



HISTOLOGICAL STUDY ON GILL OF *Tilapia guineensis* EXPOSED TO SODIUM DODECYL SULPHATE

Oghenetega Judith Ibekwe¹ * and Sixtus Eberechukwu Ibekwe²

¹Department of Pure and Industrial Chemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

²Department of Microbiology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria.

Email address:

*Corresponding author: sixtus.ibekwe@yahoo.com and sixtus.ibekwe@uniport.edu.ng

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ABSTRACT

Oil spill environments are cleaned up using dispersants - sodium dodecyl sulphate (SDS). Dispersants are chemicals containing surfactants that break liquid substance like oil into small droplets. Surfactants are dispersive chemicals having polar moiety and non-polar moiety. Acute toxicity of SDS was carried out on *Tilapia guineensis* by exposing solutions of SDS concentrations using semi-static agitation procedure. Histological staining was done as biological attempt to explain chemical reactions between tissues and dyes, and as health concern to investigate effects of SDS on *Tilapia guineensis*. Gills were preserved to enable smearing, dissecting and cutting of the tissue into sections of 5-20 microns. Sectioning and embedding were achieved with microtome and molten paraffin which supported tissue cutting. Tissues were stained with Haematoxylin and eosin which rendered cells conspicuous under the microscope. Control showed normal gill histology of *Tilapia guineensis* whereas there was congestion and narrowing of intra-tubular space within gill in 40 mg/L and 80 mg/L after 12 h exposure to SDS. This effect could be attributed to severe damage due to different concentrations of SDS resulting to expansion of lymphatic channels and cell membrane vasculature, disruption of micro-ridge, and cell degeneration. The same result was observed at 80 mg/L after 24 h exposure but with more severity. These results suggest that SDS at these concentrations have been found to be toxic to living tissues and the environment.

1. INTRODUCTION

Dispersants are chemicals containing surfactants or compounds that act to break liquid substance like oil into small droplets. Surfactants are detergents having a polar moiety and a non-polar moiety [10]. Dispersants are known to be toxic based on concentration. The data available on the toxicological effects of dispersants in plants,

mammals, fishes and microbes have been recorded (8, 4). The dispersant- sodium dodecyl (SDS) has been used in oil spill cleanup because it is able to break up oil into finer or tiny particles, though has effects on organisms based on the concentration and the time of application and duration of exposure [2, 3, 6].

Bioassay involving acute toxicity test of SDS was done on test organism *Tilapia guineensis* by exposing the organisms to solutions of different concentrations using the semi – static agitation test procedure as recommended by Department of Petroleum Resources [1].

The histological investigation involved analysis of gill to find out the effect of SDS on organs and tissues of *Tilapia guineensis*. Histological staining was carried out as a biological attempt to explain chemical reactions between tissues and dyes. Staining reaction shows concern on the state of health of living animal based on the understanding that all biological appearances are a reflection of chemical reaction. Gills were preserved in 10% formol saline to reduce cells thickness and enable smearing, dissecting and cutting of the tissue into sections of 5-20 microns in order to prevent distortion of cells and preserve the relationships of the cells in the tissue. Sectioning and embedding were achieved in microtome and molten paraffin which supported the tissue cutting. Gill- organ for respiration in fish were stained with Haematoxylin and eosin stains which rendered the tissues and cell constituents prominent and conspicuous under the microscope wherein Haematoxylin stains the nucleus blue and eosin stains the cytoplasm pink. The stained tissue on the slide was mounted on Canada balsam under a cover slip and then examined [5].

2. MATERIALS AND METHOD

2.1 Experimental Location

The experiment was done at the Environmental Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria. The test dispersant-SDS was obtained from dealers in Port Harcourt who supply them to the Oil companies.

2.2 Sampling and Test Animals

Fingerlings of *Tilapia guineensis* were sourced from African Regional Aquaculture Centre (ARAC)

at Aluu near University of Port Harcourt, Rivers State, Nigeria. They were caught with cast nets and were transferred immediately into 25 litre open plastic containers containing the habitat water. A total of 300 fish were caught and were used for the experiment. The weights and lengths of the test animals were determined with analytical balance and metre rule respectively. It was ensured that there was not more than a two-fold difference between the minimum and maximum sizes (as body length) of the specimen used [4].

2.3 Acclimatization and Selection of Test Organisms

Test organisms were first acclimatized ten (10) days at room temperature (28 ± 2 °C) in properly aerated glass aquaria. *Tilapia guineensis* was fed with fish feed obtained from ARAC. The acclimatization water was replaced with fresh water from the organism's habitat daily. Controlled lighting system was supplied (12 h of light and 12 h of darkness). Ten (10) test organisms of almost equal size were randomly caught with hand nets from acclimatization tanks and gently transferred into the test vessel. There was no hand contact with fish during the selection so as to avoid stress due to handling. Only healthy and active *Tilapia guineensis* were selected [4].

2.4 Semi-static Renewal Bioassay

Bioassay involving acute toxicity tests on *Tilapia guineensis* was done by exposing test organisms to test solutions containing different concentrations of SDS using the semi – static agitation test procedure as recommended by DPR [1]. Five different concentrations of test toxicants (20, 40, 60, 80 and 100 mg/L SDS) were prepared in triplicate using habitat water of the organism as diluents and stirred for 5 min for proper mixing and subsequently stirred at intervals of 5 min. Ten (10) healthy, active *Tilapia guineensis* were gently introduced into bioassay vessels (glass aquarium of 50 cm x 30 cm) at different toxicant concentrations.

Controls containing habitat water and 10 test organisms were prepared without SDS. Each of the test concentrations were labelled appropriately. At the end of each day, the media were replaced with fresh one at the same toxicant concentration and this continued each day for four days. Dead organisms were removed at the end of each exposure period in order to prevent contamination of live organisms by bacteria from dead and decaying organisms. Mortality was recorded after 24, 48, 72 and 96 h exposure period [8].

2.5 Steps in Histological Study

2.5.1 Fixation: Organs and tissues used in this study were fixed immediately so as to preserve the various constituents of the cell in their normal micro anatomical positioning and prevent them from any degenerative or autolytic changes. Fixation also harden the tissue providing it a constituency similar to paraffin in which it was embedded later for easy cutting into thin section. The above purposes were achieved by use of chemical substances, called fixative. The fixative used here was 10% formol saline. The size of tissue used in this study has 3mm by 3mm and the time allowed in 10% formol saline was 6-12 h [5].

2.5.2 Dehydration: Since the tissue contains water which is not mixable with paraffin. The water content of the tissue was removed by the process of dehydration using ascending graders of alcohol (50%, 70%, 90%, 95%) and lastly (100%) absolute alcohol. The tissue ideally passed slowly through ascending concentration of alcohol because if it was put directly into absolute alcohol, water would rush out suddenly, disturbing and damaging the cell structure. For tissues like one of this study, the time allowed in each grade of alcohol is 1-2 h and in absolute ethanol, it was allowed twice for one to 2 h each time [5].

2.5.3 Clearing: Since alcohol was also not miscible with paraffin, the tissue was infiltrated with such

chemical substances which not only replaces alcohol but also could mix with paraffin. Xylene which could make the opaque tissue transparent and also mixable with paraffin was used as clearing agent. The tissue was passed twice in xylene for one to 2 h each time.

2.5.4 Impregnation/infiltration: The tissue was finally placed in molten paraffin wax at a constant temperature of 60°C in a paraffin bath and it was change for about two hours each time.

2.5.5 Embedding: Special 'L'-shaped metal block was taken and filled with molten paraffin wax and then the tissue was placed in immediately with forceps. The surface to be cut must be facing downward. The paraffin was allowed to cool so that a thin scum of solid paraffin was formed on the bottom of the metal blocks, after which it was immersed in water. Upon cooling, the L- metal blocks were removed from the solidified paraffin. The paraffin block and the tissue inside it have the same consistency and were ready to be sectioned [5].

2.5.6 Microtomy: For microscopic study the sections were cut extremely thin i.e between 5 microns and 15 microns for which a special instrument called microtome was used. The type use here was rotary microtome.

2.5.7 Staining: For different elements of the cells to be rendered conspicuous, certain dyes were used to give them contrasting colours for easy recognition. The dyes used in these histological preparations were haematoxylin and eosin (HE). The haematoxylin stained the nucleus blue while eosin stained the cytoplasm pink [5].

3. RESULTS AND DISCUSSION

3.1 Histological Analysis of gill

As concerns mortality, SDS is highly toxic even at

low concentration. Despite the indubitable usefulness of oil dispersants - SDS, the potential toxicological effects of SDS on living beings especially fish must be taken into consideration. Histological analysis of organs and tissues involves staining which is usually a biological attempt to explain chemical reactions between tissues and dyes. Staining reactions reflect the state of health of living animal or plant tissues based on the understanding that all biological appearances are reflections of chemical reactions [5]. The result presented here described the toxicological effects of SDS on *Tilapia guineensis*.

Controls were assumed to be normal tissues or organs which reflect the state of good health of animal organs and tissues. Deviations from these were considered pathological (diseased) disorder and all disease states were abnormal physiological reactions.

Gill Histology of *Tilapia guineensis* Exposed SDS after 12 hours

Control (a) shows normal gill histology of *Tilapia guineensis*. There was congestion of the intra-tubular space and narrowing of the intratubular space within the gill in the 40mg/L (b) and 80mg/L (c) SDS administered samples as shown in plate 1.

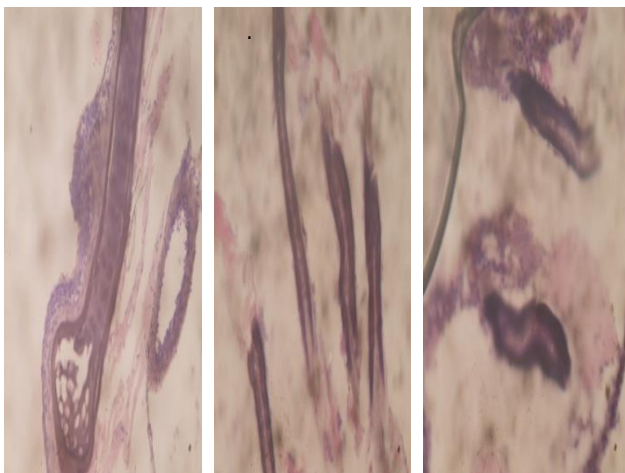


Plate 1: Gill: Control (a), 40mg/L SDS (b), 80mg/L (c) SDS after 12 h

Gill Histology of *Tilapia guineensis* Exposed SDS after 24 hours

Control showed normal gill histology of *Tilapia guineensis*. There was congestion of the intra-tubular space and narrowing of the intra-tubular space within the gill in the 80mg/L with more severity after 24 h SDS administration as shown in plate 2. This could be attributed to severe damage due to this concentration of the dispersant which included the expansion of the lymphatic channels and cell membrane vasculature, disruption of the micro-ridge, and cell degeneration.

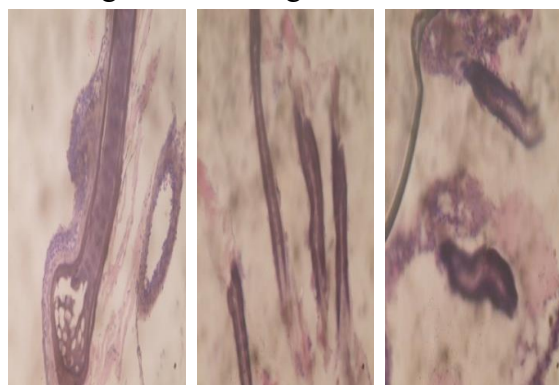


Plate 2: Gill: Control (a), 80mg/L (c) SDS after 24 h

Gill Histology of *Tilapia guineensis* Exposed SDS after 72 hours

Control (a) showed normal gill cytoarchitecture. The test shows 40mg/L (b) and 80mg/L (c) all administration after 72 h. There is severe ulceration of the tissue architecture. This is shown in plate 3 [5].

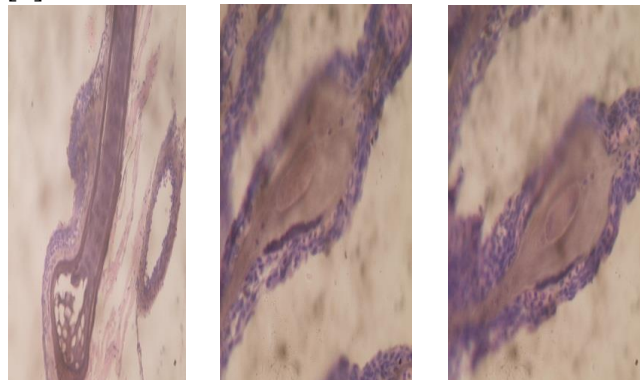


Plate 3: Gill: Control (a), 40mg/L SDS (b), 80mg/L (c) SDS after 72 h

Dispersants are chemicals that contain surfactants or compounds that act to break liquid substance such as oil into small droplets [10]. Surfactants (surface-active agents) are deter size chemicals having a polar moiety (hydrophilic or lipophobic) and a non-polar Moiety (hydrophilic or lipophilic). Dispersants are known to be toxic based on concentration.

The data available on the toxicological effects of dispersants in plants, mammals, fishes and microbes have been recorded. Dispersant-sodium dodecyl (SDS) have effects on these organisms based on the concentration and the time of application. The result presented here described the toxicological effects of this dispersant on test organism *Tilapia guineensis*. SDS is known to cause injurious effect on humans and animals. Those harmful effects depend on the intensity of concentrations and length of exposure. Some of the effects of SDS in fish-*Tilapia guineensis* are illustrated here.

Histological staining is usually a biological attempt to explain chemical reactions between tissues and dyes. Staining reaction reflects the state of health of living animal or plant tissues based on the understanding that all biological appearances are a reflection of chemical reaction [5]. From histomorphological result of test organism -*Tilapia guineensis* exposed to various concentrations of SDS showed that control appeared normal while the exposed was distorted, defigured or altered. Controls were assumed to be normal tissues which reflect the state of good health of animal tissues. Deviations from these were considered pathological (diseased) disorder. And all disease states were abnormal physiological reaction.

For all the gills after 12 h exposure at concentration of 40 mg/L, there is congestion of the intra-tubular space and narrowing of the intra-tubular space. This could be attributed that the gills showed severe damage due to these concentrations of SDS which included the expansion of the

lymphatic channels and cell membrane vasculature, disruption of the micro-ridge, and cell degeneration. The same result was observed at 80 mg/L after 24 h exposure but with more severity. This study is in agreement with Rosety et. al., [8] on gill head (*sparus aurata*) exposed to various concentration of SDS and they discovered induced morphological changes in the spleen and kidney with a significant inhibitory effect on fertilization success.

Dispersants have been found to be toxic to the environment and this has been discovered by careful toxicity testing which represent one set of tools that can be used to evaluate possible adverse ecological effects. Tests appropriate to specific circumstances were selected and that the tests were conducted in such a manner as to be useful in supporting remedial solution. The dispersant SDS showed toxic effect to the test organism *Tilapia guineensis* at different concentrations. Hence SDS should be regarded as toxic dispersant at various concentrations and exposure times.

4. CONCLUSION

The results of acute toxicity testing which is one set of tools that can be used to evaluate possible adverse ecological effects suggests that SDS is highly toxic even at low concentration and have been found to be toxic to living tissues and the environment. Despite the indubitable usefulness of oil dispersants -SDS, the potential toxicological effects of SDS on living beings especially fish must be taken into consideration.

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