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THE INVESTIGATION OF HAEMATOLOGICAL PARAMETERS OF *Clarias gariepinus* (AFRICAN CATFISH) EXPOSED TO KEROSENE

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ABSTRACT

Juveniles of the African Catfish, Clarias gariepinus were exposed to refined petroleum product {kerosene (0, 0.2%, 0.4%, 0.6%, 0.8% and 1.0%)} in replicate for in situ static bioassay studies. Twelve (12) aquaria glass tanks was also used for the study. The test organisms were acclimatized in the laboratory for two weeks (14 days) in the glass aquaria to laboratory conditions. The blood samples was analyzed for Erythrocyte count, Mean Corpuscular Haemoglobin Concentration (MCHC) was determined, haemoglobin content and haematocrit value or packed cell volume (PCV) was expressed as % of total volume white blood cell count was calculated using recommended standards. The acute exposure of Clarias gariepinus to kerosene exhibited a wide range of behavioral responses. These include pronounced gasping for breath, erratic swimming behavior uncoordinated movement, Inverted positioning and occasional darting up and down the water column. On the basis of 96hrs LC_{50} values, kerosene was more toxic to the juvenile of Clarias gariepinus. The toxicity factor calculated indicated that kerosene was toxic to Clarias gariepinus at 1.0%. The PCV, Hb and WBC content of toxicant exposed fish groups showed a progressive fall (23.40±0.00 to 19.00±0.00), (7.70±0.14 to 6.5±0.71) and (21.93±0.00 to 19.82±0.28) respectively while MCH and MCV showed a progressive rise (19.80±0.00 to 20.13±0.00) and (59.29±0.14 to 60.40±0.00) respectively. MCHC showed decrease at increase concentration of the toxicant from (33.33±0.00) at 0.2%) of toxicant to 31.57± 0.00 at 1.0% concentration of the toxicant. The physico-chemical parameters at the different concentration of the toxicant showed significant differences from that of the control ($p<0.05$).

Keywords: *Haematology, Clarias gariepinus, refined kerosene, Bioassay, Toxicology*

INTRODUCTION

The harmful effects of petroleum pollution results from physical fouling of the water and the intake of water soluble and insoluble hydrocarbons by aquatic biota (Rodrigues *et al.,* 2010). Only a relatively small fraction dissolves and becomes bioavailable. The water-soluble fraction (WSF) of crude oil and their derivations contains a mixture of polycyclic aromatic hydrocarbons (PAH), monoaromatic hydrocarbons often referred to as BTEX (Benzene, Toluene, Ethylbenzene and Xylenes), phenols and heterocyclic compounds, containing nitrogen and sulphur and heavy metals (Di Toro, 2001). Some petroleum-derived hydrocarbons are toxic to a wide spectrum of animals because they preferentially accumulate in lipidic compartment like cellular membrane, distributing the physiochemical and physiological membrane properties. The accumulation of soluble petroleum

hydrocarbons in fish is extremely rapid (Omeregie and Ufodike, 2000; Anyakora *et al.,* 2005). These pollutants tend to accumulate more in organisms than in the environment, so for this reason fish can be used as a bio-indicator to evaluate the environmental contamination level of these hydrocarbons (Anyakora *et al.,* 2005). Exposure of fish to sublethal concentration of pollutants may impose considerable physiological stress, which results to a number of manifestations such as reduced growth, impairment in reproduction, vulnerability to diseases, predatory performance or reduced capacity to tolerate subsequent stress and even reduced locomotion. Hematological analysis provides quick screening methods for assessing fish health as changes in blood parameters are often quick response to environmental or physiological stress. There is a range of measure for these physiological indicators of fish species to eradicate trauma inflicted on fish by various stressors. (Anyakora *et al.,* 2005).

Exposure of fish to different pollutants such as, industrial effluents, herbicides, pesticides and heavy metals- results in many biochemical alterations in the blood parameters. These charges are attributed to direct response of structural damage to red blood cell membranes resulting in hemoglobin synthesis (Di Toro, 2001). Despite the large number of reported spills of various scales occurring in the country particularly in the Niger Delta region, very little is known of the hematological charges that exposed fish may suffer under exposure to diesel or refined oil (kerosene). In view of this problem, it has become necessary to study the changes, especially the haematology of *Clarias gariepinus* fingerlings exposed to petroleum product (kerosene) so as to reveal the dangers of these spillage pollutants on the water bodies.

MATERIALS AND METHODS

The study area was carried out in the Department of Environmental Management and Toxicology Laboratory, Michael Okpara University of Agriculture, Umudike. The test reagent (kerosene) was obtained from the Master Energy Filling Station located at Aba Road Umuahia, Abia State. Twelve (12) aquaria glass tanks was also used for the study. Coppens feed which was used to feed the experimental *Clarias gariepinus* was purchased from a reliable and reputable fishfeed store. The test organism post-fingerlings catfish (*Clarias gariepinus*) weighing about 70.0g±2.0 to 82.0g ± 2.0 were purchased from the fish farm in the Department of Fisheries and Aquatic Resource Management, Michael Okpara University of Agriculture Umudike. The organisms were selected for the test because of its sensitive nature and response to test

substances (Reish and Oshida, 1986). The test organisms were collected in the morning hours between 7:30am – 8:30am when the temperature is low enough to prevent heat stress. The number of experimental organisms that were collected for sampling ranged between 250-300. The test organisms were acclimatized in the laboratory for two weeks (14 days) in the glass aquaria to laboratory conditions. During the acclimatization period, the test organisms were fed with fish pellet called "coppens" and thereafter water was renewed within the energy of 48 hours to avoid accumulation of waste materials that may become toxic for the test organisms (*Clarias gariepinus*). The blood samples which were used for the investigation of haematological parameters of the test organisms were collected from the caudal artery, using 21g and $^1/_5$ (0.8 x 40mm) syringe and transferred into a 8ml glass containing Ethylene Diamine Tetracetic Acid (EDTA) as an anticoagulant. Erythrocyte count, Mean Corpuscular Haemoglobin Concentration (MCHC) was determined; haemoglobin content and haematocrit value or packed cell volume (PCV) was expressed as % of total volume white blood cell count was calculated using recommended standards. Blood was collected from the test samples (fish) from each of the sample stations. The blood investigation was carried out at the Department of Veterinary Laboratory, MOUAU.

Mathematically, Mean Corpuscular Volume (MCV) in um^3/cell = $\frac{\text{PCV}(\%) \times 10}{RBC\ (10^6 mm^3)}$

Mean Corpuscular Haemoglobin (MCH) in P_g/cell = $\frac{\text{Haemoglobin (g/l)} \times 10}{RBC\ (20^6 mm^3)}$

Mortality assessment was done every 12 hours over the 96 hours (4 days) period. The post-fingerlings of the test organisms (*Clarias gariepinus*) exposed during the bioassay was taking to be dead when there was no body movement of fishes and evidence of hopping or movement even when probed with glass rod. When fishes were about to die, their swimming rate reduces and often death, there was yellowish coloration which was around the operculum. The number of death recorded during the bioassay period was recorded against time. Toxicological dose-response data involving quantal response (mortality) was analyzed by the use of probit analysis using SPSS 14.0 (Fig 1). Sublethal toxicity was carried out for 30 days for the fish species in order to investigate the sublethal effect of the test reagent (Kerosene). Sublethal concentration of test reagent was extrapolated as fractions; $1/10^{th}$, $^1/_{100}{}^{th}$ of the LC_{50} concentration.

The physicochemical parameters that were considered during the water quality assessment includes the following; Temperature, Salinity, Hydrogen ion Concentration (pH). Acute toxicity test of the toxicant (kerosene) showed that *Clarias gariepinus* exhibits differential responses on different concentration. On the basis of 96hrs LC_{50} values, kerosene was more toxic to the juvenile of *Clarias gariepinus*. The toxicity factor calculated indicated that kerosene was toxic to *Clarias gariepinus* at 1.0%. The acute exposure of *Clarias gariepinus* to kerosene exhibited a wide range of behavioral responses. These include pronounced gasping for breath, erratic swimming behavior uncoordinated movement, Inverted positioning and occasional darting up and down the water column. This can be attributed to nervous reaction of the organism to the irritating effects of the toxicants and disturbance in physiological mechanism.

RESULTS AND DISCUSSION

Table 1: Mean ± Standard deviation of interaction effects of toxicant concentration (kerosene) on hematological parameters of *Clarias gariepinus*

	Control	0.2	0.4	0.6	0.8	1.0
RBC	a 4.45±0.02	b 3.94±0.00	d 3.34±0.24	e 3.10±0.00	c 3.78±0.01	f 2.98±0.00
PCV	a 30.60±0.28	b 23.40±0.00	c 22.00±1.41	d 20.00±0.00	b 23.40±0.85	e 19.00±0.00
HB	a 8.60±0.00	b 7.70±0.14	c 5.50±0.71	d 6.75±0.35	e 7.50±0.13	f 6.50±0.71
WBC	a 23.10±0.00	ab 21.93±0.00	bc 20.68±0.00	bc 20.66±0.71	abc 21.41±1.49	c 19.82±0.28
MCH	a 19.41±0.00	b 19.80±0.00	c 20.79±0.06	d 22.88±0.00	e 20.11±0.00	f 20.13±0.00
MCV	a 56.34±2.83	ab 59.29±0.14	c 61.50±1.41	d 68.62±0.14	e 60.32±0.00	f 60.40±0.00
MCHC	a 27.92±0.00	b 33.33±0.00	c 30.93±0.71	d 35.00±0.00	e 31.66±0.00	f 31.57±0.00

Keywords: a, b, c, d, e, f, ab, abc and bc = Means with same superscript is not significantly different at P= 0.005 level of significance, RBC = Red Blood Count, PCV = Packed Cell Volume, HB = Heamoglobin, WBC = White Blood Count,

MCH = Mean Corpscular Haemoglobin, MCV = Mean Corpscular Volume, MCHC = Mean Corpscular Haemoglobin Count

The haematological values obtained are presented in table 1 Hb values ranged from 5.50±0.71 to 8.60±0.00 (g/100ml), RBC values ranged from 2.98 ± 0.00 to 4.45 ± 0.02 ($x10^6mm^3$), PCV values ranged from 19.00 ± 0.00 to 30.60 ± 0.28% MCV values ranged from 56.34 ± 2.83 to 68.62 ± 0.14nm^3 MCH values ranged from 19.41 ± 0.00 to 22.88 ± 0.00 Ng/cell, MCHC values ranged from 27.92 ± 0.00 to 35.00 ± 0.00% WBC values ranged from 19.82 ± 0.28 to 23.10 ± 0.00 ($x10^6mm^3$). The significant changes observed in table 2 were decreased in the number of red blood cells (RBC) with increased concentration of the toxicant from 3.94 ± 0.00 at concentration 0.2 percent to 2.98 ± 0.00 at concentration 1.0%. The PCV, Hb and WBC content of toxicant exposed fish groups showed a progressive fall (23.40±0.00 to 19.00±0.00), (7.70±0.14 to 6.5±0.71) and (21.93±0.00 to 19.82±0.28) respectively while MCH and MCV showed a progressive rise (19.80±0.00 to 20.13±0.00) and (59.29±0.14 to 60.40±0.00) respectively. MCHC showed decrease at increase concentration of the toxicant from (33.33±0.00) at 0.2%) of toxicant to 31.57± 0.00 at 1.0% concentration of the toxicant.

The decrease in PCV and RBC in this work indicates that fishes exposed to kerosene were anaemic. These results are in agreement with that reported by Adams and Duncan (2002) decrease in PCV and RBC in catfish exposed to pulp and paper mill effluents. The increase in MCV, MCH and decrease in PCV, RBC and Hb values confirmed the occurrence of haemolytic anaemia in fish and exaggerates disturbances that occurred in both metabolic and haemopoietic activities of fish due to toxicant exposure. It is known that leucocyte cells are normally lower in healthy fishes and could be used as a significant indicator for stress element (AIP, 2010). MCHC decreased from 33.33± 0.00 at 0.2% to 31.57± 0.00 at a higher concentration of 1.0%. Similar findings were also reported by Omoregie and Ufodike (2000) working on the haematological parameters of *Oreochromis niloticus* treated with crude cassava leaf extract.

Table 2: Effect of Kerosene on the Mortality of *Clarias gariepinus*

Treatment concentration	ohrs	24	48	72	96hrs		
T_1 0.2% A_1	10	-	-	-	-		
T_2 A_2	10	-	-	-	-		
T_3 0.4% B_1	10	-	-	-	2	20%	After 74hrs
T_4 B_2	10	-	-	-	3	30%	
T_5 0.6% C_1	10	-	-	1	1	20%	After 72hrs
T_6 0.6% C_2	10	-	-	1	2	10%	
T_7 0.8% D_1	10	-	1	-	-	10%	After 48hrs
T_8 0.8% D_2	10	-	2	-	-	20%	
T_9 1.0% E_1	10	2	-	-	1	30%	After 24hrs
T_{10} 1.0% E_2	10	1	-	-	1	20%	
Control K_1	10	-	-	-	-		
K_2	10	-	-	-	-		

Keywords

T_1 0.2% A_1 = Treatment 1 with 0.2% concentration of toxicant with first replicate

T_2A_2 = Second replicate with 0.2% concentration of toxicant

T_3 0.4% B_1 = Treatment 2 with 0.4% concentration of toxicant with (B_1) first replicate

T_4B_2 = Second replicate with 0.4% concentration of toxicant

T_5 0.6% C_1 = Treatment 3 with 0.6% concentration of toxicant with (C_1) first replicate

T_6 0.6% C_2 = Second replicate with 0.6% concentration of toxicant

T_7 0.8% D_1 = Treatment 4 with 0.8% concentration of toxicant with (D_1) first replicate

T_8 0.8% D_2 = Second replicate with 0.8% concentration of toxicant

T_9 1.0% E_1 = Treatment 5 with 1.0% concentration of toxicant with (E_1) first replicate

T_{10} 1.0% E_2 = Second replicate with 1.0% concentration of toxicant

Control K_1 = Treatment 6 with 0% concentration of toxicant with (K_1) first replicate

K_2 = Second replicate with 0% concentration of toxicant

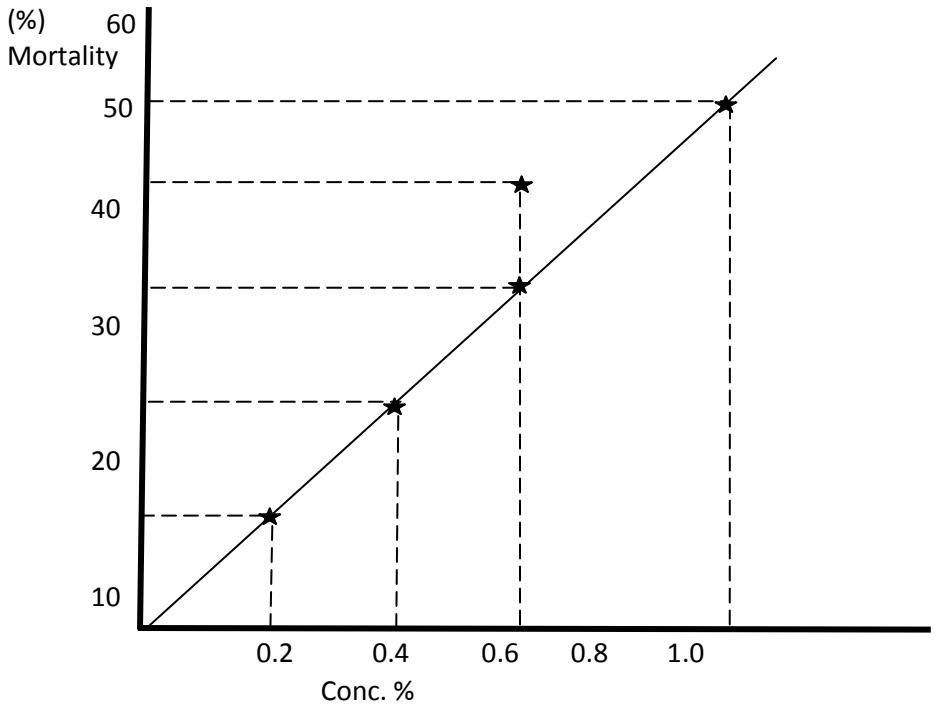

Fig 1: Toxicological dose-response (mortality)

The laboratory investigation in this research revealed the concentration of kerosene that will cause death of fish and also the concentration suitable for fish culture. The physico-chemical parameters at the different concentration of the toxicant showed significant differences from that of the control ($p<0.05$). Temperature, pH were within the normal range while alkalinity fell outside the normal range; therefore it was also evident that the decreased in the alkalinity value influenced the hydrochemistry of the water thereby resulted in fish death. The blood parameters (Hb, WBC, PCV, MCV, MCH, RBC and MCHC) also showed significant difference from that of the control.

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