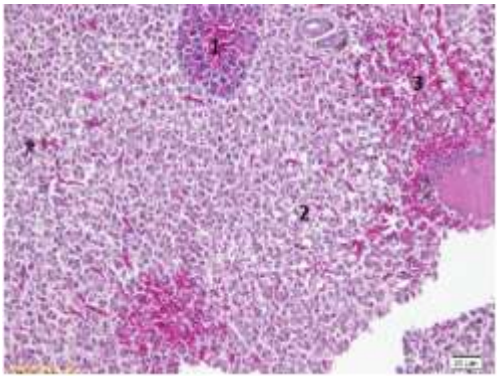

Figure 2. Hepatic Necrosis. (1). Ductus choleductus, (2). Liver epithelial cells undergo necrosis, and (3). Hemorrhage. HE X200

The level of liver damage is categorized into three, mild levels, it is fatty liver which is characterized by cell swelling. Moderate damage is congestion and hemorrhage, while severe levels are characterized by necrosis (Darmono, 1995). When it is viewed from the statement, the level of liver damage in this research was included in the category of moderate and severe. Because from observations at every station there was necrosis. Histopathological necrosis was characterized by the appearance of cell boundaries and cell nuclei that are unclear or even disappear. According to Lu FC and Sam Kacew (2003) states that the liver is very vulnerable to the influence of chemicals and become the main target organ of toxic substances. This happens because most of the poisons or toxic substances that enter the body after being absorbed by cells will be brought to the liver by the portal vein of the liver, so that the liver has the potential to experience damage.

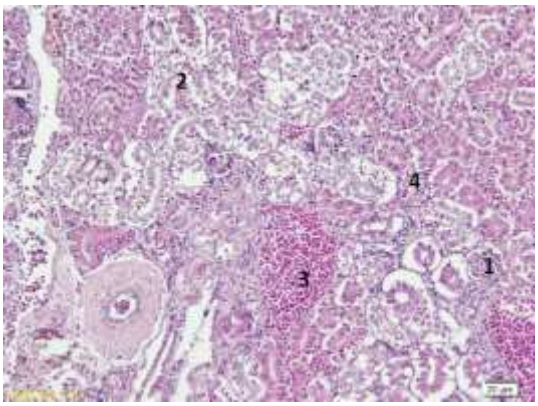

Figure 3. Nephritis. (1). Glomerulus, (2). Tubular necrosis, (3). Congestion and hemorrhage, and (4). Mononuclear cell infiltration (lymphocytes and macrophages). HE X200

Based on Figure 3, there has been a change in the histopathological condition of the kidney organ of the tilapia, it was by diagnosing nephritis, with symptoms of tubular necrosis, congestion, hemorrhage, infiltration of mononuclear cells (lymphocytes and macrophages). The damage is caused by the presence of heavy metal content of lead in the waters, which accumulates in the kidney of tilapia. In accordance with the statement of Sudarmaji et al. (2006), heavy metal lead (Pb) can cause malfunctioning of the renal tubules, vascular sclerosis, atrophic tubular cells, fibrosis and glomerular sclerosis. The result can cause aminoaciduria and glucosuria, and if exposure continues, chronic inflammation of the kidneys (nephritis) can occur. According to Kidney Health Australia (2015), states that nephritis is a term used to describe a group of diseases that cause glomerular inflammation or inflammation. This inflammation reduces the kidney's ability to filter waste. Nephritis is mostly caused by the immune system that responds to foreign substances such as poisons.

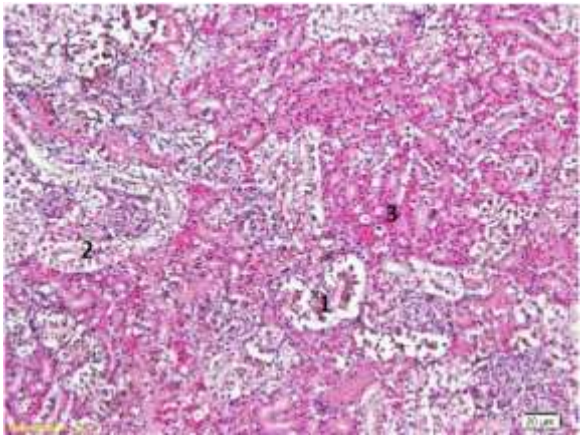

Figure 4. Nephrosis. (1). Glomerular necrosis, (2). Tubular necrosis, and (3). Hemorrhage. HE X200

Histopathological observations on kidney organs of tilapia conserved in Sukabumi BBPBAT aquaculture ponds, showed that the necrosis of tubules and glomerulus was damaged, congestion and hemorrhage (figure 11). According to Plumb (1994) that necrosis can be caused by trauma, biological agents such as viruses, bacteria, fungi and parasites, chemical agents or the occurrence of disruption of blood supply in a particular area. It can also occur due to mistreatment during the preparation of organs to be fixed. Because as is known in the heavy metal and water quality tests, in the Sukabumi BBPBAT aquaculture ponds, the waters are in good condition with controlled water conditions and the water quality values are still in accordance with the quality standards.

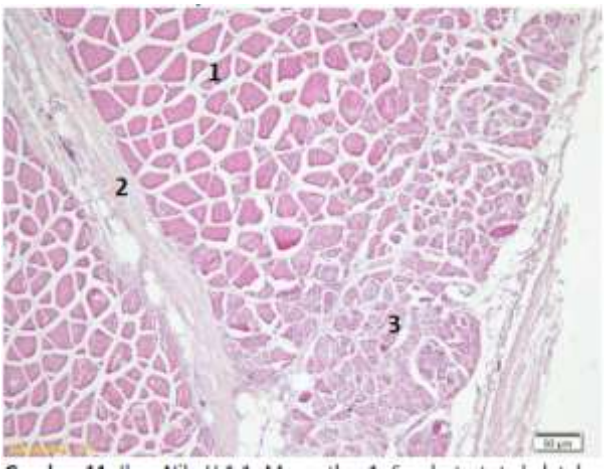

Figure 5. Myopathy. (1). Skeletal muscle fibers, (2). Fibrous connective tissue, and (3). Skeletal muscle fiber necrosis. HE X100

Based on Figure 5, tilapia muscle showed skeletal muscle necrosis. In accordance with the statement of Hibiya and Fumio (1995), Pathological changes that occur in muscles include changes in muscle fibers, changes in the nucleus of muscle cells, cloudy swelling, hyaline degeneration, granular degeneration, fat degeneration to muscle fibrous necrosis. Degeneration and necrosis of muscle cells can be caused by a lack of essential material (for example, oxygen or pantothenic acid), lack of energy sources that disrupt metabolism, mechanical heating or electrical injury, accumulation of abnormal substances in cells caused by viruses, bacteria , parasites, toxic chemicals, nutritional imbalances and mild irritants (Feist 2003).

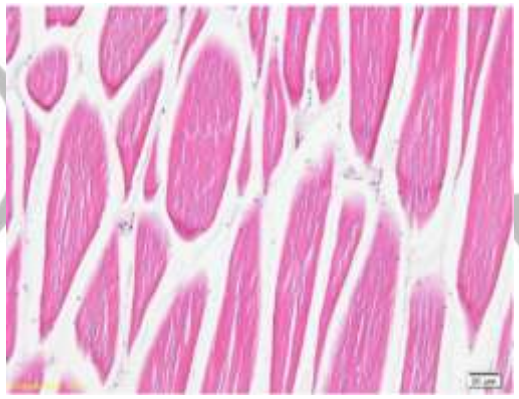

Figure 6. Tilapia H.5b. There were no specific abnormalities. HE X200

On the other hand, the muscle organs are different from before, the results of histopathological observations on the muscle organs of tilapia conserved in the aquaculture ponds of the Sukabumi Freshwater Aquaculture Fisheries Center, showed that no specific abnormalities were found. This is because the waters where tilapia live are in good condition with controlled water conditions and the water quality values are still in accordance with the quality standards.

Conclusion

Based on the results of research on the effects of lead bioaccumulation (Pb) on the histopathological conditions of the liver, kidneys, and muscle of tilapia (Oreochromis niloticus) from Floating Net Cages in Saguling Reservoir in West Java, it can be concluded that:

1. Saguling Reservoir waters at each observation station were contaminated with lead (Pb) with the same concentration that was equal to <0.05 ppm.
2. Cultured tilapia from Floating Net Cage in Saguling Reservoir has accumulated lead with varying concentrations. At station 1, liver (0.345 ppm) and kidney (0.672 ppm) exceeded the quality standard, at station 3 it showed only liver (0.577 ppm) that exceeded the quality standard, while at station 2 all organs, both liver, kidney and muscle, showed Pb concentration value that exceeded the quality standard, it was in the liver of 0.415 ppm, kidney of 1.513 ppm, and muscle of 0.331 ppm.

3. Histopathological conditions of the liver, kidneys and muscle of tilapia have experienced various damages, with damage including in the liver experiencing hepatic necrosis, in the kidney organ experiencing nephritis and nephrosis, as well as in muscle experiencing myopathy.

References

[1] Ahmed Q, Khan D, Naeema E, "Concentration of heavy metals (Fe, Mn, Zn, Cd, Pb, and Cu) in muscle, liver dan gills of adult *Sardinella albelia* (Valenciennes 1847) from gwadar water of Balochistan, Pakistan", *Federal Urdu University Art Science and Technology. Journal of Biology.* 4(2)(2014) 95-204.

[2] Cloutier. RG, M. Muris, J. Locat, C. bourg, "Retention of heavy metals in the post'96 flood sediment layer deposited in the Saguenay river, Quebec, Canada", *in Contaminated sediments: characterization, evaluation, mitigation/restoration, and Management Strategy performance, ed. West Conshohocken, Pa: ASTM International* (2003) 310-321.

[3] Darmono, "Metal in the Biological System Organism", *UI press, Jakarta* (1995).

[4] Feist S. Thain J. Forlin C, "Molecular or Cellular Process and The Health of The Individual, In A. J Lawrence and K. L Hemingway (Eds.), Effects of Pollution on Fish", *Blackwell Publishing, UK* (2003).

[5] Government regulations. "Government Regulation of the Republic of Indonesia No. 82 of 2001 about Management of Water Quality and Control Water Pollution". *Jakarta. Indonesia* (2001).

[6] Hibiya T dan Fumio T, "An Atlas of Fish Histology: Normal and Pathological Features, Second Edition", *Kodansha Ltd. Japan* (1995).

[7] Kidney Health Australia. "Nephritis-Glomerulonefritis". *The Australian Kidney Foundtion as Kidney Health Australia* (2015).

[8] Lu FC and Sam Kacew, "Basic Toxicology: Fundamentals, Target Organs, and Risk Assessment, Fourth Edition", T*aylor & Francis e-Library,* (2003).

[9] Ministry of Environment, "Saguling and Cirata Reservoirs are Contaminated by Heavy Metals", *Jakarta, Indonesia (2003).*

[10] Nandang Priyanto, Dwiyitno, Farida Ariyani, "Heavy Metals Residues (Hg, Pb, Cd, and Cu) in Fish, Water, and Sediment at Cirata Reservoir", West Java, *Postharvest and Biotechnology of Marine and Fisheries Journal,* Vol. 3 No. 1 (2008) 69-78.

[11] *National Standardization Agency of Indonesia. "Limit of Heavy Metals in Food", SNI 7387:2009, Indonesia, (2009).*

[12] Palar. H, "Heavy Metals Pollution and Toxicology", *Rineka cipta, Jakarta, (2004).*

[13] Regional Environmental Management Agency. "Water quality monitoring Data of Citarum River at Nanjung in 2013 " *Bandung, Indonesia.* unpublished *(2013).*

[14] Sudarmaji, J. Mukona, Corle. I. P, "Toxicology of Heavy Metals from Dangerous and Toxic Substances and Their Effects on Health" , *Journal of Environmental Health*, Vol 2, No. 2 (2006) 129-142.

[15] Suyanto A, Sri Kusmiyati, Ch. Retnaningsih, "Residual Heavy Metals in Fish from Contaminated Water in North Coast of Central Java", *Food and Nutrition Journal* Vol 01 No 2 (2010) 33-38.

[16] Tugiyono, Nuning Nurcahyani, R. Supriyanto, Mala Kurniati, "Biomonitoring Sugar Factory Wastewater Treatment in PT. Gunung Madu Plantation Lampung with Biomarker Analysis: Physiological Index and Histological Changes in Tilapia Liver (*Oreochtromis Niloticus* Linn)". *Science Jurnal University of Lampung,* Vol. 15, No. 1 (2009) 42-50.

[17] Wikiandy. N, Rosidah, Titin. H, "Textile Industrial Waste Pollution Impact Upon Organ Structure Damage on Fish In Upper Citarum River", *Fisheries and Marine Affairs Journal,* Vol. 4 No. 3 (2013) 215-225.

[18] Zainuri, M., Sudrajat, dan Siboro, E.S. "Heavy Metals Content of Lead in Baronang Fish, Seagrass, Sediment, and Water in the Coastal Areas of Bontang, East Kalimantan", *Journal of Marine Affairs,* Vol. 4, No. 2 (2011) 102-118.