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RESEARCH ARTICLE

ACUTE AND SUB-LETHAL TOXICITY TEST ON *Oreochromis niloticus* EXPOSED WITH TANNERY WASTEWATER.

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Abstract

This study aims to analyze the value of LC_{50}-96 hours of tannery waste exposed to *Oreochromis niloticus* and see changes in the level of oxygen consumption and the opercullar respiratory rate. LC_{50}-96 hours using Whole Effluent Toxicity (WET) method - static test. For the measurement of oxygen consumption level and operculum movements were conducted at 1.85% and 3.69% of wastewater exposure. Observations were made for 30 days after the acclimatization period. Data analysis used regression and correlation analysis and ANOVA significance test. The results showed LC50-96 hours was 25.85%. The level of oxygen consumption *of O. niloticus* at concentration of 1.85% and concentration 3.69% were decreased in the duration of exposure and the control treatment was increased with P value <0.05. While the frequency of the opercullar respiratory rate at concentration of 1.85% and 3.69% were increased also in control experiment during 30 days of tannery wastewater exposure, with the P value <0.05. The longer of the exposure time and the higher the concentration of wastewater, the decreasing of oxygen consumption level and the increasing of opercullar respiratory rate.

Introduction:-

Background:-

The tannery industry processes raw animal skin into finished skin. The tannery industry is one of the industries that releases large amounts of wastewater. The wastewater has high solubility and a toxicant material. Tannery waste usually uses tanners in the form of chromium (Cr). The raw materials used are animal skin (cattle, buffaloes, goats and others) primarily obtained from slaughterhouses.

Fish as an indicator has a major role in determining the level of water pollution as it can respond with great sensitivity to changes in the water environment. *O. niloticus* is one of the aquatic biota which react to the physical changes of water and the presence of pollutant compounds dissolved within certain concentration limits because this fish are very sensitive to environmental changes (Sudarmadi, 2007).

Toxicity level of a wastewater to aquatic biota can be seen from the value of Lethal Concentration 50 (LC_{50}) and changes in animal behavior test known as subletal toxicity test. LC_{50} is the concentration causing death in 50% of experimental animals. LC_{50} is important to know the hazard limits of a material.

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Sublethal toxicity test is a contamination test to organism that affects the metabolism of the organism but does not cause death (Al-Attar, 2005). Effects of toxic to the organs of the fish cause disruption to the physiology and histology of the organism (Mondon et al 2001). Physiology effect of fish can be observed in the means of metabolic processes (Fujaya, 2004). The metabolic process of living things can be estimated by measuring the amount of oxygen consumed by living things per unit of time, known as the level of oxygen consumption, if the metabolic process in fish is disturbed it will lead to reduced ability of fish to consume oxygen (Tobin, 2005). The fish opercullar respiratory rate is a process of swallowing the water with its mouth and pressing it through the gill and then out through the hole under the operculum (Pough et al, 2005). The relation between the level of oxygen consumption and the movement of the operculum is that if the fish is exposed to the wastewater will cause damage to the gills, the fish operculum movements increase due to the ability of fish to consume oxygen will decrease (Kramer, 1987).

A study conducted by Sivakumar et al (2016), on the influence of the tannery wastewater in *Danio rerio* freshwater fish for 5 days, showed that the oxygen consumption level in the control treatment was increased from 6.02 mg/O_2/g.hr to 6.26 mg/O_2/g.hr and at a concentration of 15% wastewater was decreased from 5.64 mg/O_2/g.hr to 4.52 mg/O_2/g.hr. A study conducted by Sreenivasan and Moorthy (2011) on the influence of tannery wastewater on tilapia fish showed in addition of 5% wastewater concentration, the level of oxygen consumption decreased.

Therefore, this research was conducted to see the toxic level of tannery wastewater including acute toxicity LC_{50}-96 hours and changes of oxygen consumption level and changes of opercullar respiratory rate of *O. niloticus* fish which exposed with the wastewater.

Materials And Method:-

Wastewater used in this study was obtained from tannery waste which produced leather approximately 500 kg in one time production with wastewater load ± 12-16 m^3. The wastewater is discharged to Batang Anai River after being processed at Wastewater Treatment Plant (WWTP). Testing the characteristics of wastewater was conducted in the environmental laboratory of the Department of Environmental Engineering Andalas University. Result of wastewater characteristic test showed that wastewater quality is exceeded government regulation on effluent standard for tannery waste industry. The results of the of tannery wastewater quality can be seen in Table 1.

Table 1:-Effluent Characteristic of Tannery Wastewater

No	Parameter	Tannery wastewater quality	
		Effluent quality	Effluent standard (Government Regulation) based on Permen LH No. 5 Year2014
1	BOD (mg/L)	53,27	50
2	COD (mg/L)	113,2	110
3	TSS (mg/L)	64	60
4	Total Chrome (Cr) (mg/L)	1	0,6
5	Fat and grease (mg/L)	11,44	5
6	Total Nitrogen (mg/L)	12,45	10
7	Amnonia (mg/L)	1,94	0,5
8	Sulfide (mg/L)	1,23	0,8
9	pH	9,4	6,0-9,0

In this study the animal test used was *O. niloticus* (*Oreochromis niloticus*). The average size of *O. niloticus* is 2-3 gram weight, ± 1 month old and size 4-5 cm (Halappa and David, 2009). *O. niloticus* were caught from fish breeding in Bangek River, Koto Tangah Subdistrict, Padang City. For acute toxicity test LC_{50}-96 hours, 120 fish was used in the range finding test and120 fish were used in the definitive test (OECD, 2003). In this study, 12 glass aquariums was used with size of 35x30x30 cm with amount of 10 fish in each aquarium with the consideration that the volume of one liter water to 0.8 grams of fish weight (APHA, 2014). As for subletal toxicity test 45 fish were observed to see the changes of oxygen consumption level and the opercullar respiratory rate, with each aquarium consist of 5 fish with dimension of 35x30x30 cm.

Acclimatization of fish was conducted for fish to be adapted to the the laboratory condition for 7 days (APHA, 2014). At the time of acclimatization, fish were given food in the form of pellets and the aquariums were equipped with an aerator with flow rate of 3.5 L / min. *O. niloticus* are fed every 3 times a day in the morning (08.00 am), noon (12.00 pm) and afternoon (16:00 pm) (Sutisna and Sutarmanto, 1999). The amount of feed given is 3% of the fish weight per day (Suyanto, 2009). Water used for this research was dechlorinated water. The replacement of test water was also done every four days (Viodhini and Narayanan, 2009). The environmental factors measured during the acclimatization were dissolved oxygen (DO) and temperature measured using DO meter Lutron DO-5510 DO meter and pH level measured with pH meter with brand of PH-009 (1).

Acute toxicity test LC_{50}-96 hours:-

The acute toxicity performed in a short time period of 96 hours. The test method is Whole Effluent Toxicity (WET) - static test with aeration and without water replacement. This method is done by inserting the fish into the aquarium containing wastewater samples and aquades. The fish were left exposed to the sample with various concentrations. There are two stages in this toxicity test including range finding test and definitive test.

Range Finding Test:-

The range finding test aims to determine the limits of the range of crisis concentration of the wastewater used for the determination of LC_{50}, which is the highest concentration in which the test animal does not experience death, and the upper threshold concentration is the lowest concentration causing 100% mortality. Range finding test was performed using a concentration range from zero to 100% of wastewater concentration. The initial consumptions used were 6.25%, 12.5%, 25%, 50%, and 100% (USEPA, 2002). This test yields a temporary LC_{50} value which is used to minimize the next stage of concentration.

Definitive Test:-

This test is an advanced stage of Range Finding Test with the same treatment but conducted with new concentrations obtained from the results of LC_{50}-96 hours from Range Finding Test. This test will inform to which concentration of toxic material that causes 50% of test animals to die. The study was conducted with duplo and the observations were performed at hours 0, 24, 48, 72, and 96 covering the measurements of temperature, pH, DO and dead test animals recorded.

There are several ways to obtain LC_{50}-96 hours values, namely graphical methods, probit, Spearmen-Karber, and Trimed Spearmen-Karber (USEPA 2002). The test results may be accepted if 90% of the test animals at the controls at the end of the observation are alive. If survival is smaller than 90% then the test should be repeated.

Oxygen Consumption and Opercullar Respiratory Rate:-

The wastewater concentration used in the sublethal test were 1/7 and 1/14 of the LC_{50}-96 hours value (hallapa, 2009) which were 1.85% and 3.69%. This tests were performed using 9 aquariums, 3 aquariums for control, 3 aquariums for concentrations of 3.69% and 3 aquariums for concentrations of 1.85% . Replacement of the test solution was carried out every 4 days (Vinodhini and Narayan, 2009) or when the water is already cloudy to maintain pollutant concentration and keep the DO. Subletal toxicity test in this study was conducted for 30 days and observed in day 0, 10 20 and day 30 of the study. The experiments were carried out by triple repetition of each concentration used. The oxygen consumption level in this study was observed by measuring the dissolved oxygen using the Iodometric method and also direct measurement using DOmeter. While the measurement of the opercullar respiratory rate was done by using counter and camera.

For the oxygen consumption experiment, one fish was taken from the aquarium to weigh, then the fish was put into a 1000 ml erlenmeyer that contains wastewater which was already aerated for 30 minutes. The DO was measured before the fish was put into erlenmeyer (DO_0). The erlenmeyer were covered with wrap paper and aluminum foil. The fish was left for one hour, after one hour 50 ml was taken for the measurement as the result DOt (DO end of observation). The calculations based on the formula of Pavlovskii (1964):

$$OC = \frac{(V) \times (DO_0 - DO_t)}{W \times t}$$

OC = Oxygen consumption (mg O_2/g.hour)
V = Water Volume (L)
DO_o = Initial DO (mg.O_2/L)
DO_t = DO in the end of observation (mg.O_2/L)

W = Fish weight (g)
t = Time period (hours)

Meanwhile, to calculate the change of frequency of opercullar respiratory rate (Muthukumar et al, 2009). The wastewater was inserted from the aquarium into a 1000 ml beaker glass which was aerated, then put the fish from the aquarium into the beaker glass. Let the fish in the beaker glass for 10 minutes, in order to adapt to the new environment. Calculate the number of operculum movements for 1 minute every 20 minutes using counter and camera. The result of the video was inserted into the Movie Maker app, the video is slowed, so it can be calculated its operative motion (Kishiya et al, 2015). Furthermore, regression analysis and correlation and Anova significance test were calculated to see the relationship and significance between wastewater concentration and exposure time of tannery wastewater to the level of oxygen consumption and opercullar respiratory rate in *O. niloticus* fish.

Results And Discussion:-

Acute Toxicity Test Results LC50-96 hours:-

The results of *O. niloticus* analysis for acute toxicity test were fulfilling the water quality that good for life of fish which can be seen from parameters of water quality measurement including pH, DO, and temperature (USEPA, 2002). The result of pH measurement during range finding test and definitive test were approximately ± 7.0 which meet the range of 6.0 - 9.0 that support for fish to grow, while DO levels ranged from 5-6 mg/L, whereas in the definitive test DO levels ranged from 5.3-6.2 mg/L . This level of DO is suitable for fish environment with requirement larger than 4 mg/L. Temperatures during the range finding test ranged from 27^{o}C-28.1°C, while the acclimatization during definitive test ranged between 27^{o}C-28^{o}C. The acclimatization state in the range finding test and definitive test is still within the range of the life span of *O. niloticus*, which is 25^{o}C-30^{o}C.

Range finding test result:-

From the data obtained mortality of fish happened in two or more concentrations within 96 hours experiment as seen in Table 2, so the value of LC_{50} can be determined by probit method (USEPA, 2002). With probit program, there was obtained LC_{50} value of 20,400% with highest concentration 42,42% and lowest concentration of 7,4%. The lowest and highest concentration limits are further used for the concentration on the definitive test.

Tabel 2:-Fish Mortality during Range Finding Test

No	Wastewater exposure (%)	Fish Mortality Percentage (%)
1	0	0
2	6,25	30
3	12,5	45
4	25	55
5	50	60
6	100	75

Definitive Result:-

The definitive test was performed after the range finding test using a concentration range of wastewater that caused 50% fish mortality based on preliminary test with the range of concentrations used ranging from 7.4% -42.22%. In order to facilitate the dilution of the waste then selected the concentration of 0% (control), 7%; 15%; 25%; 35%; and 45% as the wastewater concentration on the definitive test. The percentage of deaths in the baseline test can be seen in Table 3

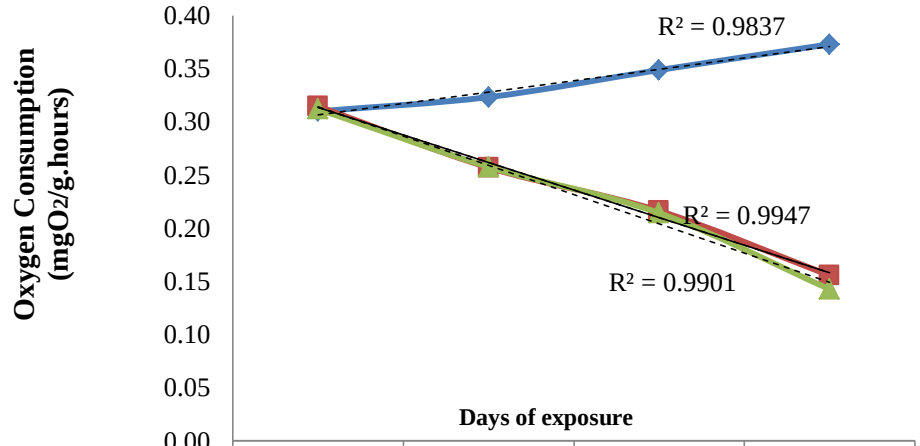
Tabel 3:-Fish Mortality in Definitve test

No	Wastewater exposure (%)	Fish Mortality Percentage (%)
1	0	0
2	7	20
3	15	45
4	25	50
5	35	55
6	45	60

Based on the result of the research, it can be concluded that the leather waste of tannery industry is classified as toxic for *O. niloticus*. This is evidenced by the percentage of obtained LC_{50} value of 25.85%. The results of this study are also supported by research that has been done previously by Rizki Masfufah et al (2007) which proves that the tannery waste industry is toxic for *Daphnia carinata king* with a concentration of 0.3% or 3000 ppm. In addition, research conducted by Priyanto (2006) also shows that the tannery wastewater is also toxic to *Lemna sp* with concentration of 0.1%.

Oxygen Consumption:-

The level of oxygen consumption is an important parameter to observe the effect of a toxic substance on fish because the toxic substances that enter the fish body will interfere with the respiratory process (Tobin, 2005). The level of oxygen consumption can be measured by calculating the ratio of dissolved oxygen at the beginning and end of the observation using winkler titration. The relationship between the level of oxygen consumption of *O. niloticus* to exposure time can be seen in Figure 1.



	0	10	20	30
Controlled test	0.31	0.32	0.35	0.37
1,85%	0.31	0.26	0.22	0.16
3,69%	0.31	0.26	0.21	0.14

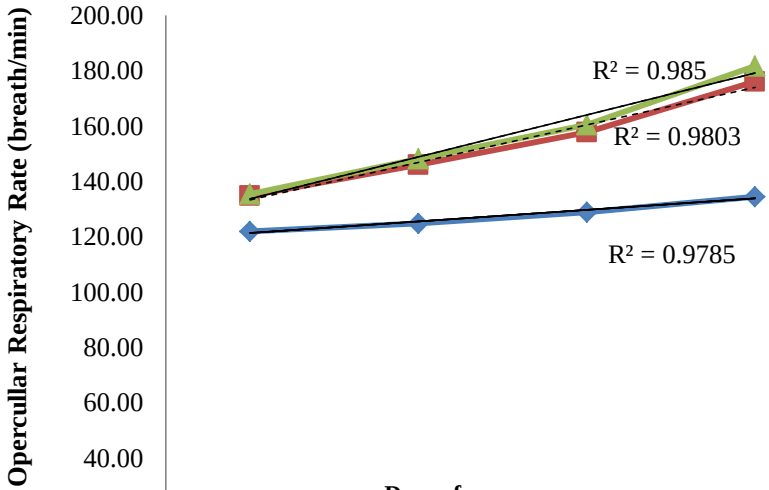
Figure 1:-Oxygen Consumption Level of *O. niloticus* within 30 Days of Exposure

The correlation between oxygen consumption of *O. niloticus* in 30 days of exposure In the control test, the concentration of 1.85% and 3.69% obtained was 0.99, it can be concluded that the duration of exposure and concentration of wastewater has a very strong relationship. Decrease in the level of oxygen consumption occurs when *O. niloticus* is exposed tannery wastewater. This is because the gill tissues of organisms that rapidly accumulate toxic substances is the gill tissue. The location of the gills that are directly related to the environment and the thin structure make the gills very vulnerable to changes in environmental conditions. If the gill tissue is disturbed, then the respiratory process will be disrupted, so the ability of fish to consume oxygen to be reduced.

The results of the analysis can be verified by performing the Anova test on Variation of exposure concentration to oxygen consumption level and duration of exposure to oxygen consumption level which resulted $P<0.05$. This proves that there are differences in the value of fish consumption level on control, 1.85% and 3.69% of wastewater concentration, and also a significant difference in the level of oxygen consumption levels on exposure to days 0, 10, 20 and 30 days.

The results of this study are in accordance with the study of Sivakumar et al (2016), on the effect of tannery waste on *Danio rerio* freshwater fish for 5 days. The results showed the level of oxygen consumption increased in the control experiment after 5 days, while there was decrease in the oxygen consumption level in the fish with wastewater exposure. Sreenivasan and Moorthy (2011) studied the influence of tannery waste against tilapia fish. In addition of 5% of wastewater concentration caused of the level of oxygen consumption decreased.

Opercullar Respiratory Rate:-



	0	10	20	30
Controlled test	121.89	124.67	128.89	134.44
1,85%	134.78	146.00	157.78	176.00
3,69%	135.33	148.11	160.33	181.67

Figure 2:-Opercullar respiratory rate of *O. niloticus* exposed with Tannery Wastewater

In the Figure 2 it can be seen that there was an increase in the respiratory rate of fish due to tannery waste exposure. Correlation were 0.98 to 0.99 between respiratory waste versus duration of exposure in various concentration of waster exposure which means a very strong relationship. The respiratory rate tend to increased by the longer exposure, caused by gill is the first organ directly related to toxic material in the waters, with wide and open surface, hence causing this part become main target for the toxic substances present in the waters. If the gills in the fish are damaged, then the fish will be difficult to breathe and gill movement becomes unstable, which is seen from the respiratory rate of *O. niloticus*(Wong, 2000). Bivariate analysis showed the $P<0.05$ for the significance test on exposure concentration to respiratory rate and duration of exposure to respiratory rate of fish. Exposure to the tannery's wastewater affects the gills of *O. niloticus* which will affect the process of metabolism and growth of fish.

Conclusion:-

Based on the research result of acute toxicity (LC_{50}-96hours) test in *O. niloticus* exposed with tannery wastewater was obtained 25,85%. The level of oxygen consumption of *O. niloticus* from day 0 to day 30 were increased in the control experiment, while in the experiments with tannery waste exposure, the oxygen consumption of *O. niloticus* were tend to decreased. The opercullar rexpiratory rate of *O. niloticus* for 30 days tend to be higher in the higher concentration of tannery wastewater exposure. Further research can be conducted to see the histopathological changes in the fish exposed with tannery waste.

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References:-

1. Al-attar, A.M. (2005). Changes in Haematological Parameters of the Fish, Oreochromis niloticus Treated with Sublethal Concentration of Cadmium. *Pakistan Journal of Biological Sciences*, 8 (3), 421-424.
2. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF). (2014). *Standard Methods for the Examination of Water and Wastewater, 22nd*. Washington DC. American Public Health Association.
3. Badan Standar Nasional (2004). *SNI 06-6989.14-2004 tentang Air dan limbah, bagian 14: Cara Uji Oksigen Terlarut secara Yodometri*. (in Bahasa Indonesia)
4. Fujaya, Y. (2004). *Fisiologi Ikan*. Jakarta: Rineka Cipta. (in Bahasa Indonesia)
5. Halappa, R., dan David, M. (2009). Behavioural Responses of the Freshwater Fish, Cyprinus carpio (Linnaeus) Following Sublethal Exposure to Chlorpyrifos. *Turkish Journal of Fisheries and Aquatic Science*s. (9), 233-238.
6. K. Mishra, A., dan Mohanty, B., (2008). Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, Channapunctatus (Bloch). *Journal Environmental Toxicology and Pharmacology*, 26, 136–141.
7. Kishiya, A.D., Solomon, J.R., Umar, A. A., U.A., dan kishiya, H.S. (2015). Influence of temperature on the respiratory rate of Nile Tilapia, *Oreochromis niloticus* (*Pisces: Cichlidae*) in the laboratory. *Journal Department of Biological Sciences, University of Abuja*, 8 (1), 27-30.
8. Kramer, D.L. (1987). Dissolved Oxygen and Fish Behavior. *Journal Environmental Biology of Fishes*, 18 (2), 81-92.
9. Mondon, J.A., S. Duda and., B.F. Nowak, (2001). Histological, Growth and 7-Ethoxyresorufin o-deethylase (erod) Activity Responses of Greenback Flounderrhombosoleatapirina to Contaminated Marine Sediment and Diet. *Journal Aguat Toxicol*, 54 (2), 231-246.
10. Muthukumar, G., Krishnan, K.R., dan Anbalagan, R. (2009). Adaptive Changes In Respiratory Movements Of An Air Breathing Fish *Tilapia Mossambicus* Exposed To Endosulfan. *Jurnal of Industrial Pollution Control*, 25 (1), 1-8.
11. Padmanabha, A., H.R.V, Reddy., Khavi1, M., Prabhudeva, K.N., Rajanna, K.B., dan Chethan, N. (2015). Accute Effects of Chlorpyrifos on Oxygen Consumption and Food Consumption of Freshwater Fish (*Oreochromis mossambicus*). *International Journal of Recent Scientific Research*, 6 (4), 3380-3384.
12. Pavlovskii, E.N. (1964). *Technique for The Investigation of Fish Physiology*. Israel: Program Scientific translation Ltd.
13. Peraturan Menteri Negara Lingkungan Hidup Nomor 05. (2014). *Tentang Baku Mutu Air Limbah*. (in Bahasa Indonesia)
14. Sivakumar, P., Dr. M. Kanagappan., dan Dr. S.Sam Manohar Das. (2016). Effect of Tannery Effluent on Oxygen Consumption and Accumulation of Toxic Metal in Freswater Fish Danio Rerio (Halminton). *Journal of Pharmaceutical, Chemical and Biological Sciences*, 3 (4), 601-607.
15. Sreenivasan, R. S., dan., P Krishna Moorthy. (2011). Biochemical Stress of Chromium in Tannery Effluents on the Fresh Water Fish Tilapia mossambica (Pisces). *International Journal of Biological & Medical Research*, 2 (3), 616-620.
16. Sutisna, D.H. dan., R. Sutarmanto. (1999). *Pembenihan Ikan Air Tawar*. Yogyakarta: Kanisius. (in Bahasa Indonesia)
17. Suyanto, R. (2009). *Ikan Nila*. Yogyakarta: Penerbit Kanisius. (in Bahasa Indonesia)
18. United States Environmental Protection Agency (USEPA). (2002). *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition.*
19. UPTD Padang Panjang. (2016). *Profil UPTD Penyamakan Kulit Padang Panjang. UPTD Penyamakan Kulit*. Padang Panjang. (in Bahasa Indonesia)
20. Vinodhini, R. and Narayanan, M. (2009). Heavy Metal Induced Histhopatoloical Alterations in Selected Organs of the *Cyprinus carpio* L (Common Carp). *Journal Internationa J. Environ. Res*, 3 (1), 95-100.
21. Vutukuru, S. S., Ch.Suma., K. Radha Madhavi., Juveria, J., Smitha Pauleena, J., Venkateswara Rao., and Y. Anjaneyulu. (2005). Studies on the Development of Potential Biomarkers for Rapid Assessment of Copper Toxicity to Freshwater Fish using *Esomus danricus* as Model. *International Journal of Environmental Research and Public Health*, 2 (1), 63-73.
22. Wong, C.K. (2000). Morphological and Biochemical Changes In The Gills Of Tilapia (*Oreochromis mossambicus*) To Ambient Cadmium Exposure. *Journal Aquatic Toxicology*, 48, 517-572.