



Histopathological and Hematological Alterations in *Cat fish* Exposed to Sublethal Concentrations of Naphthalene, a Polycyclic Aromatic Hydrocarbon

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Abstract: The effect of naphthalene on selected haematological and histopathological parameters as well as relative growth rate in the tropical African catfish was evaluated. Healthy juvenile fish ($n = 90$) weighing 19.7 ± 1.8 g were exposed to sublethal concentrations of naphthalene over a period of 35 days after which haematological and histopathological parameters were analyzed. The median lethal concentration LC 50 of naphthalene was determined to be $6600 \mu\text{g/L}$ in Catfish with estimated safe level ranging from 0.066 to $330 \mu\text{g/L}$. Sublethal concentrations of naphthalene led to significant declines in red blood cell (RBC) counts, haemoglobin concentration and haematocrit. The erythrocyte indices showed mixed results with mean corpuscular haemoglobin concentration (MCHC) showing significant elevation while changes in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were not significant. Naphthalene was immunotoxic in exposed fish leading to significant elevations in circulating white blood cells (WBC). There was also a significant increase in platelet (PLT) count in naphthalene exposed fish. Growth rate significantly reduced in a dose response pattern. While there was no observed histopathological alteration in the liver of exposed fish, haemorrhage with blood coagulation was observed in the gill sections. There were changes in the haematological parameters. The significant reduction in (RBC) and the reduced growth rate of catfish shows that naphthalene is of environmental concern due to its toxicity.

Keywords: Histopathological, Hematological, *Cat fish*, Naphthalene, Sublethal

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds consisting of two or more fused rings. PAHs are of environmental concern due to their persistence and toxicity. They are also considered to be mutagenic and carcinogenic [30]. PAHs find their way into the environment through natural sources such as volcanoes, forest fire, and biosynthetic process. PAHs could also be emitted by diverse anthropogenic sources including vehicle exhausts, oil spillage and urban sewage [30].

Naphthalene is a toxic air and water pollutant widely used as an intermediate in the production of phthalic anhydride, surfactants and pesticides [15]. It is also found in many other environments, especially soils [14]. Naphthalene is rather a special compound in terms of its properties and chemical structure. It is a flammable white solid with the formula C_{10}H_8 and the structure of two fused benzene rings, with melting and boiling points of 80.5 and 218°C , respectively [15]. Naphthalene has been a target compound in

environmental studies examining volatile organic compounds (VOCs), and PAHs [15]. While known as a common and widespread air and water contaminant for many years, naphthalene received relatively little attention prior to the finding of its carcinogenicity in rats in 2000 [1]. In the aquatic environment, naphthalene is particularly hazardous due to their combination of mobility, toxicity, and general environmental hazard [14].

Naphthalene exposure has been shown to increase oxygen consumption by benthic invertebrates and reduced photosynthesis in *Chlamydonas angulosa* [22]. The aim of this work was to evaluate the effect of naphthalene on some haematological parameters of the tropical catfish as well as study the possible histopathological alteration in liver and gills of exposed fish. The tropical catfish was chosen for this work because of its abundance in tropical climes as well its hardiness and ease of handling. This study will contribute to the toxicological database and also improve on the ecological risk assessment of naphthalene in aquatic environments.

2. Materials and Methods

2.1. Chemicals

All reagents used were of analytical grade. Naphthalene was obtained from Sigma Aldrich (Germany). Acetone was obtained from BDH chemicals (UK).

2.2. Animals

Juvenile catfish (*C. gariepinus*) weighing 19.7 ± 1.8 g were obtained from a commercial fish farm in Aba, south east Nigeria. The fish were acclimatized for 2 weeks in dechlorinated tap water prior to experimentation. The fish were fed twice daily ad libitum with commercial fish feed. Fecal matter and uneaten food were removed daily to prevent contamination of the water.

2.3. Acute Toxicity Study

Prior to the acute toxicity study, a combination of existing data (from the scientific literature) and range finding test were used to determine the appropriate concentrations of exposure. Based on the pre-LC₅₀ data, the 96 hour LC₅₀ was determined for naphthalene according to the revised OECD guidelines for testing of chemicals [18]. Triplicate sets of 7 fish each were randomly exposed to varying concentrations of naphthalene. Acetone was used as solvent carrier. The fish were placed in 30L plastic tanks holding 20L of dechlorinated tap water. Another set of 7 fish was also maintained with equal amount of tap water (and solvent carrier) but without the test chemicals and considered as the solvent control. Fish was not fed throughout the experiment and lethality was the toxicity end point. Fish were visually examined daily and considered dead on cessation of opercular movements and lack of movement when probed. Dead fish were removed and the mortality recorded at intervals of 24, 48, 72 and 96 h. The 96 hour LC₅₀ value of naphthalene for the fish was determined by probit analysis.

2.4. Sublethal Toxicity Study

Stock solutions of naphthalene were prepared by dissolving naphthalene in distilled water taking acetone as solvent carrier. Test solutions were prepared by dilution of stock solutions in tap water. During sublethal studies, fish were exposed to 1/2 and 1/4 of the LC₅₀ value (corresponding to treatment levels 1 and 2). A solvent control was included in the experimental design. Fish were kept in groups of 10 in 30L plastic tanks containing the test solutions. Experiments were performed in triplicates. Period of exposure lasted 35 days.

2.5. Assays

At the end of the exposure period, fish were anaesthetized using non-chemical method by hypothermia. Blood was then collected from the immobilized fish by caudal vein puncture method as described by Argungu *et al.* [2] using a 5ml sterile disposable syringe with a 22 gauge needle. The blood was transferred to EDTA tubes and transported to the lab for analysis.

The liver and gill tissues were dissected and placed in 10% formal saline solution prior to histopathological investigation.

2.6. Haematology

Haematological parameters were determined using automated Sysmex XE-5000 haematology analyser, Japan.

2.7. Relative Growth Rate (RGR)

RGR was determined using the method of Thaller *et al* (2014):

$$RGR = \frac{w_t(\text{final weight}) - w_i(\text{initial weight})}{w_i(\text{initial weight})} \times 100$$

2.8. Statistical Analysis

Results were expressed as mean \pm standard error. Data from the different treatment groups were compared by a one-way analysis of variance (ANOVA) followed by a Scheffes test to determine statistically different groups. All differences were considered significant at $p < 0.05$. Statistical analysis was performed using Microsoft Excel and the SPSS statistical package (ver. 24.0 SPSS Company, Chicago, IL, USA).

3. Results

By probit analysis, the LC₅₀ of naphthalene in catfish was determined to be 6600 $\mu\text{g/L}$. No mortality occurred in the control. One hundred percent mortality was recorded after 96 h at the highest naphthalene concentration (25600 $\mu\text{g/L}$) tested.

The results of estimated safe levels of naphthalene as calculated by multiplying the 96 hour LC₅₀ with different application factors are given in Table 1. The estimated safe levels of naphthalene in *C. gariepinus* varied from 0.066 to 330 $\mu\text{g/L}$.

The results showed that exposure to sublethal concentrations of naphthalene affected some of the haematological parameters. There was a statistically significant reduction in the red blood cell count of exposed fish. The observed reduction appeared to be dose-dependent with a higher dose of the chemical (3300 µg/L) causing a greater reduction in the reduction compared to the lower dose (1650 µg/L). There was also an observed reduction in haemoglobin concentration and haematocrit. The impact of naphthalene on the erythrocyte indices showed mixed results. While there was an increase in MCHC, there were no observed changes in MCV and MCH. There was a dose-dependent increase in WBC and PLT counts.

The result for relative growth rate is shown in Figure 2. After an exposure period of 5 weeks, there was a significant decline in the RGR of exposed fish as compared to the control.

The histopathology results of the liver and gill are shown in plates 1 to 4. The liver and gill from the solvent control (plates 1 and 3 respectively) exhibit normal architecture. The

liver of fish exposed to the second level of treatment (plate 2) showed normal architecture. The gills of exposed fish at the second level of treatment (plate 4) exhibited haemorrhage accompanied with blood coagulation.

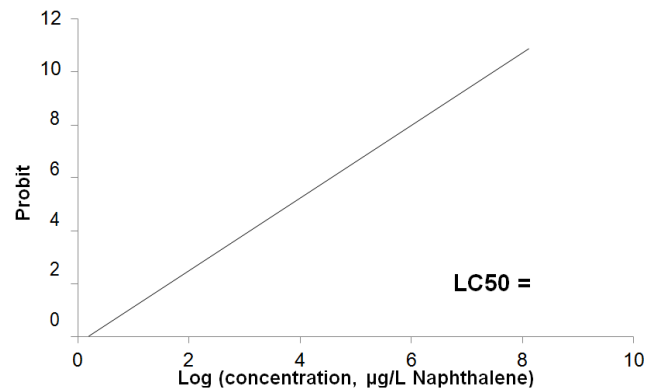


Figure 1. Shows the linear regression curve of log₁₀ concentration versus probit of naphthalene induced mortality on catfish. $y = 1.3712x - 0.24$. The LC₅₀ was 6600 µg/L.

Table 1. Estimate of safe levels of Naphthalene at 96 hour exposure time in *C. gariepinus*.

PAH	96h LC ₅₀ (µg/L)	Method	AF	Safe level (µg/L)
Naphthalene	6600	Sprague (1971)	0.1	3.9968
		CWQC (1972)	0.01	66
		NAS/NAE (1973)	0.01-0.00001	66 – 0.066
		CCREM (1991)	0.05	330
		IJC (1977)	5% of 96h LC ₅₀	330

Table 2. Haematological parameters of *C. gariepinus* exposed to sublethal concentrations of naphthalene. Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$.

Parameter	Control group	Naphthalene 1650 µg/L	Concentration 3300 µg/L
RBC	2.86±0.09 ^a	2.07±0.04 ^b	1.84±0.53 ^c
Hb	100.25±3.9 ^a	87.75±2.95 ^b	86.25±2.97 ^b
Hct	37.52±2.38 ^a	31.32±2.13 ^b	27.5±1.97 ^c
MCV	132.8±1.71	138.7±4.6	133.3±3.85
MCH	38.75±0.19	39.2±0.53	39.35±0.61
MCHC	272±3.4	285±3.07	302.25±5.6
WBC	90.87±4.2 ^a	121.25±3.32 ^b	118.98±1.9 ^b
PLT	20±1.2 ^a	19.5±2.7 ^a	42±2.1 ^b

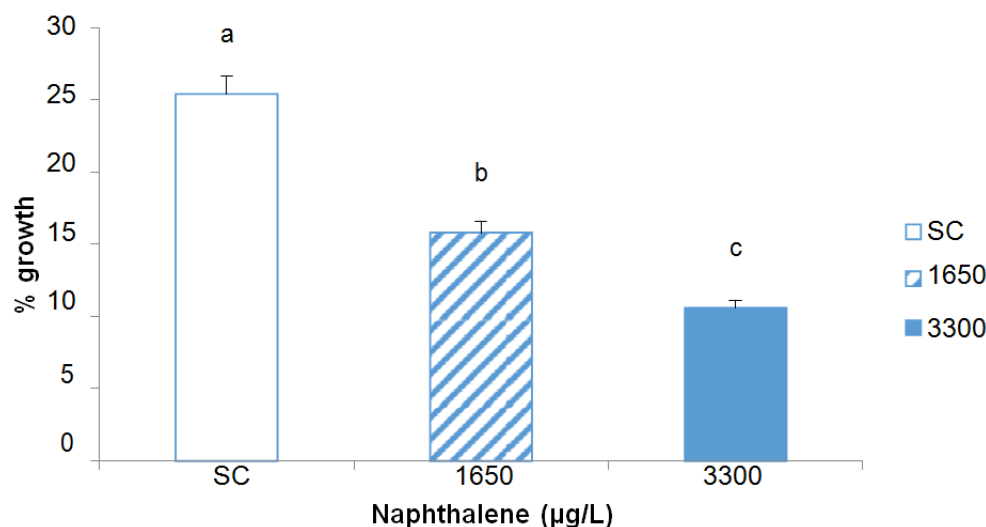


Figure 2. Relative growth rate of *C. gariepinus* exposed to naphthalene. Means not sharing the same letter (a, b or c) are statistically different at $p < 0.05$.



Figure 3. Photomicrograph of a section of the gill from the control showing normal impression. H&E mag x10.



Figure 4. Photomicrograph of a section of the gill from the group exposed to naphthalene showing blood coagulation (BC).

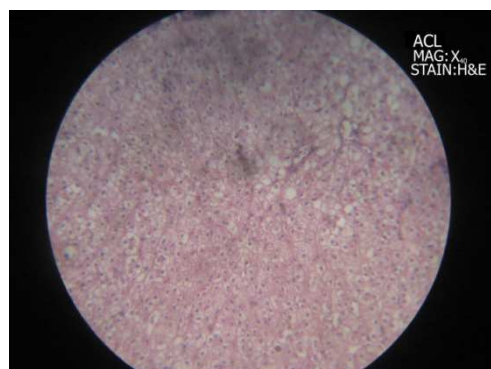


Figure 5. Photomicrograph of a section of the liver from the control group showing normal impression.



Figure 6. Photomicrograph of a section of the liver from the group exposed to naphthalene, showing normal impression.

4. Discussion

The median lethal concentration LC_{50} typically represents the anticipated toxicity of a chemical. It is the concentration of a chemical that is lethal to 50% of the animals under a toxicity study. In the present work, naphthalene was observed to have an LC_{50} value of 6600 $\mu\text{g/L}$ in the Catfish (*C. gariepinus*.) The LC_{50} data for naphthalene in fishes (under static renewal systems) have been determined by several investigators and some of them include: *M. fluviatilis* [21]; 5180 $\mu\text{g/L}$ for Milkfish, *C. chanos* [19]; 5400 $\mu\text{g/L}$ for Climbing perch, *A. testudineus* [16]; 7210 $\mu\text{g/L}$ for Catfish, *C. gariepinus* [25]; 500 $\mu\text{g/L}$ for Rainbow fish; 2830 $\mu\text{g/L}$ for Florida pompano and *T. carolinus* [24]. The findings in this work and that of other mentioned researchers show that LC_{50} values of naphthalene varies with the species of aquatic organisms. These variations could be as a result of differences in metabolism of the different organisms. Furthermore, environmental factors including physico-chemical parameters, size and regional influences could also play crucial roles in determining acute toxicity of naphthalene [19].

The estimated safe level for naphthalene in the tropical catfish varied from 0.066 to 330 $\mu\text{g/L}$. There is controversy over the acceptability of safe levels in the field of ecotoxicology due to the large variations in safe level values. These variations are as a result of the different methods for determining safe levels of toxicants [20]. The major shortcoming in calculation of application factor (AF) is its dependence on LC_{50} values [10]. The extrapolation of laboratory data to field data is not always meaningful, hence the difficulty in deciding the acceptable concentration that may be considered “safe” based on laboratory experiments [20].

The tendency of toxicants to elicit anaemia is well studied. Dey et al. [6] revealed in a recent study that *A. testudineus* on exposure to 2 levels of naphthalene concentration exhibited a significant decrease in RBC in comparison to the control. The decrease in RBC count was shown to be dose dependant as seen in the present study. Usually, a decrease in RBC count is indicative of poor iron metabolism [5]. In an earlier work, the authors also observed a similar effect with *A. testudineus* exposed to anthracene, a related polycyclic aromatic hydrocarbon [7].

As with RBC count, many authors have demonstrated declines in haemoglobin concentration of aquatic organisms exposed to toxicants, especially polycyclic aromatic hydrocarbons. Haemoglobin is a conjugated protein containing heme as prosthetic group and globin as the apoprotein [4]. Haemoglobin content can serve as a sensitive indicator of alterations in ecological conditions. Chavez-Veintemilla [3] observed that haemoglobin concentration in the tambaqui exposed to phenanthrene was the most affected blood parameter in the fish. The author attributed the decrease in haemoglobin concentration to a reduction in its synthesis or by increases in its destruction. In a related work, Mehrnaz et al. [13] reported significant reductions in

hemoglobin levels of *A. latus* exposed to PAHs.

Chemical pollutants have in addition to altering RBC counts and haemoglobin content, been shown to have a negative impact on observed haematocrit. Dey *et al.* [7] reported a significant decline in haematocrit in *A. testudineus* under naphthalene exposure. Reduction in haematocrit which measures the volume of packed red blood cells relative to the whole blood can be caused by the deformation of erythrocytes or reduced erythrocyte synthesis. Furthermore, reduced haematocrit value is indicative of impaired metabolic activities and lower adaptive capability [8].

Erythrocyte indices are useful in understanding the etiology of anemias. Anemias are classified, according to the size of the erythrocyte, as being normocytic (normal MCV), macrocytic (increased MCV), or microcytic (decreased MCV). [29]. While there was a significant increase in MCHC, there were no observed changes in MCH and MCV on exposure to naphthalene.

The present study showed that naphthalene was immunotoxic to *C. gariepinus*. There was an observed increase in the levels of circulating white blood cells in exposed fish. Leukocytes are part of the immune system and participate in both the innate and humoral immune responses. They circulate in the blood and mount inflammatory responses to toxicants, injury or pathogens [26]. Ramesh and Saravanan [23] reported an elevation in WBCs in *L. rohito* exposed to deltamethrin at sublethal concentrations.

Platelets are known to play important roles in wound healing and the inflammatory process, usually through interaction with immune cells. Platelets are however not cells, but rather cytoplasmic fragments derived from megakaryocytes [28]. Under stress conditions that cause physical injuries to vascular tissues, platelet counts are increased to mitigate haemorrhage [7, 28].

Growth is an integrated physiological response involving external conditions (food quality and quantity, water quality) and internal physiological status (health, stress, reproductive state) [17]. PAHs and other injurious chemicals have been shown to inhibit growth rate. Chavez-Veintemilla [3] reported significant reductions in specific growth rate in tambaqui exposed to very low concentrations of phenanthrene after only two weeks of exposure. Jee *et al.* [9] also reported significant decrease in the average mass gain of flounder exposed to both high and low concentrations of phenanthrene for a period of 14 and 28 days. Reductions in relative growth rates can be attributed to reduced food uptake by the animals as a result of naphthalene intoxication Jee *et al.* [9].

Histopathological changes are widely used as biomarkers to evaluate the health status of aquatic organisms exposed to toxicants. In the present study, sections of the liver tissues were observed to assess the level of alterations in the liver cells and gills as a result of PAH exposure. The liver plays a crucial role in fish metabolism. It is also the storage site for many metabolites, especially glycogen [11]. Fish exposed to naphthalene showed no visible alterations as the control group. However, the gills which are in close contact with the external environment showed observable haemorrhage

accompanied with blood coagulation.

5. Conclusion

The study showed the effect of different concentration of naphthalene exposed to catfish. There were reduction in red blood count, haemoglobin, haematocrit, mean corpuscular volume and also coagulation of blood in the gill of the catfish.

Naphthalene causes changes in hematological parameters in *Cat fish* and also inhibits growth rate. It is thus of environmental concern due to its toxicity.

6. Recommendation

There is need for further research to study the metabolism and biotransformation pathways of naphthalene in *Cat fish*. The information obtained from this study will help to assess the sublethal effects of PAHs in general and naphthalene in particular and to set up water quality criteria for control policies and conservation strategies in tropical region.

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