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Toxicity of Copper (11) Tetraoxo Sulphate to African Catfish (*Clarias Gariepinus*) Fingerlings

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Toxicity of Copper (11) Tetraoxo Sulphate to African Catfish (*Clarias Gariepinus*) Fingerlings

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Abstract

This study was carried out to determine the toxicity of copper sulphate to African Catfish (*Clarias gariepinus*). The range – finding test was conducted twice in order to get reliable values that could be used for the definitive test. The toxicant (copper sulphate) was introduced at varying concentrations of 10g/l, 20g/l, 30g/l and 40g/l in the first range- finding test, while it was introduced at concentrations of 2g/l, 4g/l, 6g/l and 8g/l in the second range- finding test. However, in the definitive test, the toxicant was introduced at varying concentrations of 0.1g/l, 0.125g/l, 0.15g/l, and 0.175g/l. From the definitive test, the Median Lethal Concentration of copper sulphate to *Clarias gariepinus* was found to be 0.175g/l. The study showed that *Clarias gariepinus* fingerling exhibited initial erratic movement, rapid opercular movement, skin discoloration and loss of reflex. Also, histopathological alterations such as the degeneration of gill and liver tissues were observed in the fish that were exposed to the toxicant. The water quality data were analyzed by subjecting them to one – way Analysis of Variance (ANOVA).Thus, the study indicates that copper sulphate is toxic to *Clarias gariepinus* fingerlings.

Key words: Toxicity, Copper (11) Tetraoxo Sulphate, *Clarias garipenius*

Introduction

Water pollution is any chemical, physical or biological change in the quality of water, that has a harmful effect on any living thing that drinks, uses or lives (in) it (Lenntech, 1998).).Activities resulting from human population explosion as well as advancements in science and technology have led to the direct or indirect release of contaminants into surface water bodies and ground water deposits. Although, natural phenomena such as volcanoes, algae blooms, storms and earthquakes also cause major changes in water quality and the ecological status of water, water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either undergoes a marked shift in its ability to support its constituent biotic communities or does not support a human use like drinking (Wikipedia, 2008, Katryna, 2002).

Water pollution is a major problem in the global context. The primary sources of water pollution are grouped into two categories on the basis of their point of origin. The categories are; Point and non-point sources (Wikipedia, 2008). Point sources discharge pollutants (substances or matter that cause pollution) at specific locations through pipelines or sewers into the surface water, while non-point sources are those ones that cannot be traced to a single site of discharge (Lenntech, 1998, Berka et al 2001).Examples of point sources include; factories and sewage treatment plants, while those of non-point sources include: run-off from farms and fuel discharge from motorized canoes. Contaminants that pollute water include; pathogens, particulate (suspended) matter sewage and chemicals. Chemical pollution is a common type of water pollution. It is the contamination of water by chemical substances. Such activities include agricultural and industrial work which involve the use of chemicals that run-off into water and pollute it. In Agriculture, pesticides are used to control weeds, insects and fungi.

Run-off of these pesticides poisons aquatic life. Contamination of aquatic environment by heavy metals whether as a result of acute or chronic events constitutes additional source of stress to aquatic organisms (Kori-Siakpere and Ubogu 2008, James and Little 2003). When water PH falls, metal solubility increases and the metal particle becomes more mobile. That is the reason why metals are more toxic in soft water. Both localized (point source) and dispersed (non-point source) metal pollution cause environmental damage because metals are non-biodegradable (Lenntech, 1998, Adeniji and Ovie 1981). Copper is a common heavy metal pollutant in surface waters and its toxicity is largely attributable to its cupric (Cu^{2+}) form (Olaifa et al, 2004). At low concentration, copper is an essential element for both plants and animal because it is an important component of enzymes. However, it can become harmful when large single or daily intake occurs (Olaifa et al, 2004, Okaeme, 1990). At

present, high concentrations of copper have been introduced into the environment due to anthropogenic activities such as mining, electroplating, and release of effluent from paint and pigment industries as well as textile factories and the use of pesticides.

Copper occurs in form of several compounds. The cheapest and most commonly used form is copper sulphate (Watson and Yanong, 2006). It is a fungicide that is used to control bacterial and fungal diseases of fruits, vegetables and field crops; it is also used as an algaecide and herbicide and to kill snails and slugs in irrigation as well as municipal water treatment systems (Atamanalp et al, 2008). The African catfish, *Clarias gariepinus* is a fresh water fish that is widely distributed in Africa and the Middle East. It inhabits tropical swamps, lakes and rivers, Apart from its importance in capture fisheries; it is also an important aquaculture species. Nevertheless, like other aquatic animals, the existence and well-being of this species are being threatened as a result of the pollution of the aquatic environment by copper sulphate. Hence, there is the need to study the effect of copper sulphate on *Clarias gariepinus* fingerlings and determine the maximum concentration of the compound that they can tolerate. Finally fingerlings have to be used in this study because their survival determines the future availability of table-size *Clarias gariepinus.*

Justification

The African catfish, *Clarias gariepinus,* is a common food fish which is of high commercial value in Nigeria. Apart from enhancing food security, its sales serve as a source of income for many families. However, the pollution of the aquatic environment can result in mass mortality of this fish. Apart from that, chronic exposure of the fish to pollutants can impair their feeding, growth and reproductive performance. Copper sulphate is a toxic substance that is discharged into aquatic systems through anthropogenic activities. Consequently, there is the need to determine the degree of toxicity of the chemical to *Clarias gariepinus* fingerlings. Thus, it is hoped that this research work will help to determine the effects of copper sulphate on *Clarias gariepinus* fingerlings. This will go a long way to help fisheries administrators in formulating policies that will control the use of the chemical. Hence, the availability of this fish for consumption and commercial purpose will be sustained. In addition to that, aquaculturists will be able to know the concentration of copper sulphate that should be used for therapeutic and prophylactic purposes as the chemical is also used in the treatment of external ecto-parasites in fish.

Objectives of the Study

The specific objectives of the study are to:

i. Determine the median lethal concentration (LC_{50}) of copper sulphate on *clarias gariepinus* fingerlings.
ii. Determine the effect of different concentrations of copper sulphate on the histo - pathology of the gill and liver tissues *of Clarias gariepinus* fingerlings.
iii. Determine the effect of different concentrations of copper sulphate on the behaviour (swimming and reflex) of *Clarias gariepinus* fingerlings.

Methodology

Four hundred (400) life and healthy *Clarias gariepinus* fingerlings were purchased from Ondo State Ministry of Agriculture and Fisheries, Akure, Ondo State, Nigeria and were subsequently transferred in plastic containers to the Fisheries and Wildlife Laboratory of the Federal University of Technology, Akure, where they were acclimatized without food for 72 hours in plastic tanks measuring 24cmxl5cmxl2cm. The fingerlings were weighed with an electronic Mettler Toledo weighing balance (model PM 460) while the standard length was measured with a graduated ruler. The average weight of the fish was 5.84g $\pm$ 0.5 while that of standard length was 6cm $\pm$ 1.0. This was followed by the stocking of ten fingerlings of similar sizes into each plastic tank containing 10 liters of water. Each tank represented one treatment. The experiment was conducted in five (5) treatments. Four of the treatments contained varying concentrations of copper sulphate pentahydrate ($CuSO4^{5H2O)}$ the fifth treatment (control) had zero concentration of the chemical (toxicant). Each treatment had two replicates. Thus, there were ten tanks in all. Fish Mortality was monitored and recorded every 24 hours. The inability of fish to respond to external stimuli was used as an index of death.

Two range-finding tests were conducted in order to determine the actual concentration of the toxicant (copper sulphate) needed for the definitive test. In the first range-finding test, the toxicant was introduced at l0g/L, 20g/, 30g/L and 40g/L, while the control had zero concentration (0g/L). However, in the second range-finding-test, the toxicant was introduced at 2g/L, 4g/L, 6g/L, and 8g/L. The control had zero concentration (0g/L) of the toxicant. The results obtained from the range-finding test provided a guide for determining the concentration levels to be used in the definitive test. Thus, the definitive test was conducted using copper sulphate concentration of 0.10g/L 0.125g/L, 0.15g/L and 0.175g/L. the control had zero concentration (0.g/L) of the toxicant. The result obtained

from this test was used in the graphical determination of the Median Lethal Concentration (LC_{50}).

Temperature, PH, conductivity and dissolved oxygen ($D0_2$) were determined and recorded at the beginning and at the end of the experiment. The number of dead and living test organisms in each tank was counted every 24 hours. Behavioral and morphological changes were also observed. These included: erratic swimming, discoloration and loss of reflex. The LC_{50} which is the concentration of the toxicant, estimated to be lethal to 50% of test organisms after an exposure time of 96 hours was determined by graphical method. The histology of the vital organs of the test organisms was studied in order to determine the effect of copper sulphate on them. The organs that were studied include: gill and liver. The water quality data were analyzed by subjecting them to one – way Analysis of Variance (ANOVA).

Results

The range finding test was conducted twice in order to derive reliable values for the definitive test. Some behavioral changes were displayed by the fingerlings during the two tests. These changes include: erratic swimming; (which occurred shortly after the introduction of the toxicant), discolouration and loss of reflex (which occurred before death). In the two tests, mortality did not occur in the control group. However, total mortality occurred in the remaining four treatments. Mortality was directly proportional to the concentration of the toxicant and the length of exposure of the fish to the toxicant. The afore-mentioned total mortality occurred within the first 24 hours in the first range-findings test, while it occurred within the first 72 hours in the second range-finding test. The mortality data of the two range-finding tests are presented in tables 1.0 and 2.0 below.

TABLE: 1 Percentage Cumulative Mortality of the Range Findings Test

Concentration of Copper Sulphate	24 hours %M	48 hours %M	72 hours %M	96 hours %M
C (0g/L)	0	0	0	0
Tl (l0g/L)	100	100	100	100
T2 (20g/L)	100	100	100	100
T3 (30g/L)	100	100	100	100
T4 (40g/L)	100	100	100	100

KEY: M = Cumulative Mortality.

TABLE: 2 Percentages Cumulative Mortality of the Range Finding Test II

Concentration of Copper Sulphate	24 hours %M	48 hours %M	72 hours %M	96 hours %M
C (0g/L)	0	0.	0	0
Tl (2g/L)	75	90	100	100
T2 (4g/L)	100	100	100	100
T3 (6g/L)	100	100	100	100
T4 (8g/L)	100	100	100	100

KEY: M = Cumulative Mortality.

In the definitive test, the toxicity of the toxicant increased with its increasing concentration and length of exposure of the test organism to it. Behavioural changes such as loss of reflex and skin discoloration were more noticeable in the treatment that had the highest concentration of toxicant. Mortality only occurred in the two treatments with the highest concentrations of 0.15g/L and 0.175g/L respectively. The mortality data and the changes observed in each treatment are presented in tables 3.0 and 4.0 respectively.

TABLE: 3 Percentages Cumulative Mortality of the Definitive Test

Concentration of Copper Sulphate	24 hours %M	48 hours %M	72 hours %M	96 hours %M
C (0g/L)	0	0	0	0
T1 (0.lg/L)	0	0	0	0
T2 (0.125g/L)	0	0	0	0
T3 (0.15g/L)	0	20	20	20
T4 (0.175g/L)	30	50	50	50

KEY: M = Cumulative Mortality

Table: 4 Observations during the Definitive Test

KEY: - = Not Present, + = Present, T1= 0.1g/l, T2= 0.125g/l, T3= 0.15g/l, T4= 0.175g/l and C= Control

	24 Hours					48 Hours					72 Hours					96 Hours				
	C	**T1**	T2	T3	**T4**	**C**	**T1**	**T2**	**T3**	**T4**	**C**	**T1**	**T2**	**T3**	**T4**	**C**	**T1**	**T2**	**T3**	**T4**
Erratic Swimming	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of Reflex	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+
Rapid Opercula Movement	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	+	+
Discoloration	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Release of Bubbles	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Death	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-

Table: 5 Water Quality Measurements

	Water Temperature(^{0}C)		PH		Conductivity (mv)		Dissolved (mg/l) Oxygen	
	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL
C (0.g/L)	27.5 ± 0.000	30.55 ± 0.500	7.30 ± 0.000	7.075 ± 0.050	34.0 ± 0.000	34.0 ± 0.000	6.96 ±0.025	3.94 ± 0.800
T_1 (0.1g/L)	27.5 ± 0.000	30.55 ± 0.500	7.31 ± 0.000	7.055 ± 0.050	36.0 ± 0.000	34.0 ± 0.000	6.50 ±0.000	3.73 ± 0.800
T_2 (0.125g/L)	27.5 ± 0.000	30.55 ± 0.500	7.00 ± 0.000	7.055 ± 0.000	36.0 ± 0.000	34.5 ± 0.000	6.20 ±0.120	3.39 ± 0.300
T_3 (0.15g/L)	27.5 ± 0.000	30.55 ± 0.500	7.00 ± 0.000	7.060 ± 0.000	36.0 ± 0.000	35.0 ± 0.000	5.94 ±0.000	3.270±0.150
T_4 (0.175g/L)	27.5 ± 0.000	30.55 ± 0.500	6.99 ± 0.000	7.065 ± 0.050	36.5 ± 0.500	34.5 ± 0.000	5.61 ±0.115	3.11 ± 0.700

Table: 6 Final Water Parameters and their Significant Different

Parameters	Control	T_1	T_2	T_3	T_4
Temperature	30.55 ± 0.500^a	30.55 ± 0.000^a	30.55 ± 0.000^{ab}	30.55 ± 0.000^b	30.55 ± 0.00^b
PH	7.075 ± 0.050^a	7.055 ± 0.050^a	7.055 ± 0.050^{ab}	7.075 ± 0.050^{ab}	7.075 ± 0.050^b
Conductivity Dissolved	34.00 ± 0.00^a	34.00 ± 0.00^a	34.50 ± 0.50^{ab}	35.00 ± 0.00^b	34.50 ± 0.00^b
Oxygen	3.94 ± 0.800^a	3.743 ± 0.800^{ab}	3.39 ± 0.300^b	3.27 ± 0.150^c	3.11 ± 0.800^c

Means and standard error on the same row with the same superscript are significantly different at P> 0.05.

At the end of the experiment, there were changes in the water parameters. The most noticeable changes were, however, observed in the dissolved oxygen content, which reduced drastically. The results of the water quality measurements are shown in table 5.0. The gills and the liver of *Clarias gariepinus* fingerlings were observed and examined to assess the histological effect of copper sulphate which was applied to the fish at different concentrations. The results on the slides shows that there was, no damage whatsoever on the liver and gills of the fish in the control experiment. On the contrary there were varying degrees of damages and degenerations to the gills and liver of the fish in other concentrations with increasing damages from the lowest concentrations to the highest concentration.

The gills showed a slight vacuolation (vacuole formation) in the fish gills of fingerlings in O.lg/L and 0.l25g/L of copper sulphate solution. The liver of *Clarias gariepinus* fingerlings exposed to O.lg/L and 0.125g/l. showed a slight cellular change with small space formations. At higher concentrations of O.15g/L and ().175g/l. of copper sulphate, there was pronounced damages to the gill filaments with fragmentation of the lamella in O.175g/L solution of the toxicant. Also, the cellular changes within the liver cells were significant with large vacuole formation in the liver tissues and distortion in the cells.

This experiment therefore indicates that copper sulphate has a direct and significant impact on effect on the internal organs of *Clarias gariepinus* fingerlings particularly the liver and the gills.

Discussion

Contamination of the aquatic environment by heavy metals, whether as a consequence of chronic or toxic events constitutes additional sources of stress for aquatic organism. Copper occurs naturally within the environment at low concentration, it is an essential element both for plants and -animals. However, large doses can be harmful. Copper sulphate is used as a fungicide to control bacterial and fungal diseases of fruits vegetable, nut and field crops. It is highly toxic to fish. Fish species are most sensitive to aquatic pollutants during their early life stages. Consequently, it is necessary to monitor the impact of copper sulphate on different fish. In this study, the fish that were exposed to the highest concentration of copper sulphate displayed erratic swimming. This is similar to the result obtained by Ayotunde and Ofem (2008), where adult *Oreochromis niloticus* exposed to pawpaw seed powder swam erratically.

Furthermore, it was discovered in this study that the *clarias gariepinus* fmgerlings eventually exhibited loss of reflex, skin discolouration and rapid opercular movement. This agrees with the work of Ayotunde and Ofem (2008), in which adult tilapia *{Oreochromis niloticus)* were reported to have exhibited loss of reflex, molting discoloration and air gulping. Thomas and Rice (2005) suggested that increased opercular movement may be caused by decreased efficiency in oxygen uptake or transport; thus, the rapid opercular movement displayed by *C; gariepinus* fingerlings in this study is an indication of reduced oxygen uptake. The marked decrease that occurred in the dissolved oxygen levels of the treatments further buttresses the fact that therapid opercular movements resulted from reduced oxygen uptake. Lloyd (1961) reported that the toxicity of several poisons to rainbow trout increased inversely to the concentration of water. Therefore the decrease in the dissolved oxygen recorded in this study could have increased the toxicity of copper sulphate to *Clarias gariepinus* fingerlings.

Conclusion

This study was carried out by exposing *Clarias* ***gariepinns*** fmgerlings to copper sulphate. The fmgerlings that were exposed to the toxicant (copper sulphate) exhibited loss of reflex, rapid opercular movement and discolouration. Histopathological alterations were also observed in the gill and liver tissues. The toxicity of the test media could not have occurred as a result of the PH and temperature because their values obtained before and after the experiment were tolerate to the test organism. The dissolved oxygen, however, decreased tremendously. This could have increased the toxicity of the toxicant and consequently contributed to the mortalities that were recorded during this experiment. In conclusion, copper sulphate is highly toxic to *Clarias gariepinus* fingerlings.

Recommendation

Although, copper sulphate is a useful chemical that serves many purposes, it has to be used with caution because of its potential as toxicant. It can have chronic and acute effects on fish. Also, consumers may be exposed to hazards by consuming copper sulphate-contaminated fish. Thus, there is the need to carry out further works in order to determine the Maximum Admissible Toxicant Concentration (MATC) of copper sulphate to *Clarias gariepinus* fingerlings

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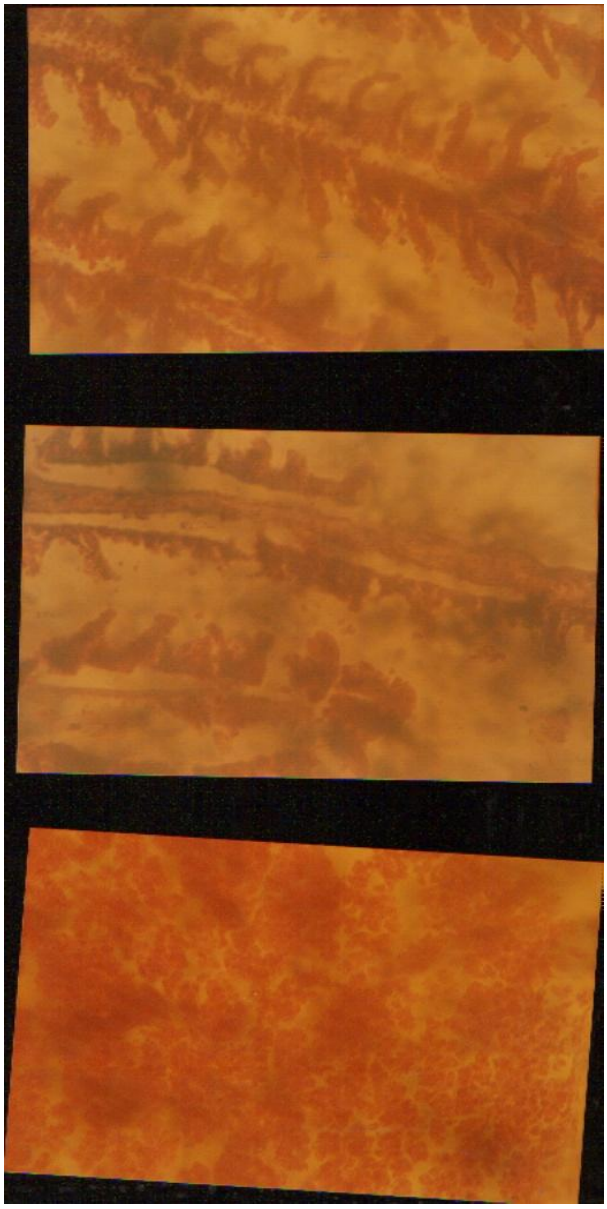
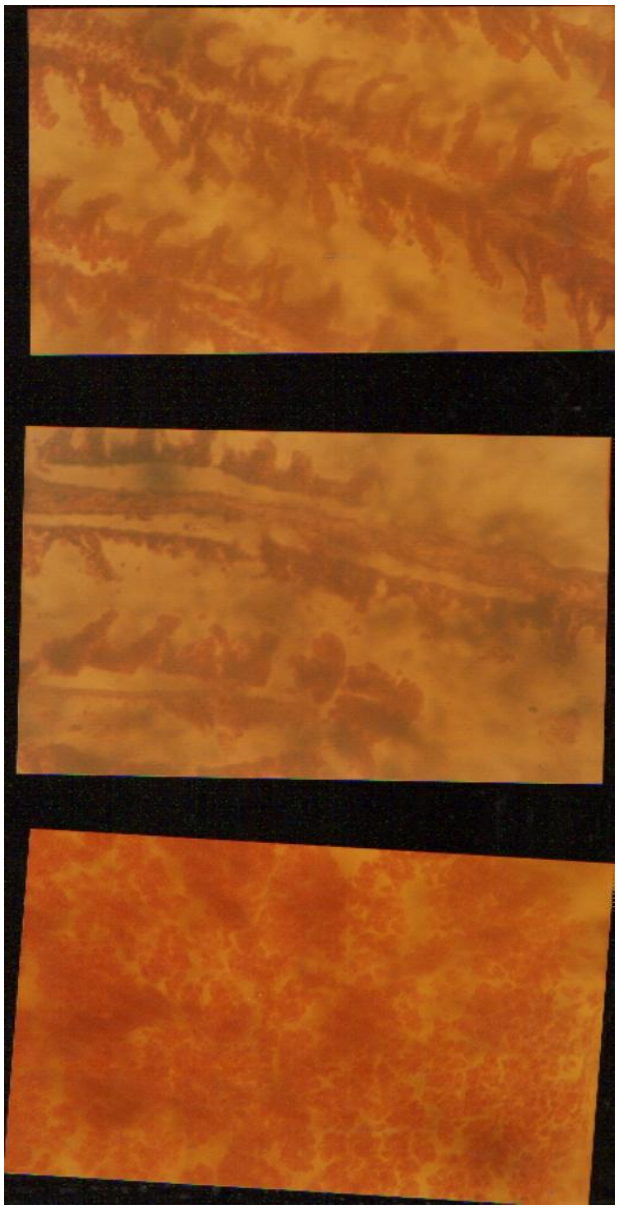


The gill of the fingerling in the control experiment showing no histopathological alteration.

The gill of the fish in treatment 1 (0.10g/L of the toxicant) showing slight vacuolation.

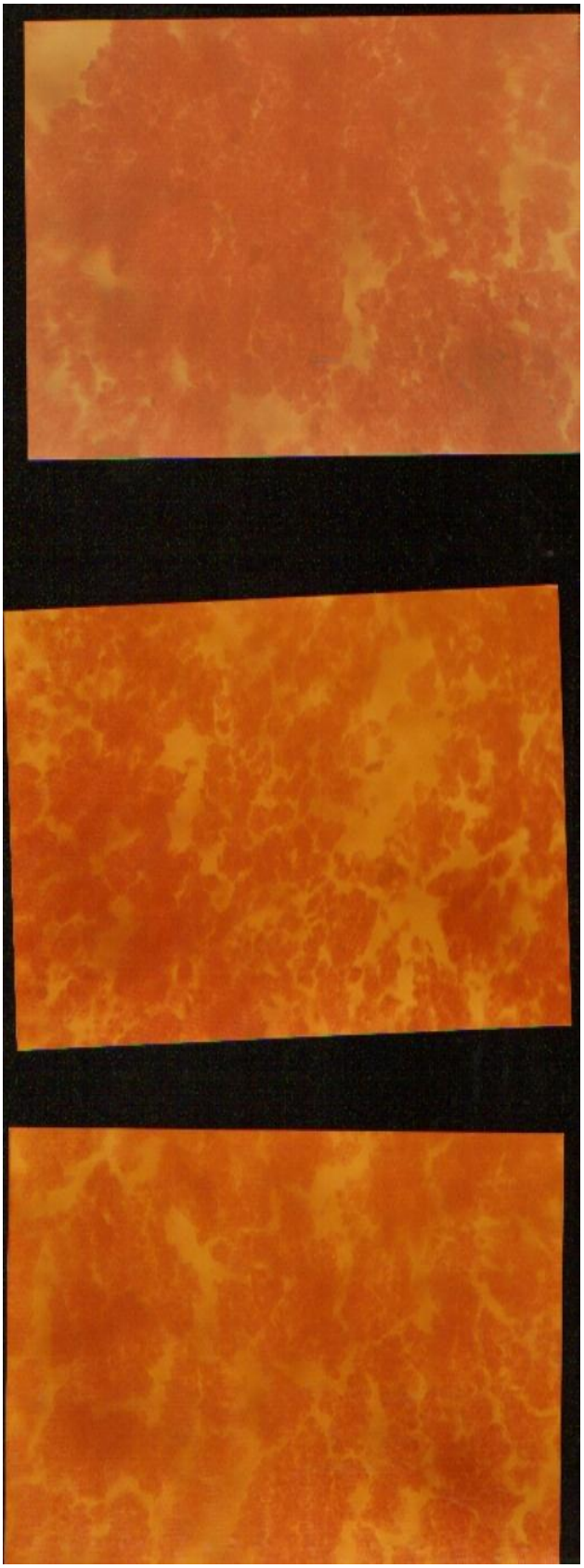
The gill of the fish in treatment 2 (0.125g/L of the toxicant) showing slight vacuolation.



The gill of the fish in treatment 3 (0.15g/L of the toxicant) showing extensive vacuolation.

The gill of the fish in treatment 4 (0.175g/L of the toxicant) showing extensive vacuolation of the tissue and the fragmentation of the lamella

The liver tissue of the fish in the control experiment showing no histological alteration.



The liver tissue of the fish in treatment 1 (0.10g/L of the toxicant) showing small space formation

The liver tissue of the fish in treatment 2 (0.125g/L of the toxicant) showing vacuole formation

The liver tissue of the fish in treatment 3 (0.15g/L of the toxicant) showing large vacuolation.