

Histopathological Effect of the Different Concentration of Crude Oil and Its Products on the Gill Apparatus of Catfish (*Heterobranchus bidorsalis*)

Ugbeyide Juliet Avwersuoghene^{1,*}, Nwamba Helen²

¹Department of Biological Sciences, Edwin Clark University, Kiagbodo, Nigeria

²Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Nigeria

Email address:

jullyakpo2000@yahoo.com (U. J. Avwersuoghene), nwambahelen@yahoo.com (N. Helen)

*Corresponding author

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Abstract: This project is aimed at assessing the Histopathological effect of the different concentration of crude oil and its products on the gill apparatus of catfish (*Heterobranchus bidorsalis*). Three concentrations (1.0, 3.0 and 5.0 M/L) of the petroleum products Premium Motor Spirit (PMS), Dual Purpose Kerosene (DPK) and Bonny-Light Crude oil (BLCO) were made. To each concentration, three adults of *H. bidorsalis* were incubated for 1, 24 and 96 hours. Triplicate values of samples were recorded in each of the different concentration of petroleum products. Three plastic containers containing three fishes each were not contaminated with petroleum product and was left as control (0.0 M/L) for the experimental work. Histology of the gill was used to determine the pathology of petroleum products on the gills. Histopathological examination following exposure to petroleum products showed elongation of gill lamellae, hyperplasia of lamellae, fusion of lamellae tips, separation of respiratory epithelium with underlying supporting tissue and altered gill rakers. Microscopical observation showed a significant difference ($P < 0.05$) in hyperplasia and elongated gill lamellae. There were no significant difference ($P > 0.05$) in heartbeats, weight and underlying supporting tissue in the different concentration of petroleum products. The various biological responses and changes observed with such histological alterations can be used as a tool to detect toxic effects of pollutants in the organs of living organism making it a good environmental stressor indicator for bioassay.

Keywords: Histopathology, Petroleum Products, Gills, Crude Oil, *Heterobranchus bidorsalis*

1. Introduction

Petroleum and its products have been recognized as a potential environmental contaminant since shortly after the beginning of the twentieth century [1]. The degree of hazardous effect of petroleum products (PPS) is dependent on the degree of their concentration, chemical components and solubility in water. An example of such effects is the distortion of gill apparatus in *Heterobranchus bidorsalis* fish [2]. Oil pollution is the release of petroleum products into the aquatic environment and pollution is a major disaster that affects aquatic life [3] which also causes massive destruction to economically important aquatic organism like *H. bidorsalis* [4].

1.1. *Heterobranchus bidorsalis*

H. bidorsalis is a known catfish, generally recognized by the barbells on each side of the mouth which mimics the whisker of a cat and thus the name “Cat fish”. This group of fish is extremely large and diverse. They are generally grouped under a single order-Siluriformes [5]. This order is made up of 31 families and *H. bidorsalis* belong to the family Clariidae (air breathing cat fish) [5]. They are members of the class Actinopterygia (ray finned fish). There are 400 genera with approximately 2200 species in the family [6]. The habitat of this catfish is fresh water ranging from cool swift running mountain streams to still water, low land lakes and ponds [5]. *H. bidorsalis* is a highly esteemed group of

fishes in tropical Africa. It commands high market value hardly in nature possesses air-breathing organs that enables it tolerate aquatic condition [7]. *H. bidorsalis* is equipped with a special respiratory organ known as the gills. The gill plays a vital role in the fish since they are the main site for gaseous exchange as well as partially responsible for osmo-regulation, acid bases balance and taste [8]. The gill of aquatic organism is the primary target of disturbance by petroleum product. The morphological change is a consequence of environmental changes [9] also an adaptive attempt in conserving some physiological functions [10].

1.2. Aquatic Oil Pollution

Pollution is made available under certain environmental conditions, thus resending a source of diffuse pollution in the water compartment [11]. The toxicity bioassay together with physiological and chemical analysis used to detect and quantify the petroleum substance present in water ecosystem allows the assessment of the effect of toxic agent on the environment [12]. Acute effects, such as mortality and immobility in short exposure time as well as chronic effect, detected in longer exposures, are important tools for the assessment of pollution effects on the structures of communities [11]. The effects of petroleum products on the gills can be assessed by population studies particularly through the evaluation of the death and survival success [13]. Oil pollution makes breathing difficult for fishes and may even lead to their starvation and death [14]. This was seen in the behavioral response of *M. Roosevelt* when exposed to 7 ppm lethal does and it is possible therefore to trace a parallel among the hyperplasia and the behavioral effect of intoxicated fishes [14]. The gills of fishes represent the primary target of disturbance of pollutants as they are in direct contact with the external medium to perform gaseous exchange [15]. This organs has linking sites to promote its regular functions and these sites may link to toxic substance with differentiated charges, triggering mechanical responses and toxic effects to the fish [16].

1.3. Eco-toxicological Effects

The 96 hours of LC₅₀ value for an accommodated fraction of fuel oil ranged from 3.2 to 56 mg/l in fish [17]. However there can be long-term effect from these exposures such as historical damages of the organs. According to Kiihnhold [18] damage caused by oil pollution are both significant and diverse. Such pollution can destroy or limit marine life, contaminates water supplies and create fire hazard [19]. Pollution causes damages in the pillar cells of fish gills [20]. Such cellular damages are often associated to high dose in which the animals are close to death [20]. Karlsson-Norrgren and Runn [21] verified an increase in the volume of the secondary lamellae of *Danio rerio*, filling in the inter-lamellar space, staring from concentration 10ug/l, resulting in the decrease of the diffusion capacity. Assessing the effect in adult *Dania rerio* females, verified that within 24 hours the products had already been incorporated by the gill, skin and parts of the tracts.

According to Ewald, [22], tissue alterations are preceded by biochemical and physiological responses and once the damage

is detected the adverse effect on the organism are incontestable. Mallrat [23] in his studies verified that the most common lesions were necrosis, hyperplasia, hypertrophy and rupture of gill tissues, lamella fusion, hyper secretion and proliferation of mucous cells, alterations in chloride cells and vascularization. According to Peleksić, and Mitrović-Tutundžić [24], there are three phases of gill alterations where there is progressive worsening of the lesions. The first phase is when the damage may be reversed under satisfactory conditions for organism. In the second phase, the lesions are more severe and difficult to reverse. In the third phase the separation of the gill structure is irreversible, jeopardizing its vital functions and eventually causing the organism to die [24].

The current study is aimed at investigating the acute toxicological effect of the water soluble fractions of Crude oil, Premium Motor Spirit (PMS) and Kerosene (DPK) on the gill apparatus of catfish (*Heterobranchus bidorsalis*) adult. It seeks to provide some needed toxicological data on the Crude oil, Premium Motor Spirit (PMS) and Kerosene (DPK) that is commercially used in Nigeria especially on a common tropical fish *H. bidorsalis*. To determine the petroleum products that has the greatest effect on the gill apparatus of *H. bidorsalis*.

2. Materials and Methods

One hundred and eleven (111) adults of *Hetrobranchus bidorsalis* with mean weight of 163.2±1.00g were transported from a private fish hatchery at Ugwuomu Emene in Enugu State Nigeria to the Phenoma Farms Head Quarters Ngwo Enugu State. These adult fishes were in one plastic container of 200 L and the water temperature was maintained at 23±1.0°C during transportation. The fishes were acclimatized for 14 days and were maintained on a 38% crude protein diet at 3% body weight.

2.1. Experimental Oil Products

Three samples of crude oil and its products were used for this study; these were Bonny-Light-Crude Oil (BLCO), Premium Motor Spirit (PMS) and Dual Purpose Kerosene (DPK). Aliquot of each of these three oil samples were introduced in triplicate to 27 plastic containers (90 L) at concentration 1.0, 3.0 and 5.0 M/L. Three plastic containers with fish were not contaminated with any oil samples (0.0 M/L) and were left stocked in Completely Randomized Block Design (CRBD) in 30 plastic containers (90 L) at 3 fish per container. Each container was then filled to 50 L mark with water.

2.2. Experimental Treatment

Two experimental periods were adopted for the study: the toxicity period, which lasted for 1 hour (60 minutes) and the recovery period of 24 hours (1440 minutes) and 96 hours' (5760 minutes) was monitored at daily internals. At the end of the toxicity period, the surviving fishes and the containers were washed thoroughly with clean water to allow for 24 and 96 hours recovery period to commence.

Heart beat of fish was counted per minutes during each experimental period. This is done by sectioning the fish

through the ventral side from the anal opening and lifting the liver by holding it with forceps there by exposing the beating heart. Mortality rate, feeding and swimming activities were observed in the experimental periods. Fish sample was dissected using scissor, razor blade, dissecting board, forceps etc. The heartbeats were recorded per minutes and the gills cut off from the fish and preserved in 10% formalin.

2.3. Histopathology of Gill

The gill was obtained by chopping, off the opercula bone from the ventral side there by exposing them. The gills were dissected, out, placed in a sample bottle containing physiological saline serving as preservatives. The Histopathological examination of the gills was carried out at UNTH Histopathological laboratory Enugu Nigeria. In the laboratory triplicates treatments of crude oil and its products and control were analyzed for the gills pathological parameters using methods as follows:

The specimen from 10% formalin was processed with automatic tissue processor. The reagents used include alcohol, which removes water from the tissue. Xylene removes alcohol debris and fat from the tissue. Paraffin wax for impregnation and also removes Xylene from tissue.

2.4. Histopathological Filtration

This process involved filtering three beakers of large size with absolute alcohol of 70, 90 and 95% respectively. Three other beakers of the same size were filled with Xylene, which does not need dilution. Another three beakers were filled with Paraffin wax. The specimens were passed through these three reagents sequentially. The tissues were then trimmed, mounted on a wooden block, sectioned with microtome and place on a slide and stained with Hematoxylin and Eosin stain. The prepared slides were taken to Applied Biology Laboratory, Faculty of Applied Natural Science, Enugu State University of Sciences and Technology (ESUT) and observed under 100 power magnification of an electric binocular microscope.

2.5. Histopathological Findings

Structural alterations and abnormalities such as elongation of gill lamellae, fusion, separation of respiratory epithelium with underlying supporting tissue, necrosis and hyperplastic of lamellae were observed [25].

2.6. Statistics Analysis

The statistics analysis of this work was carried out using Analysis of variance (ANOVA) method to determine whether there were significant differences between the treated and the control gill samples in the different concentration of petroleum products used.

3. Results

Table 1 shows mean \pm S. E of heartbeat per minutes and weight of *Heterobranchus bidorsalis* adult exposed to different concentration (M/L) of crude oil and its products at 1, 24 and 96 hours. The range of value for heartbeat was 96.7 to 66.3 beat/minutes while the range for weight was 162.3 to 81.2mg. The highest value for the weight was recorded I M/L (163.2g) in DPK at 1 hour exposure and the least value was recorded in 5 M/L (81.2g) in PMS at 96 hour exposure. There was no significant difference ($p>0.05$) in the heartbeats and weight of the fish (*H. bidorsalis*) in the different concentration of petroleum products used.

Table 2 shows mean + S. E of fused gill apparatus of *Heterobranchus bidorsalis* exposed to different concentration (M/L) of crude oil and its products. The range of value for PMS was 32.3 to 2.3 mg. The highest value was recorded in 5 M/L (32.3 mg in altered fine branches of supporting tissues) and the least value was recorded in 1 M/L (2.3 mg fused lamellae tips). The range of value for DPK was 28.7 to 1.3 mg. The highest value was recorded in 5 M/L (28.7 mg in altered fine branches of supporting tissues) and the least value was recorded in 1 M/L (1.3 mg fused lamellae tips). The range of value for BLCO was 33.3 to 2.7mg. The highest value was recorded in 5 M/L (33.3 mg in altered fine branches of supporting tissues) and the least value was recorded in 1 M/L (2.7 mg fused lamellae tips). The analysis of variance showed significant effect ($P<0.05$) in the fused gill apparatus of the different concentration in M/L of PMS, DPK and BLCO used.

Figures 1, 2 and 3 shows percentage increase in size of the underlying supporting tissues of the gill of *H. bidorsalis* exposed to different concentrations (M/L) of petroleum products. The range of value was 70 to 10%. The highest value was recorded in 5 M/L (70%) in BLCO while the least value was recorded in 1 M/L significant (10%) in DPK. There was no significance ($P > 0.05$) between the concentrations of petroleum product in the underlying supporting tissue of the exposed gill.

Table 1. Means \pm S.E of heartbeats and weight of *H. bidorsalis* exposed to petroleum products.

Time (H)	Concentration (M/L)	H. B (X \pm S. E)	Weight (X \pm S. E)	PMS (M/L)	H. B (X \pm S. E)	Weight (X \pm S. E)	DPK (M/L)	H. B (X \pm S. E)	Weight (X \pm S. E)	BLCO (M/L)	H. B (X \pm S. E)	Weight (X \pm S. E)
1	0.0	82 \pm 0.0	163 \pm 1.0	1.0	83.0 \pm 0.3	162.1 \pm 1.0	1.0	84.0 \pm 1.0	162.3 \pm 0.4	1.0	85.0 \pm 0.5	161.5 \pm 0.5
				3.0	80.6 \pm 0.3	102.7 \pm 0.4	3.0	82.0 \pm 0.9	100.0 \pm 0.5	3.0	76.3 \pm 0.3	96.0 \pm 1.2
				5.0	73.3 \pm 0.3	103.5 \pm 0.7	5.0	76.7 \pm 0.7	102.0 \pm 0.7	5.0	81.0 \pm 0.5	98.0 \pm 1.0
				1.0	93.0 \pm 0.2	95.2 \pm 0.3	1.0	83.7 \pm 1.1	85.7 \pm 0.9	1.0	67.9 \pm 0.5	90.6 \pm 0.9
24	0.0	82 \pm 0.0	163 \pm 1.0	3.0	80.6 \pm 1.7	90.1 \pm 0.5	3.0	82.7 \pm 0.9	95.5 \pm 0.7	3.0	74.7 \pm 1.1	90.7 \pm 1.2
				5.0	73.6 \pm 0.6	87.1 \pm 1.1	5.0	92.2 \pm 0.6	124.7 \pm 1.0	5.0	83.2 \pm 1.5	84.4 \pm 0.5
				1.0	66.3 \pm 1.2	104.5 \pm 0.5	1.0	86.5 \pm 0.4	103.2 \pm 0.7	1.0	96.7 \pm 0.7	85.2 \pm 0.7
				3.0	93.6 \pm 0.5	88.6 \pm 0.2	3.0	67.1 \pm 1.5	120.0 \pm 0.3	3.0	85.0 \pm 0.3	81.3 \pm 0.5
96	0.0	82 \pm 0.0	163 \pm 1.0	5.0	73.3 \pm 0.2	81.2 \pm 0.5	5.0	74.3 \pm 0.5	100.1 \pm 0.5	5.0	82.0 \pm 0.5	90.5 \pm 0.9

KEY: S. E: Standard Error, H. B: Heart Beat

Table 2. Mean±S. E of fused gill apparatus of *Heterbronchus bidorsalis* exposed to different concentration of petroleum products

oil	Concentration (m/l)	Fused lamella tips	Hyperplasia of lamella	Separation of respiratory epithelium with underlying supporting tissues	Altered fine branches of supporting tissues	Elongation of gill lamella	Altered gill rakers
CONTROL	0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	1.0	2.3±0.3	5.6±0.5	4.6±0.3	4.6±0.3	18.3±0.8	5.6±0.3
PMS	3.0	2.6±0.3	6.7±0.4	5.7±0.6	5.7±0.6	25.1±0.5	6.3±0.4
	5.0	4.3±0.3	7.6±0.3	7.1±0.3	7.1±0.1	32.3±0.1	7.0±0.5
DPK	1.0	1.3±0.3	3.7±0.3	2.7±0.3	16.0±0.5	4.7±0.3	3.0±0.5
	3.0	1.7±0.3	6.3±0.3	5.0±0.5	23.6±1.9	5.5±0.7	5.7±0.3
BLCO	5.0	3.7±0.3	6.8±0.3	5.3±0.3	28.7±0.6	6.0±0.5	6.0±1.2
	1.0	2.7±0.3	5.7±0.5	4.7±0.3	18.7±0.3	4.7±0.7	5.7±1.4
BLCO	3.0	2.9±0.5	6.7±0.3	5.7±0.3	26.0±0.8	6.0±0.8	6.0±0.5
	5.0	4.7±0.5	7.7±0.3	7.3±0.3	33.3±1.4	7.0±0.8	7.3±0.3

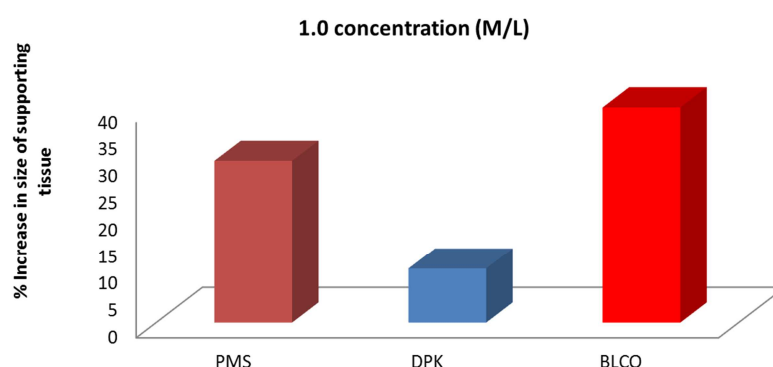


Figure 1. Percentage increase in size of underlying supporting tissues of the gill of *H. bidorsalis* exposed to 1 M/L of petroleum products.

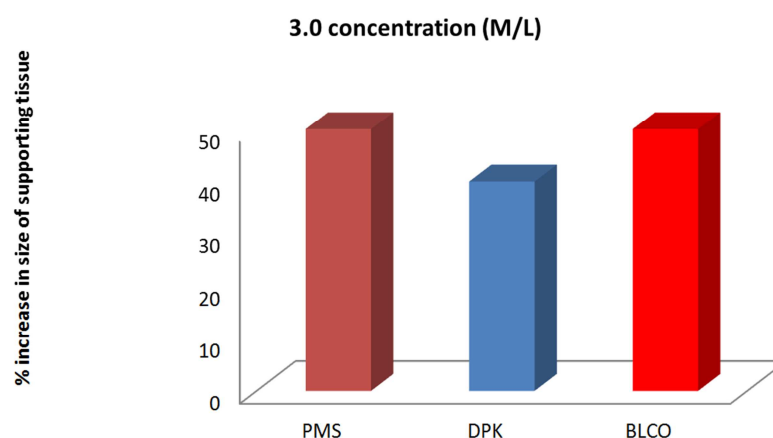


Figure 2. Percentage increase in size of underlying supporting tissues of the gill of *H. bidorsalis* exposed to 3 M/L of petroleum products.

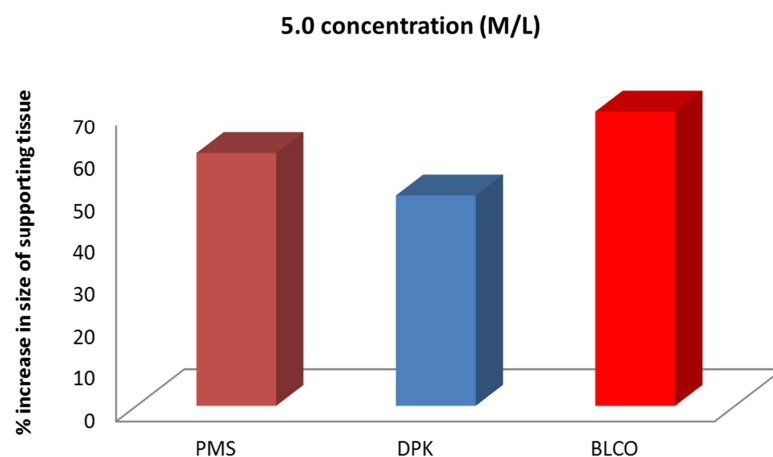


Figure 3. Percentage increase in size of underlying supporting tissues of the gill of *H. bidorsalis* exposed to 5 M/L of petroleum products.

4. Discussion

The objective of the histological assessment of the gill was to verify the possible damage caused by the exposure of the fish to petroleum samples; evidencing alterations resulting from the chronic effect. The exposure of *H. bidorsalis* to petroleum products showed increased mortality even at low concentration. This agreed with [26] in their study showing that petroleum products caused damage to gill tissues and also induced fish mortality by the excessive cell proliferation and mucus production.

Test fish behavioral changes observed in this study are attributable to the presence of crude oil products, because similar changes were not observed in the control. Increase opercula movement is an indication of respiratory difficulty. The gills are respiratory surfaces for gaseous exchange but when they are blocked by an environmental toxicant, the surfaces available for gaseous exchange are reduced, resulting in respiratory difficulty. According to Reiser, *et al.* [27], Fish gills are one of the vital organs that are affected by petroleum products. Fish gill is an organ with a large surface area, highly sensitive to the effect of toxicant. Epidemiologists have attributed respiratory morbidity in human and some animals to crude oil pollution [28]. It was also observed that affected fishes were hanging at the water column, which is an indication of oxygen deficiency in the water. According to Peters *et al.* [29], petroleum products is a complex mixture of toxic compounds with wide variability of deleterious effects in human and animal studies. Other behavioral changes noticed in the exposed fish are erratic swimming, moribund movement, jumping and lack of balance. This observation is similar to that of [30]. The toxic effects of petroleum used are not the same, they vary according to the components they are made up of. Considering petrol, because of its high rate of dissolution in water, the chemical components, viscosity and diffusion, it possesses the potential more than the other two products to be lethal and account for its high acute toxicity. Therefore the death of the fishes may have resulted from the little quantity that affects the rate of breathing there by leading to their death. This is in line with [31, 32].

Histopathological study of the gills showed structural abnormalities such as elongation of gill lamellae, fusion, separation of respiratory epithelium with underlying supporting tissue, necrosis and hyperplasia of the lamellae. This agreed with the study of [19] that refinery effluents caused gill damage in *O. niloticus*. The oxygen stress encountered by the fish which is responsible for the respiratory distress and death, was due to their inability to withstand the oxygen depletion of the water induced by the organic compounds in the water soluble fraction of the fuel. Similar oxygen stress imparted by the water soluble fraction of crude oil had been studied in the shrimp, *Palaemon adspersus* [33] and in the catfish *Clarias gariepinus*. Furthermore, the gill elongation and hyperplasia in the cultured fish may be induced by this oxygen stress.

Though there was no figure recorded for the mortality and

behavior during the toxicity phase, this is attributed to loss of oxygen because the petroleum products blanketed the water surface there by reducing the rate at which oxygen diffuse into water. On the other hand this led to stress and the result of this stress is death. This confirmed the experiment of oxygen stress imparted by water-soluble fractions of petroleum carried out by [33]. In kerosene treatment, the increasing effect in the gill apparatus may not be according to the inhibition in the synthetic pathway of the fish, rather the increasing effect may have resulted due to the injury created on the gill, which multiplies as the concentration, increased. This is in line with [17]. The result showed significant difference ($P < 0.05$) when compared to the control.

5. Conclusion

The result of this research work showed that crude oil and its products caused alterations in the gill apparatus of *H. bidorsalis*. To this effect, crude oil and its products was seen to have significant effect ($P < 0.05$) on the gill apparatus such as fusion, separation of respiratory epithelium with underlying supporting tissue, elongation of gill lamella and hyperplasia of lamella which led to mortality in *H. bidorsalis* adult. Increase in the concentration of the petroleum products caused an increase in hyperplasia of lamella. Thus from this research work, it was clearly seen that the different concentration of crude oil and its products has a significant effect ($P < 0.05$) on the Histopathological structure of gill apparatus of *Heterobronchus bidorsalis* (cat fish).

Having considered the effect of crude oil and its products on *H. bidorsalis* as it causes alteration and damage to the gill apparatus, it is obvious that adequate control measures should be taken so as to minimize the rate at which oil pollution occurs. Petroleum products do not only have adverse effect on fish but it also has effect on everything it gets in contact with. Moreover, the effect of petroleum products on fish is much more similar to the effect in man. Therefore it is advisable that man should not expose himself to this toxicant and avoid contaminated fish.

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