

## TOXICITY OF INDUSTRIAL PALM OIL WASTE WATER AND SUB-LETHAL TEST TO *OREOCHROMIS SP.*

by:

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### Abstract

Research of Toxicity of industrial palm oil waste water and sub lethal test to *Oreochromis sp.* has been conducted on February 1<sup>st</sup> to March 1<sup>st</sup> 2009 in Aquaculture Technology Laboratory, Fisheries and Marine Science Faculty University of Riau. Objective of this research is to determine the concentration of a test material or the level of an agent that produces a deleterious effect on *Oreochromis sp.* during a short-time exposure under controlled condition. In this study, static 96-h acute toxicity tests were carried out using *Oreochromis sp.* as test organisms. Probit analysis using the computer software EPA and graphical method were used to calculate the 96-h LC<sub>50</sub> depending on data suitability. Results of the 96-h LC<sub>50</sub> and Biological Safety Level of the industrial palm oil waste water were 126,06 ml/l and 1.26 ml/l respectively.

*Keywords:* Toxicity, industrial palm oil waste water, sub lethal, *Oreochromis*

### 1. BACKGROUND

One of the most critical problems of developing countries is improper management of vast amount of wastes generated by various anthropogenic activities. More challenging is the unsafe disposal of these wastes into the ambient environment. Water bodies especially freshwater reservoirs are the most affected. This has often rendered these natural resources unsuitable for both primary and/or secondary usage.

River systems are the primary means for disposal of waste, especially the effluents, from industries that are near them. These effluent from industries have a great deal of influence on the pollution of the water body, these effluent can alter the physical, chemical and biological nature of the receiving water body. Wastes entering these water bodies are both in solid and liquid forms. These are mostly derived from Industrial, agricultural and domestic activities. As a result, water bodies which are major receptacles of treated and untreated or partially treated industrial wastes have become highly polluted. The resultant effects of this on aquatic organism and the environment are usually great in magnitude.

Impacts of waste water oil palm industry include loss of native organism and water and air pollutants. High levels of pollutants from waste water oil palm industry in river water systems causes an increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), toxic metals such as Cd, Cr, Ni and Pb and fecal coliform and hence make such water unsuitable for drinking, irrigation and aquatic life. The process of pollution, especially water pollution can not be separated from the presence of industry. Indonesia is state producer biggest palm oil in the world and is generally derived from Riau Province. Without have control of the industry, it is clear that the industry will waste a lot of waste in the environment.

Waste water of palm oil as a potential pollutants to pollute the environment, because the waste water is smell, contains the value of COD and BOD and suspended solids are high.

For comparison that one tonne of palm oil will produce two and a half tons of waste. Control necessary physical treatment, chemical and biological (Said, 1996), because there is no one piece of equipment that can be directly to detect whether a polluted waters, but can only be measured by using organism (Syafriadiman, 2000). One of the cultured organism that can be used as a bio-indicator organisms are red tilapia (*Oreochromis sp*).

Red tilapia fish species classified as resistant to changing environmental conditions, and in great demand by the people of Riau. And much cultivated by the owners of oil palm plantations, especially oil fields in the trenches disposal plant. Ordinary tilapia farmed in rivers, but fish in a polluted environment can cause death. As reported in the Indonesian media on Friday 8 November 2002 issue of "Thousands of Fish Dead in Pekanbaru Siak River". This pollution occurs due to the disposal of industrial waste palm oil into the Siak River. Quantity of waste generated is 120 m<sup>3</sup> / day. Total volume of waste from each palm oil with a capacity of 30 tonnes of fresh fruit bunches / day is 600m<sup>3</sup> / d (Said, 1996).

The potential of waste of industry palm oil has caused considerable environmental pollution. For that we need to know the effects of palm oil waste is the life of red tilapia. Furthermore, the need to find safety biological level to life tilapia waste. And is a very important information to cultivate tilapia in industrial areas.

## 2. RESEARCH METHODS

Based on the results of preliminary tests it was found that the concentration range of waste water palm oil industry obtained was 125-128 ml/L for the definitive test (determining 96 hour LC<sub>50</sub>). The container used is aquarium and the aquarium cleaned and washed with 20 ppm potassium permanganate (Afrianto and Liviawati 1992), and labeled as a treatment to be used. Water solvent used taken from wells drilled Faculty of Fisheries and Marine Sciences University of Riau and aerated for 48 hours until the dissolved oxygen concentration of about 6 ppm.

Liquid waste palm oil as a toxicant derived from PTPN V VFD Oil Permai KM 55 Dayun Siak District. Volume of water for each treatment was 20 L/aquarium. Organisme had used in acute and chronic toxicity test in this study is a red tilapia (*Oreochromis sp.*). This fish purchased at hatchery of Limbungan Rumbai. The average age of the fish seed is 1 month with the size of ± 5 cm. Amount of fish per aquarium is 10 individu. Before the acute toxicity test, the fish acclimatized for 2 days.

Static methods used in the acute toxicity test in determining the death of 50% of the test organisms. The characteristics of dead fish characterized by the absence of tilapia fish movement, loss of balance and the change in the levels of growth (Rand and Petrocelli, 1985). Observations are made every 12 hours by observing the state and behavior of fish visually. Then record all the symptoms that occur in fish as well as counting the number of fish dying. 96-hour LC<sub>50</sub> value of palm oil wastewater on the Red Tilapia fish (*Oreochromis sp*) are determined using the EPA Probit Analysis Program version 1.5. Boundary Biological Safety Level (BSL) is calculated using the formula proposed by Denton et al (in Syafriadiman 2000) as follows:

$$BSL = LC_{50} \times 96 \times \text{application factor}$$

BSL = Biological Safety Level

Numbers of application factor is 0.01

Water quality parameters is measured on the growth test, especially DO, pH, temperature, ammonia and turbidity. Measuring was done once a week for a month. Determination of water quality parameters was conducted also before the fish entered to in the aquarium. Measuring of absolute weight and growth rate are :

1. Absoluteweight (*A<sub>w</sub>*),  $A_w = W_t - W_o$  (Rickers, 1975);

where: *A<sub>w</sub>* = Absoluteweight(g)

*W<sub>t</sub>* = Weight offishend of the observation(g)

*W<sub>o</sub>* = Weight offishearlyobservations(g)

2. Daily Growth Rate ( $\alpha$ )

$$\alpha = \left( \sqrt[t]{\frac{W_t}{W_o}} - 1 \right) \times 100 \% \quad (\text{Rickers, 1975})$$

where:  $\alpha$  = daily growth rate (%)  
 $W_o$  = Weight of fish early observations (g)  
 $W_t$  = Weight of fish end of the observation (g)  
 $t$  = Duration Research (days)

3. RESULTS AND DISCUSSION

The results of acute toxicity tests

Mortality of seed red tilapia (*Oreochromis* sp.) during the acute toxicity test were increased with increasing concentrations of the palm oil industry waste for exposing 96 hours (Table 1).

Table 1. Mortality of seeds of red tilapia (*Oreochromis* sp.) during the acute toxicity test

Treatment	Mortality of seeds of red tilapia (%)				Mean
	Replications			Total	
	1	2	3		
control	0	0	0	0	0
125,0	10	0	10	20	6,67
125,4	20	20	10	50	16,67
125,8	20	30	20	70	23,33
126,2	50	40	40	130	43,33
126,6	70	80	80	230	76,67
127,0	100	100	100	300	100

Table 3 shows the mortality of red tilapia seed in each treatment during the study. In this research, mortality of fish was 0% in controls (Rand and Petrocelli, 1985). Percentage mortality in this study are nearly same with the results of the research by Romi (2003) and Jumedi (2003). The sequence of tilapia seed mortality during the 96 hours from concentration of the palm oil industry high to low concentration was 127.0 ml/L, 126.6 ml/L, 126.2 ml/L, 125.8 ml/L, 125.4 ml/L and 125.0 ml/L (Figure 1). In Figure 1 shows that the lowest mortality 0% and the highest 100% on the concentration of 127.0 ml/L.

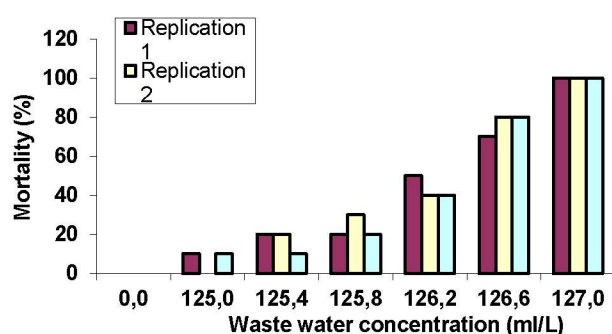


Figure 1. Histogram of seed mortality of red tilapia (*Oreochromis* sp.) during the acute toxicity test

Result of analysis of variance (ANOVA) showed that the concentration of palm oil industry waste treatment was very significant on tilapia seed mortality ( $p < 0.01$ ). Newman-Keuls test showed that the concentration of 127.0 ml/L was very significant ( $p < 0.01$ ) with other treatments. While, the concentration of 126.2 ml/L was non-significant ( $p > 0.05$ ) with another treatment. Treatment 126.6 ml/L was very significant ( $p < 0.01$ ) with controls, significantly ( $p < 0.05$ ) with a concentration of 126.2 ml/L and non-significantly ( $p > 0.05$ ) by treatment others. Concentrations of 126.2 ml/L, 125.8 ml/L, 125.4 ml/L, 125 ml/L and

controls non significantly ( $p > 0.05$ ) each other. Relationship of seed mortality of red tilapia with concentrations of oil palm waste during 96 hours is positively linearly (Figure 2).

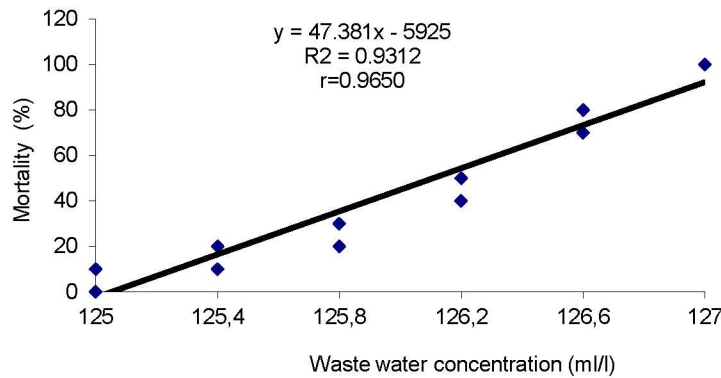


Figure 2. Relationship of Tilapia seed mortality with the concentration of waste water of palm oil industry during acute toxicity tests

Figure 2 showed the regression equation  $y = 47.38x - 5925$  with  $R^2 = 0.9312$  and  $r = 0.97$ . Contribution of toxicities palm industrial waste to changes in mortality percentage of tilapia is 93.12%, and 6.88% is influence by other factors. The result of this research also was found same result by Romi (2003) and Jumedi (2003), that have the relationship of concentration of palm oil waste with the cork fish and grouper. Closeness of the relationship between the concentration of palm oil waste with tilapiamortality may be caused by such waste toxicant power at a certain concentration level of that can shut down the test organism.

According to Said (1996), palm oil effluent has COD, BOD, suspended solids and emulsified high fat and can be organisme dead. Deposition and decomposition of organic matter that can gradually cause a decrease and an increase in pH resulting in deterioration of water quality and causes the death of test organisms. The values LC1-99 96 hours of palm industry waste water for seed tilapia fish in Table 2 and Figure 3.

Table 2. The values LC1-99 96 hours of liquid industrial waste of the oil palm for seed tilapia (*Oreochromis* sp)

LC (%)	Concentration (ml/L)
1	124,97
5	125,21
10	125,36
15	125,47
<b>50</b>	<b>126,06</b>
85	126,89
90	127,13
95	127,52
99	128,39

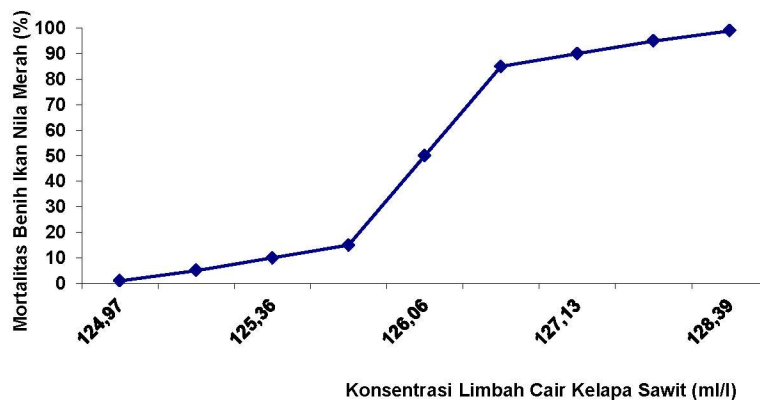


Figure 3. The values LC1-99 96 hours of wastewater oil palm industry for seed tilapia (*Oreochromis* sp.)

Table 2 and Figure 3 shows the 96-hour LC50 value is 126.06 ml/L. This value indicates that if the waste water palm oil industry entering into the waters with a concentration of 126.06 ml/L will cause the death of the seed of red tilapia (*Oreochromis* sp.) 50% for 96 hours. Value 96-hour LC50 is 126.06 ml/L, this value are  $\pm 3$  times greater than 96 hour LC50 values obtained by Romi (2003) (39.35 ml/L), for different organisms (fish larvae cork) of the same toxicant. This indicates that the waste water palm industry is more toksit on the cork fish larvae when compared with red tilapia seed. Koesumadinata and Sutrisno (1997) suggest that the sensitivity (susceptibility) organisms to toxicant is different according to the type and size of the organism.

Different waste concentrations provide a different form of response in each individual fish (Syafriadiman, 2000). Observations made during the acute toxicity test to seed red tilapia fish (*Oreochromis* sp.) there are symptoms of behavior and morphology in normal conditions, sub lethal and lethal. Toxicant in this study may change the conditions of tilapia were initially normal to be lethal. Environmental disruption caused by waste water palm oil industry has caused the fish to become stressed, so it looks to be a different response depending on the sensitivity and resistance of fish. Said (1996) states that palm oil wastewater has potential as an environmental pollutant because of the smell, it contains the value of COD and BOD and suspended solids are high and oil in water emulsion. If sewage is discharged directly into the river portion will settle, decompose slowly, consume dissolved oxygen, causing turbidity, and issued a sharp odor. These factors are thought to cause stress tilapia seed, nearly dead (sub-lethal) and the occurrence of death, especially at high concentrations.

Under normal conditions of tilapia seed as test organisms in this study movement active, agile, balanced and body morphology are not damaged. Under normal conditions of tilapia seed in this study better with normal cork fish larvae in the study Romi (2003), ie no fins and scales are separated, fish eye clean and very responsive to stimuli. So also the fish's mouth opening and operculum move regularly, and red gills.

Condition of sub-lethal in this study that tilapia seed show the movement is not balanced with direction uncertain, often spinning and blundering aquarium wall. Conditions begin to deteriorate be shown that the fish morphology characterized by the presence of scales apart. Mouth opening is very fast, operculum fast moving fish and less response to stimuli. While, characteristic of the tilapia seed dead during the study was not the moving and paused at the base of the aquarium. Morphology characterized by the body breaks down the scales loose and easily removed from the body. Fish eyes and as if to stand out, while the mouth and operculum open, and gills pale.

Biological Safety Level of wastewater palm oil industrial for red tilapia seed was found during the study was 1.26 ml/L. Value of Biological Safety Level is  $\pm 3$  times greater with the results found Romi (2003) for the same toxicant and different test organisms, namely fish larvae cork (0.40 ml/L).

#### **The results of the test sub-lethal**

The growth of red tilapia fish during the study experienced an increase on P4 concentrations except 126.06 ml/L, where the seventh day all the fish died. Growth and survival of red tilapia seed during the study in Table 3.

Table 3 shows that the seeds of red tilapia on the seventh day of the treatment concentration of P4 (126.06 ml/L) died. It is likely the fish are not able to survive for seven days in a concentration of 126.06 ml/L, due to the toxicity of waste palm oil can not be tolerated by the red tilapia and eventually die. The study of the growth of the average weight of red tilapia seed for the growth of the test is declined in the average growth of seed red tilapia with increasing concentration of palm oil effluent (Table 4)

Table 3. Growth and survival of red tilapia seed for 28 days

P	U	Growth (g)					n <sub>0</sub>	n <sub>28</sub>
		0	7	14	21	28		
Po	1	3,16	4,36	4,65	5,42	6,41	10	10
	2	3,19	4,37	4,68	5,53	6,46	10	10
	3	3,27	4,44	4,86	5,51	6,55	10	10
P1	1	2,86	3,35	3,85	4,37	5,56	10	10
	2	3,24	4,34	4,88	5,62	6,32	10	10
	3	3,21	3,29	4,63	5,45	6,18	10	10
P2	1	2,82	3,57	3,98	4,42	5,35	10	9
	2	2,98	3,60	4,36	4,98	5,57	10	9
	3	2,66	3,05	3,78	4,62	5,01	10	9
P3	1	2,38	2,68	3,45	3,91	4,35	10	8
	2	2,57	2,96	3,47	4,06	4,64	10	9
	3	2,36	2,67	3,44	3,90	4,32	10	8
P4	1	3,06	0	0	0	0	10	0
	2	2,77	0	0	0	0	10	0
	3	2,80	0	0	0	0	10	0

Description:

P : Treatment No : Total number offish seed baseline

U : Replication N28 : The number offish seedafter 28days

Po : control P1 : Concentration ofoil palm waste1.26ml/L

P2 : Concentration ofoil palm waste31.52ml/L P3 : Concentration ofoil palm

waste63.03ml/L P4 : Concentration ofoil palm waste126.06ml/L

Table4. The absolut growth weight ofseedsredtilapia(Oreochromissp)

Replication	Growth of the absolute weight (g)			
	Control	1,26 ml/L	31,52 ml/L	63,03 ml/L
1	3,25	2,70	2,53	1,97
2	3,27	3,08	2,59	2,43
3	3,28	2,97	2,35	2,62
Total	9,8	8,75	7,47	7,02
Mean	3,27	2,92	2,49	2,34

Table 4 shows that the Mean growth in the absolute weight of red tilapia seed is highest on the control treatment is equal to 3.27 g, and continued to decline until on the treatment a concentration of 63.03 ml/L is equal to 2.34 g. The percentage decrease on the concentration of 1.26 ml/L, 31.52 ml/L, 63.03 ml/L and controls were 10.70%, 23.85%, and 28.44% respectively. This decrease was probably caused by the poor condition of the water as well as the toxicity material contained in the waste water palm industry. The relationship between the fish growth of the absolute weight with waste water palm industry concentration were negative linear (Figure 4).

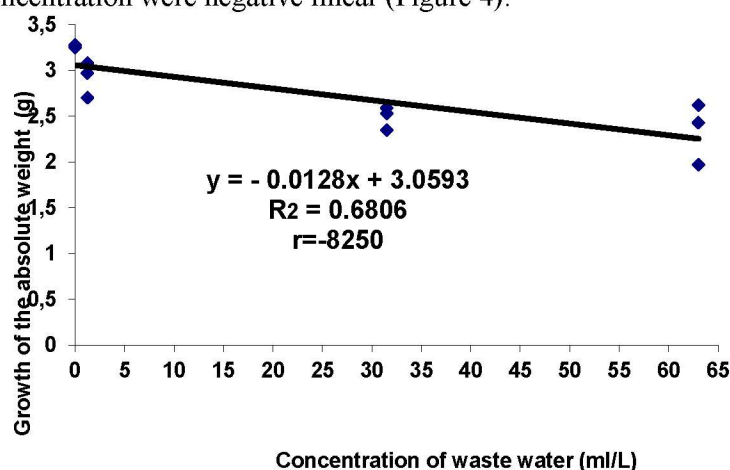




Figure 4. Relations absoluteweightgrowthof redtilapiaseed with the concentration of waste water palm industry for sublethaltest

Figure 4 shows the regression equation  $y = -0.0128x + 3.0593$  with a value of  $R^2 = 0.6806$  and  $r = -0.8250$ .  $R^2$  values indicate the concentration of 68.06% contribution of oil palm waste to an absolute weight change in the growth of red tilapia seed during the study. Decrease in absolute weight growth was also found in the study Jumedi (2003). Absolute weight loss was caused by toxic materials that can affect the condition of the water of the test fish. Based on the analysis of variance (Anova) that the concentration of waste oil does not give a significant influence ( $p < 0.01$ ) on the growth of red tilapia seed absolute weight (*Oreochromis sp.*). As per the provision of test results anava concentration of palm oil waste at different exposure time of 96 hours gives highly significant effect ( $p < 0.01$ ) on mortality of red tilapia seed. Newman-Keuls test result that absolute control treatment had different weight highly significant ( $p < 0.01$ ) of the treatment concentration 31.52 ml/L and treated with a concentration of 63.03 ml/L, did not differ by treatment with 1.26 ml/L. Treatment concentration of 1.26 ml/L had different ( $p < 0.05$ ) between treatment concentration of 31.52 ml/L to 63.03 ml/L. While the control treatment did not differ ( $p > 0.05$ ) by treatment with concentration 1.26 ml/L. Value of daily growth rate of red tilapia seed was found to decrease with increasing concentration of palm oil effluent. Where the higher concentrations of the lower palm oil waste daily growth rate of red tilapia seed (Table 5).

Table 5. Daily Growth Rate of Seeds Red Tilapia (*Oreochromis sp.*)

Replication	Daily Growth Rate (%)			
	Control	1,26 ml/L	31,52 ml/L	63,03 ml/L
1	2,56	2,40	2,31	2,18
2	2,55	2,42	2,26	2,13
3	2,51	2,37	2,29	2,18
Total	7,62	7,19	6,86	6,49
Mean	2,54	2,40	2,29	2,16

Table 5 shows that the average daily growth rate of red tilapia seed in controls was 2.54%, on treatment with a concentration of 1.26 ml/L at 2.40%, on treatment with a concentration of 31.6 ml/L of 2,29%, and the treatment with a concentration of 63.1 ml/L of 2.16%. The presence of this daily growth rate decreased, probably due to the water conditions are not conducive to the growth of red tilapia seed (Table 5). The relationship of the daily growth rate of red tilapia seed against palm oil effluent concentration is negative linear (Fig. 5)

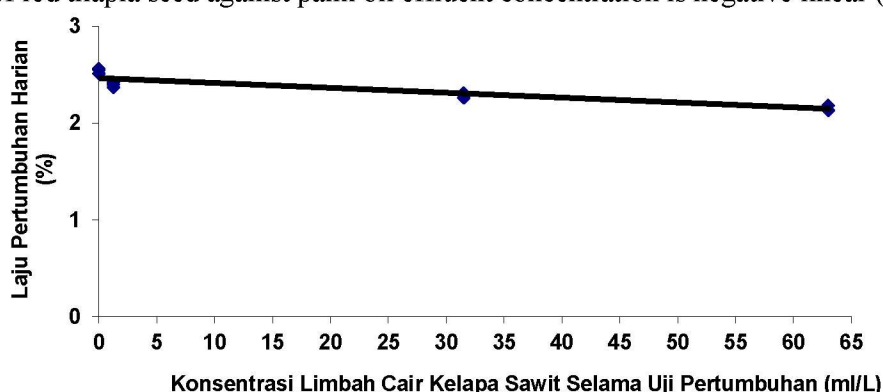


Figure 5. Daily growth rate relationship to the concentration of oil palm waste during the study.

Figure 5 shows the regression equation  $y = -0.005x + 2.4666$  with a value of  $R^2 = 0.8487$  and  $r = -0.9213$ . Based on the regression equation is then an increased concentration of one unit, it will reduce red tilapia daily growth rate of 0.005 times. Of the value of  $R^2$  is known

that the concentration of oil palm waste contribution to the change rate of daily growth of red tilapia seed at 84.87%, while the rest is influenced by other factors with a strong negative relationship closeness ( $r = -0.9213$ ). Decrease in daily growth rate was also found in the study Jumedi (2003). Absolute weight loss was caused by toksit materials that can affect the condition of the waters of the test fish.

Based on the analysis of variance (Anova) it was found that there was a significant influence ( $p < 0.01$ ) between the concentration of palm oil effluent to the daily growth rate of red tilapia seed (*Oreochromis sp.*). Then further test Newman - Keuls explained that the control treatment; 1.26 ml/L, 31.52 ml/L and 63.03 ml/L daily growth rate differences are very real to each other.

#### Water quality parameters

Values of water quality parameters during the study was almost the same in each treatment, ie range between are temperatures 26-29°C, pH 5-8, DO 1.2 - 6.4 ppm, turbidity 0-65 NTU and Ammonia 0.04 - 0.840 ppm respectively. All the parameters range is still good for the growth of fish. Arie (2000) explained, the optimal temperature for growth of fish is range between 25-30°C. Then, Boyd (1979) also explained that the tropical areas, the temperature range between 25-32°C and is still viable for the growth of aquatic organisms.

pH values during the study ranged between 5-8, pH recording at research baseline is 5, this value be due to mixing of sewage water between water pH 6 as solvent with waste water palm oil pH 4. Values of pH during research is fluctuated, the same condition, Romi (2003) found that the pH value also fluctuates in the his research, in the same toxicant conditions, ie waste water palm oil with different of fish, ie the fish larvae cork. Despite this, however the range of pH during the study is still quite good for fish life. According to Brown (1980), the pH of the water is less than 4 or more than 11 can cause the death of fish. While the ideal pH for the life of aquatic organisms ranges from 6.5 to 8.5 (Pescod, 1973).

Therefore, values DO (Dissolved Oxygen) is recorded during the study are ranged between 5 to 6.6 ppm. Values DO in the control treatment container is 6.0-6.6 ppm, while in the treatment concentration 1.26 ml/L was recorded the DO ranged between 5-6 ppm. Increasing the concentration of palm oil wastewater was reaches the concentration 126.06 ml/L can cause impairment values DO. Then, the impairment DO values can lead to increased the mortality of red tilapia seed (*Oreochromis sp.*) up to 100%.

Concentration of ammonia can also cause increasing the percentage of deaths the tilapia fish seed. Range of ammonia is recorded during the study was 0.04 to 0.840 ppm. Boyd (1979) states that level toxicities of NH<sub>3</sub> in the short-term exposure period is 0.6 to 2.0 ppm. Afrianto and Liviawaty (1992) states that concentrations of ammonia low can cause the tissue damage gills, making fish hard to take oxygen from the environment.

#### 4. CONCLUSION

These results of the research indicate that palm oil waste was very significantly ( $p < 0.01$ ) on percentage of mortality seed tilapia (*Oreochromis sp.*). Value 96-hour LC<sub>50</sub> in the acute toxicity test was 126.06 ml/L, and value a Biological Safety Level (BSL) the waste water palm oil industry is found 1.26 ml/L. Then, concentration of the palm oil waste was very significantly ( $p < 0.01$ ) on value of growth rate and absolute weight rate of tilapia fish seed during 28 days.

The increasing of waste water palm oil concentration will be caused the lower the absolute value of the weight growth and daily growth rate of seeds of red tilapia (*Oreochromis sp.*). Value water quality parameters for acute toxicity testing and growth test was be bad, when the concentration of palm oil industry waste was be increased. Therefore, for testing of acute toxicity and growth test must be ever good the condition of water quality in the experiment media.



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