Acute Toxicity of Antifoam Polydimethylsiloxane on African Catfish (Clarias gariepinus, Burchell 1822) Juveniles

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Abstract
The study investigated the acute toxicity of antifoam solution (Polydimethylsiloxane) on the Juveniles of *Clarias gariepinus* (mean weight 5.5g ± 0.8g) using static bioassays over a period of 96hrs. The histological changes in the gills and liver were determined after the exposure to the acute concentrations of the chemical (0.0mg/l, 750mg/l, 1000mg/l, 1250mg/l, 1500mg/l) Water quality variables (pH, Temperature, Total Dissolved Solids, Conductivity and Dissolved Oxygen) monitored during the acute toxicity test revealed a decrease in dissolved oxygen (7.8 to 4.4 mg/l), and pH (7.5 to 3.5), while an increase in conductivity (18.2 to 95.8µs) and total dissolved solids (11.8 to 63.0 ppm) were recorded when compared with the control. Certain behavioral changes including erratic swimming, jumping, incessant gulping of air and loss of balance, were observed before death, which increased with increase in antifoam concentration. The main histological changes observed in gills exposed to antifoam include fused secondary lamella, cyst in secondary lamella, distorted epithelium darkening of the hyaline cartilage and thickening of the epithelium. The liver of control group exhibited a quite normal architecture while the fish exposed to antifoam showed vacuolation and necrosis. The data indicated that the antifoam had significantly affected some water quality variables, histology of gill and liver tissue of the test fish. The implication of these findings is that the antifoam has negative effect on the test fish which may partly help explain the decline in wild fishery resources, especially in areas where there are oil drilling activities.

Keywords: Acute, Clariasgariepinus, Toxicity, Histology, Antifoam.

1. INTRODUCTION

Aquatic pollution as a field of study has gained a lot of attention over the decades majorly because of the vital role the aquatic environment plays in the human lifecycle[1]. Pollution of the aquatic environment by toxic substances is a growing cause of concern in developing countries throughout the world such as Nigeria. Nigeria being one of the top oils producing country in Africa is faced with challenging issues of environmental pollution[2].The Niger Delta region in Nigeria has the largest Delta in Africa and one of the largest wetlands in the world. Niger Delta ecosystem is of high productivity that supports important local and commercial fisheries [3]. Despite the oil and gas exploration and production in the Niger Delta area of Nigeria, the area is classified as the most severely damaged ecosystem in the world[4]. During oilfield exploration, additives such as rheological modifiers, antifoams and shale inhibitors are frequently added into drilling fluids to reduce shale swelling and foam formation. However, some drilling fluids contain additives which are environmentally unacceptable and are particularly hazardous to aquatic organisms [5].

The term anti-foam agent and de-foamer are often used interchangeably. They are a chemical additive that reduce and hinder the formation of foam in industrial processes. Foams pose serious problems such as; prevent the efficient filling of containers and clog the machine. Antifoams are used in many industrial processes such as wood pulp, paper, paint, and plastic manufacturing industry[6].As reported by[7], high concentration of Silicone antifoam caused detrimental effects on sparged cultures which applies to large scale animal.According to Histological characteristics of species organs are used to express both endogenous and exogeneous impacts of pollutants on the organism as a result of pollution.As reported by [8]and [9], studies on toxicity of fish under controlled condition provide thorough evaluation of behavioural, physiological and morphological changes that correspond to the effects of pollutants on the biota. Therefore, standard toxicity tests should be performed in the laboratory to predict the effect of such chemical in the aquatic ecosystem[10].The study of the acutetoxicity of antifoam on common aquatic organisms has been scarcehence the need for this study.

2. MATERIALS AND METHODS

2.1Collection of Test Organisms and chemical

One hundred healthy life postfingerlings of *Clarias gariepinus* (catfish) of average weights 5.5g±0.8g and standard lengths 4.67cm ±1.0cm was obtained from Bel farm in Aluu, Port-Harcourt. The fish were transported during the early hours of the day in plastic aerated containers of 50 litres capacity to the Animal House where the experiment was conducted.The acclimatization lasted for a period of two weeks (14days)[11]. The water tanks were cleaned and water renewed regularly. Feeding commenced from day two at 3% body weight using foreign feed of 40% crude protein. Feeding was done twice daily and leftover food was siphoned out. During the acclimatization period, the fish were gradually subjected to dilution water until they

could survive in the dilution water without showing signs of stress such as discolouration or unusual behaviour. Feeding was stopped 24hours prior to the commencement of the experiment. Both male and female were used for the experiment without discrimination. The test chemical was purchased from Epoxy Oil Firm, Port-Harcourt, Rivers state.

2.2 Acute Toxicity Test

Acute 96hr bioassay was conducted with two replicates for each concentration using static non-renewal bioassay[12]. A preliminary range finding test was carried out prior to the actual toxicity test to determine the initial safe concentration to use, which is the concentration of test solution needed to cause mortality to the fingerlings within 96hours [13]. From the range finding test, five treatments which gave 0.0mg/l (control), 750mg/l (Treatment1), 1000mg/l (Treatment2), 1250mg/l (Treatment3) and 1500mg/l (Treatment 4). Water quality parameters of the test tanks were monitored using standard methods[14]. Observations were made during the first 6hrs and 12 hours subsequently for 96hours. The behaviour and general conditions of the fish were observed from the time of introduction and mortality was used as a measure of toxicity. Fish which lost their equilibrium, floated ventral side up and did not respond to touch were considered dead and removed immediately.

A gill arch of the right side of each fish was collected and fixed in Bouin's fluid for24h, dehydrated in graded ethanol concentration and embedded in paraffin wax. Sagittal sections (5µm thickness) were cut and mounted on glass slides. Sections of the liver were deparaffinized in xylene, hydrated in ethanol and stained with haematoxylin-eosin (HE). The liver was quickly dissected, sliced into 3mm thick slabs and immersed in Bouin's fixative for 24h, dehydrated and embedded in paraffin. Histological sections (5µm of thickness) were cut and stained with HE.Changes induced by treatment in the gill and liver tissues were viewed at x400 magnification in an Olympus light microscope and photographed[15].

3. RESULT

3.1Physio-chemical Parameters

The data on the physicochemical water variables are presented in Table 1. Water quality parameters monitored during the experiment varied slightly when compared with the control. The temperature was observed to have remained relatively constant ranging from 27.2^0C to 28.1^0C, while dissolved oxygen reduced from 7.8mg/l in Treatment4 to4.4mg/l in Treatment1. The pH values indicated that the medium changed from being slightly alkaline to acidic that is, from 7.5 to 3.5 which implies that the medium was not optimum for fish growth which has been reported to be 6.5-8.5[16]. Total Dissolved Solid (T.D.S) and conductivity increased in value from Treatment4 to Treatment.

Table 1:Physio-Chemical Parameters of the different concentrations for Acute Toxicity Test

Parameters	**Control 0.0 mg/L**	**Treatment1 750mg/L**	**Treatment2 1000 mg/L**	**Treatment3 1250mg/L**	**Treatment4 1500mg/L**
pH	7.5±0.13[a]	5.5 ±1.49[b]	4.6 ±1.47[b]	4.0±1.86[b]	3.5± 1.50[b]
DO	7.8 ±0.25[a]	5.9 ±1.60[b]	5.6 ±1.15[b]	4.5 ±1.48[b]	4.4± 1.32[b]
Temp.(°C)	27.2 ± 0.62[a]	27.4 ±0.35[a]	27.3 ±0.59[a]	28.0 ±0.71[a]	28.1±0.53[a]
TDS (mg/L)	11.8±1.70[a]	29.5 ±1.89[a]	36.6 ±1.92[a]	46.6 ±1.91[a]	63.0±1.77[a]
Conductivity (Us/cm)	18.2±1.39a	44.8 ±2.83[a]	47.1 ±2.87[a]	66.1 ±2.92[a]	95.8±2.67[a]

Note: Values in each row with the same superscript are not significantly different at P>0.05

3.2 Mortality:

The mortality of *Clarias gariepinus* juveniles exposed to the antifoam *Polydimethylsiloxane*is shown in table 2. There was no mortality in the control group while mortality increased as concentration among the treatment groups increased. The lowest concentration which was 750 mg/L recorded 20% mortality while the highest concentration 1500 mg/L had 90% mortality rate. The ANOVA revealed a statistically significant difference between treatments for fish mortality at P <0.05. The 96 hours LC50 which is the lethal concentration that kills 50% of the test organism in 96hrs, was observed at 1023mg/L concentration, as shown in Fig 2.

Table 2: Mortality of *Clarias gariepinus* exposed to acute concentrations of Polydimethylsiloxane

Treatments (Conc. in mg/L)	**Number of Fish/Tank**	**Total Mortality**			**Probit Mortality**	**Percentage Mortality**
		Replicate 1	**Replicate 2**	**Replicate3**		
0.010	0	0	0	0	0.00	0
75010	2	1	1		4.16	20%
100010	3	2	3		4.75	40%
1250	10	6	5	3	5.52	70%
150010	8	6	4		6.28	90%

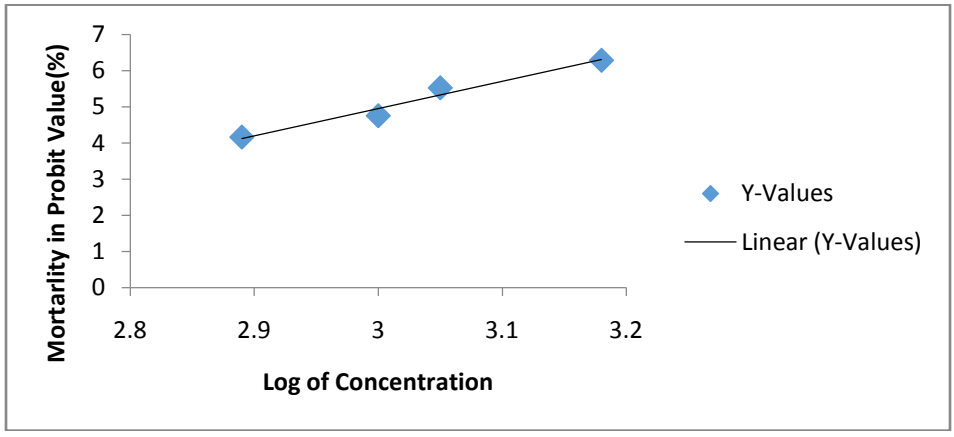


FIG 1: Graphical representation of the mortality in probit value of *Clarias gariepinus* exposed to Antifoam Solution.

3.3Behavioural Responses

Different behavioural responses were exhibited by the test fish during the exposure period. The responses include, loss of equilibrium, rapid swimming, and attempt to jump out of the tank, air gasping followed by opercula movement until responses to stimuli ceased completely. It should be noted however, that no behavioural change occurred in the control tank. On visual examination, most of the dead fish has severed tail fin, haemorrhaged gill and erosion of the head. The skins of some dead fish were whitish and were too soft having lost their protective linings. Signs of respiratory stress before death were shown by the widely opened mouth and opercula plates of the dead fish.

3.4 Histological Changes

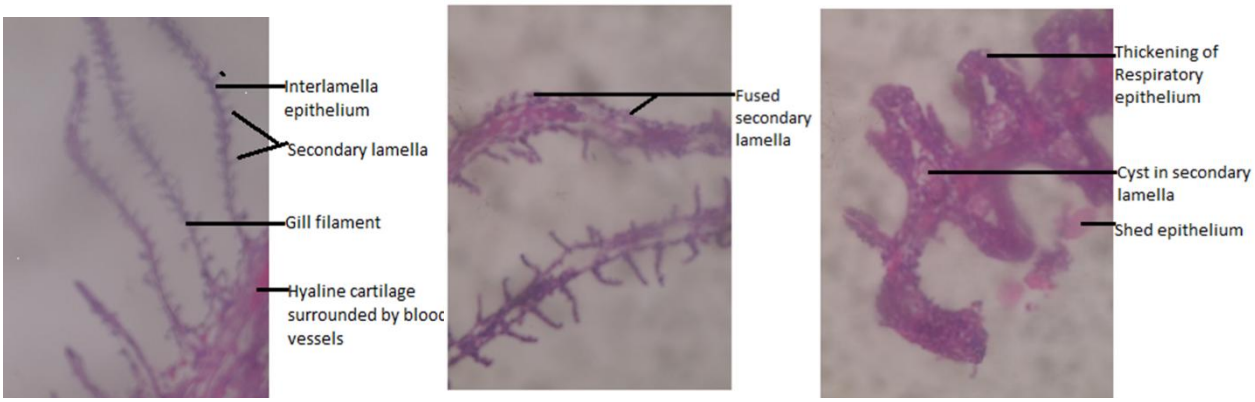
3.4.1 Gills

The histological sections of the gills of *C. gariepinus* fingerlings exposed to antifoam solution for 96hours and the control are presented in Plates 1 The gill hemibranchs of the unexposed fish consists of long thin filaments, the primary lamellae, which project from the arch, like the teeth of a comb. The surface area of the primary lamella is further increased by the formation of regular semi lunar folds across its dorsal and ventral surfaces by the secondary lamellae. The gills of fish exposed to 750mg/l of the concentration showed fused secondary lamella. The gills of fish exposed to 1000mg/l of antifoam showed cyst in secondary lamella and distorted epithelium. The gills of fish exposed to 1250mg/l of the antifoam showed darkening of the hyaline cartilage and thickening of the epithelium. The gills of the fish exposed to 1500mg/l of the antifoam had more pathological signs than those of the lower concentrations which includes separation of respiratory epithelium.

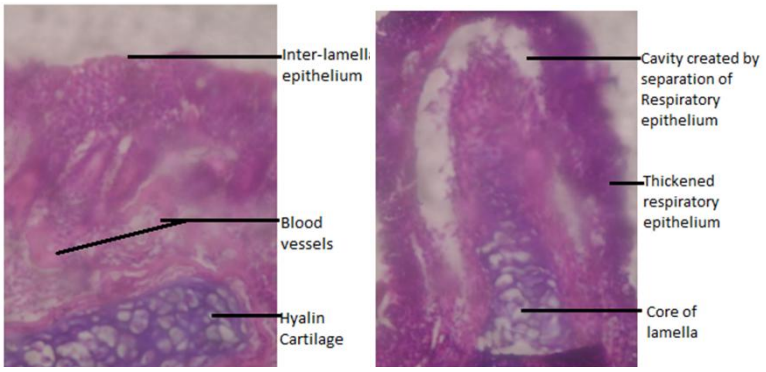
3.4.2 Liver

The liver of fish in the control had no apparent significant histological changes(Plate 2) The hepatocytes and central vein were normal. Most common pathological signs observed on the liver were necrosis of the hepatocytes and vacuolation. The liver of fish exposed to 750mg/l of the antifoam showed mild vacuolations (Plate 4.7). The liver of fish exposed to 1000mg/l showed generalized severe vacuolations and distortion of the central vein (Plate 4.8). The liver of fish exposed to 1250mg/l showed necrosis of the hepatocytes and vacuolations (Plate 4.9). The liver of fish exposed to 1500mg/l showed degenerated hepatocyte, and widespread severe vacuolations (Plate 4.10)

Plate 1. Showing the gills of *Clarias gariepinus*exposed to different concentrations of antifoam solution.

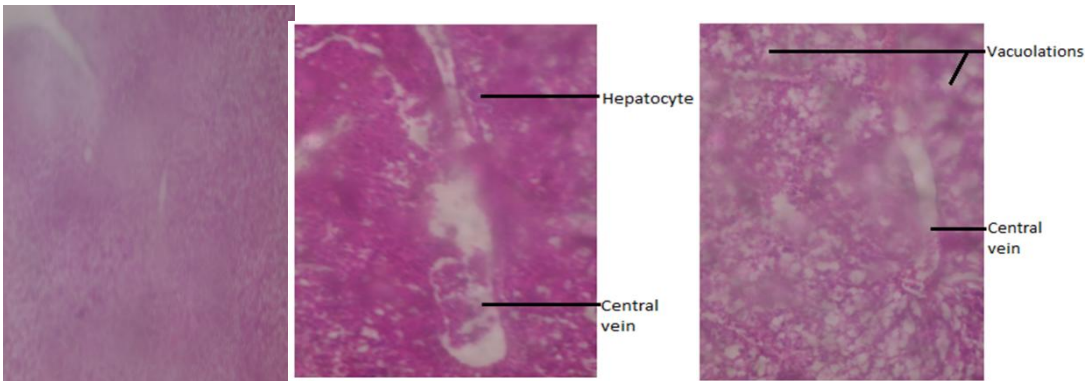


Control(0mg/l) 750mg/l antifoam solution 1000mg/l antifoam solution

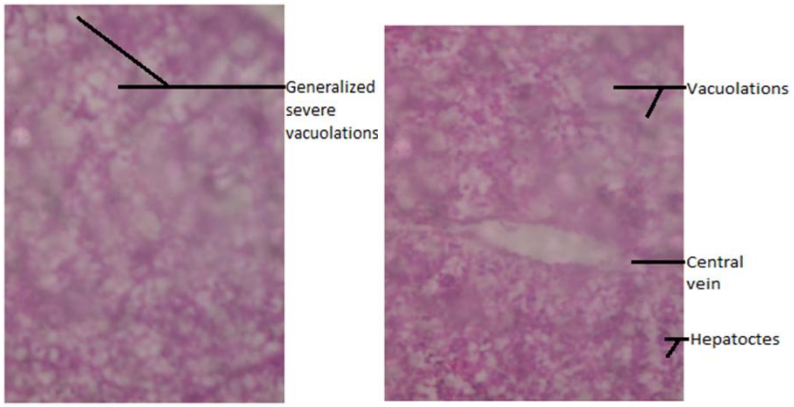


1250mg/l antifoam concentartion 1500mg/l antifoam concentration.

Plate 2 showing the liver of *Clarias gariepinus* exposed to different concentrations of antifoam**.**



Control (no antifoam) 750mg/l antifoam concentartion 100mg/l antifoam concentrion



1250mg/l antifoam concentration 1500mg/l antifoam concentration.

4. Discussion

4.1 Physic-Chemical Variables

During this study some physic-chemical variables of the test media fluctuated significantly during the toxicity test. The values were normal for toxicity test[17]. There was a negative relationship between pH and dissolved oxygen values. Fish health, growth and reproductionare affected by physio-chemical parameters such as temperature, pH, dissolved oxygen, pH, electric conductivity and total dissolved solids are important factors which affect[18].In case of dissolved oxygen, the treatments showed a dose dependent decline in concentration it had earlier reported that when toxicants are introduced into an aquatic system, it might decrease dissolved oxygen concentration, which may lead to asphyxiation[19]. The pH values of the different treatments varied from concentration to concentration and where lower than the standard given by [20] for fish survival in fresh water which is 6.5-8.5. The decline in pH levels may be due to chemical composition of the antifoam[21].The water quality variables particularly pH and Total dissolved solids may have contributed to the variation in behavioural pattern, opercula ventilation rate and the mortality of the test fish during the study period[22].

4.2 Behavioral Responses:

The behavior of *Clarias gariepinus*, was affected by the presence of the toxicant this was evident in the erratic swimming on exposure.In addition, morphological changes were observed in *Clarias gariepinus* exposed to higher concentration of during this study, this was observed in skin colour changes, whitish lesions, heavy secretion of mucus and may have resulted due to the toxic property of the chemical.Metals in effluents can either increase or decrease morphological changes, depending on the effects of the reacting metals[23].[22] reported vigorous movement, increased opercular movement and jumping in *Clarias gariepinus* exposed to leaf extract (*Hypoestesforskalei)*. The impact of toxicant on the fish can clearly be seen from the erratic behaviour reported in this study and previous studies. Mucus are natural defensive secretions by the fish but excessive secretions are observed when trying to reduce absorption of the offending toxicant[24]. However, this has been reported to reduce the fish respiratory activity[25].

4.3 Mortality (LC50)

The increasedmortality rate observed was dose dependent which indicates the possible danger and effect of the toxicant on fish species and points out the impact on the natural environment negatively. Previous reports such as [26] and[27] are in agreement as well. The implication on the ecosystem is that the discharge chemical substance indiscriminately could bring about bioaccumulation, fish kill and possible loss of our natural biodiversity [28]. The LC50 reported by[29] was 15mg/L at 15 ^{o}C when he tested the effect of surfactants on fish samples. [30] who studies the 96 h LC value of bisphenol reported a value of 6.48 mg/ L.The toxicity bioassay reported by [31]showed that the 96 hours LC50 was 40.768 mg/l when he tested the acute toxicity of paraquat on *Oreochromis niloticus*.

4.4 Gills

Changes in physiological functions of tissues and organs of organisms can be caused by introduction of toxicants. The expression across the organ studied depicts the effect of exposure of *C.gariepinus* to antifoam. This agrees with [32] who reported that pollutants are most times organ specific in reaction.The gills are organs for respiration, osmoregulation and excretion of nitrogenous wastes materials in fish [33]. They are particularly sensitive to change in the quality of water since they remain in close contact with external environment and are considered the primary target organ of the toxicant[34]. Changes in gill tissues in the present experiments were congestion of the primary lamellae and hyperplasia of branchial plates. The changes were indicative of lowered oxygen supply to the test fish, resulting in negative respiratory responses. Damages of the gills indicated that impairment in gaseous exchange efficiency of the gill lamella and hyperplasia were observed and this is similar to the observation of [35]. Observed gill lamella changes in gill filaments are probably due to increased capillary movement [36]. Alterations in the gills like fusion of some secondary lamellae are examples of defence mechanisms which result in the increase of the distance between the external environment and the blood serving as a barrier to the entrance of contaminants [34]. As a consequence of the increased distance between water and blood due to epithelial lifting, impaired oxygen uptake is evident. However, fishes have the capacity to increase their breathing rate, to compensate low oxygen uptake[37]. Different authors showed the histological changes in gills of different fishes exposed to various toxicants [38] observed hydropsy, vascular degeneration and severe necrotic changes in the secondary gill tissues of fish *Labeorohita* exposed to the sub lethal concentrations of chlorpyrifos. Shorter and irregular gill lamellae, severe vacuolations, fusion and complete destruction of lamellar were observed in guppy *Poecilia reticulata* exposed to chlorpyrifos[39]. [40]observed histological effects of deltamethrin on the gill (necrosis, fusion of secondary lamellae, lifting of the lamellar epithelium, oedema, and epithelial hyperplasia) of common carp after chronic exposure at concentration of 0.029 and 0.041 mg/l. This gill damage reduced proper gaseous movement in the respiratory organs which is between the organs and the surrounding water, thus leadings to air gulping by the stressed fish.

4.5 Liver

Liver is a major organ which plays a fundamental role in the detoxification, uptake and biotransformation of foreign compounds in the body[41]. It is also one of the most affected organs by contaminants in water which results in different levels of damage[34]. Liver of the fish exposed to antifoam showed degenerative changes. These may be attributed to the direct

toxic effects of pollutants on hepatocytes, since liver is responsible for detoxification in an organism. The liver of the test fish had vacuolated cells which is evidence of degeneration of fatty cells. Vacuolation of hepatocytes is a common effect observed in fish when exposed to a variety of toxic chemicals[42] . The vacuolization observed in the present study are in agreement with [43]. Necrosis of some portions of the liver tissue that were observed may have resulted from the excess work required by the test fish to get rid of the toxicant during the process of detoxification from its body. The inability of fish to regenerate new liver cells may have led to necrosis. These findings are similar with the result of [44]. Significant changes such as hyperplasia, disintegration of hepatic mass and necrosis were found in *Labeorohita* exposed to cypermethrin [45]. [46] observed degeneration of hepatocytes in rainbow trout after cypermethrin exposure. The liver tissues of fish *Gambusia affinis* exposed to sub-lethal concentrations of deltamethrin had necrosis[47]. Also, the swelling of hepatocytes in the present investigation were similar with the findings of [48] in *Heteropneustesfossilis* treated with Malathion.

5. Conclusion

It is concluded that exposure of fish to chemicals such as antifoam caused irreparable changes in various vital organs which made the fish less fit for better survival. Contrary to the claims that the antifoam is not a toxicant, is evidenced by the very low LC_{50} of this antifoam.Physio-chemical parameters of the water as observed were also affected by the antifoam which lead to behavioral distress like restlessness, jumping, gulping of air and loss of balance of the test fish. These changes can alter various physiological activities of the fish. Thus, the changes observed in the gills and liver of the freshwater fish *Clarias gariepinus* indicate that the fish were responding to the direct effects of the toxicants as much as to the secondary effects caused by stress. Therefore, the discharge of antifoam (Polydimethylsiloxane)could lead to pollution of an ecological system, thus posing great threat to aquatic organisms such as fish and such should be discouraged

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