

Haematological Studies on the Effect of Crude Oil on Juvenile African Catfish

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Abstract

This research looked at how crude oil from an oil exploration business in Delta State affected juvenile African catfish's blood, gills, kidney, heart, and liver (*Clarias gariepinus*). 25 juvenile African catfish were employed in the study, divided into 5 groups (5 per treatment) and used for the study. The juveniles were exposed to different concentrations of crude oil (0%, 0.1%, 0.3%, 0.5%, and 1%) for a period of 9 days. At the end of the test period, haematological activities were carried out on the blood of the juveniles. The findings revealed a difference ($p < 0.05$) in haematological parameters between the control and exposed groups, which, according to previous research, indicates a decrease in oxygen supply, resulting in a decrease in respiratory response and oxidative stress. Haemoglobin of 0.1% crude oil contaminated group showed a decrease in activity when compared to the control group. Lymphocytes showed an increase across all contaminated groups when compared to the control group. All of the juveniles kept in the control stock exhibited no degeneration. In conclusion, this research has shown that even low amounts of crude oil can cause haematological abnormalities in the fish's blood, kidney, heart, liver, and gills.

Keywords: *Crude oil; Haematology; Clarias gariepinus; Juveniles.*

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Background to the Study

It is generally agreed that petroleum exploration has boosted Nigeria's economy. However, the associated activities of petroleum exploration have negative effects on Niger Delta environment (Kamalu, and Nwokocha, 2011). Wrong disposal of petroleum-derived hazardous waste streams, such as oily and toxic sludge, equipment failure, oil spills, operational discharges, and sabotage of petroleum facilities are all important environmental hazards (Ite *et al.*, 2013). According to Jernelov (2010), the mortality of oil and its compounds is unquestionable because exposure to hydrocarbons induces internal organ harm. The Nigerian economy relies heavily on catfish production. It provides a source of income, lowers the unemployment rate, and boosts the economy's Gross Domestic Product (GDP). It gets a greater price than tilapia in most countries since it may be sold live at the market and has a market value two to three times that of tilapia (Emokaro, 2010).

It is often agreed that the early life stages of fish are more sensitive to xenobiotics, and are mostly used to detect and estimate legally applicable measurements of pollutants and their effects on aquatic ecosystem (Incardona *et al.*, 2011). Haematological changes in fishes exposed to contaminants have been proposed and used as biomarkers for pollutants such as petroleum products (Eseigbe *et al.*, 2013).

Haematology is the study of the number and form of the blood's cellular constituents such as leucocytes, erythrocytes and platelets (Neal and Kathryn, 2016). Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to the blood. They serve as a pathological reflector of the health of animals that have been exposed to toxicants and other conditions (Olafedehan *et al.*, 2010). Animals with low white blood cell counts are at danger of disease infection, whereas those with high counts are capable of producing antibodies during the phagocytosis process and have a high level of disease resistance (Soetan *et al.*, 2013) and enhanced adaptability to local environmental and disease prevalent conditions (Kabir *et al.*, 2011; Okunlola *et al.*, 2012 and Iwuji *et al.*, 2013). The red blood cell is involved in the transport of oxygen and carbon dioxide in the body, according to Isaac *et al.* (2013). As a result, a lower red blood cell count indicates a decrease in the amount of oxygen carried to the tissues as well as the amount of carbon dioxide returned to the lungs (Ugwuene, 2011; Soetan *et al.*, 2013 and Isaac *et al.*, 2013).

Due to its ability to detoxify and store hazardous substances, the liver is commonly utilized as an environmental biomarker (Stori *et al.*, 2014). One of the bodily organs in charge of excretion and controlling the water balance in fish is the kidney (Byron, 2014). Blood is pumped by the heart (Graham and Dickson, 2004). The gills may immediately take in oxygen from the air (Byron, 2014).

It is widely known that several research has been done on the contact of African Catfish (*Clarias gariepinus*) with pollutants. No research has been conducted to yet on the effects of crude oil generated by this particular oil company; Warri Refining and Petrochemical Company (WRPC) on the blood, liver, gills, kidney, and heart of juvenile African Catfish. Due to the company's proximity to a water body, it is vital to look at its consequences.

Materials and Methods

Specimen Collection/ Acclimatization

Twenty-five healthy 6 weeks old juveniles of *Clarias gariepinus* with an average weight of 0.2kg were purchased from a local fish farm in Delta State (AB farms, Agbarho) and transported in an open gallon half filled with clean tap water. Five juveniles each were kept in five separate constructed bioreactors of 25 litres capacity containing 5kg soil and 20litres of water within Department of Petroleum Resources limit.

Ethical Clearance

All animals were kept in conventional laboratory settings with free access to balanced pellet diet and water. The experiment methodology was authorized by the Federal University of Petroleum Resources, Effurun (FUPRE), Nigeria ethics committee (FUPRE/ECC2019/SC/EMT001) and complied with all ethical standards for the use of animals in research.

Experimental *Clarias Gariepinus*

Experimental *Clarias gariepinus* was classified into five groups designated thus;

Group A: *Clarias gariepinus* cultivated in 0% crude oil impacted soil

Group B: *Clarias gariepinus* cultivated in 0.1% crude oil impacted soil

Group C: *Clarias gariepinus* cultivated in 0.3% crude oil impacted soil

Group D: *Clarias gariepinus* cultivated in 0.5% crude oil impacted soil

Group E: *Clarias gariepinus* cultivated in 1.0% crude oil impacted soil

Hematological Studies

The fish were sacrificed and blood samples were collected via the caudial vein puncture as described by Lawrence et al, (2020). Blood samples were collected with sterile 5ml syringe and 21G needle. The needle was introduced on the ventral midline, between the anal opening and the beginning of the anal fin to assess the caudal vein beneath the vertebral column. The collected blood was then dispensed into a plastic tube containing EDTA (Ethyl Diamine Tetraacetic Acid) as anticoagulant for haematological analysis.

The use of plastic syringe is a necessary precaution with fish blood, because contact with glass results in decreased coagulation time (Lawrence et al., 2020). The haematological properties were determined using an automated machine.

Analysis Statistical

All data were evaluated using the Steel and Torrie method of Analysis of Variance (ANOVA) (1960). Duncan's Multiple Range Test was used to examine whether there was a significant difference between the treatment means at 5% confidence level (Duncan, 1955).

Results

The results obtained from the haematological examination of the blood samples collected from the juveniles exposed to crude oil and control are presented in Table 1. From the results of haematological studies presented, it is observed that the haematological indices of groups exposed to crude oil were changed when compared to control ($p < 0.05$)

Table 1: Haematological Properties of *Clarias Gariepinus* Cultivated in Crude Oil Contaminated Sediment

GROUP NAME	HB	PCV	WBC	NEU	LYM	MON	EOS	BAS	RBC	MCHC	MCV	MCH	P/T
0%	7 ±0.1 ^a	22±0.2 ^a	23520± 1140 ^a	2±0.03 ^a	93±0.1 ^a	1±0.02 ^a	3±0. 1 ^a	0.04±0. 002 ^a	1±0.03 ^a	43±0.1 ^a	139±0. 2 ^a	52±0.4 ^a	242±1.0 ^a
0.1%	6±0.03 ^b	19±0.2 ^b	23960±379 ^a	4±0. 1 ^b	88±0.2 ^b	3±0. 1 ^b	6±0. 1 ^b	0.2±0.005 ^b	1±0.00 1 ^a	47±0. 3 ^b	138±0. 9 ^a	63±0. 6 ^b	209±3. 2 ^b
0.3%	9±0. 1 ^c	25±0.3 ^c	67440±787 ^b	5±0. 1 ^c	88±0.3 ^b	3±0.1 ^b	3±0.03 ^a	0.1±0.004 ^c	2±0.02 ^b	36±0.2 ^c	144±0.4 ^b	52±0.2 ^a	364±7.3 ^c
0.5%	7±0. 1 ^a	21±0. 4 ^d	44480±15 39 ^c	8±0. 2 ^d	83±0.1 ^c	3±0. 1 ^b	6±0.05 ^b	0.2±0.004 ^b	1±0.03 ^a	41±0.3 ^d	141±0. 2 ^c	58±0.3 ^c	386±6.9 ^d
1%	7±0.1 ^a	24±0.2 ^c	45540±855 ^c	6±0. 2 ^c	83±0.4 ^c	4±0. 1 ^c	7±0. 1 ^c	0.3±0.01 ^d	1±0.02 ^a	37±0.4 ^c	143±1. 2 ^b	48±0. 3 ^d	230±4. 0 ^c

Education and Science November, 2022 Results are means of five determinations ± SEM. Values on the same column carrying different superscripts are significantly different (p < 0.05)

Key: Hb = Haemoglobin, PCV = Packed Cell Volume, WBC = White Blood Cell, RBC = Red blood cell, NEU = Neutrophil, LYM = Lymphocyte, MON = Monocyte, EOS = Eusinophil, BAS = Basophil, WBC = White blood cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, P/T = Platelet

Behavioural Observation of the Juveniles

Erratic swimming, projecting of their heads to gasp for air, sudden quick movement and summersaulting were observed in the juveniles exposed to crude oil.

Discussion

Hematological parameters serve as a pathological indicator of the health of animals that have been exposed to contaminants (Olafedehan et al, 2010). Red blood cells, white blood cells, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and haemoglobin are all haematological components that play a part in the physiological status of the organism (Doyle, 2006).

There was significant change (P<0.05) of haematological parameters of *Clarias gariepinus* exposed to crude oil when compared to control. This is in disagreement with Gabriel et al (2004) who reported a non-significant difference in hematological values for *Clarias gariepinus* exposed to toxicants which opposes the observation in this study.

The haematology results in the present study agrees with Akinrotimi et al. (1971) who reported effects of transportation stress on haematological parameters of Blackchin Tilapia *Sarotherodon melanotheron*. In the light of the present study, it is obvious that the exposure of *C. gariepinus* to 0.1% crude oil contaminated sediment caused a significant decrease in the concentration of haemoglobin. In *C. gariepinus* treated to malachite green, a reduction in haematocrit was observed, indicating an anemic response (Ololade and Ogini, 2010). The

current study also supports (Kumar and Banerjee, 2016) findings of a considerable decrease in haematocrit and haemoglobin concentration in *Heteropneustes fossilis*. In *C. gariepinus*, a considerable decrease in haemoglobin values indicates severe anemia. The destruction or suppression of erythrocyte formation could be the cause of anaemia or haemodilution as reported by (Abdel-Wahab et al., 2020).

When compared to the control group, white blood cell levels increased in all crude oil polluted groups. The rise in leucocyte synthesis as a defense mechanism against the breakdown of erythrocytes, which function in the generation of antibodies and chemical compounds that serve as a defense against infection, could be ascribed to the large increase in white blood cell levels (Chaplin, 2010).

Packed red cell volume values reduced in 0.1% and 0.5% crude oil contaminated groups compared to control group, and this is traceable to different fishes having different blood parameters unlike human blood that is constant (Trifonova et al., 2018). The findings of this investigation corroborate those of (Andem and Esenowo, 2015), who saw comparable results when *C. gariepinus* was exposed to a sublethal dose of formaldehyde. The results of statistical analysis suggest that various blood parameters have decreased, which could be an indicator of anemia, which is defined by a lack of haemoglobin, packed cell volume, and erythrocytes. Haematocrit readings may be beneficial as a general predictor of fish health, according to teleost haematology research, because fish fed iron-deficient diets or exhibiting anemia all had lower haematocrit (PCV) values (Witeska, 2015).

Asides from chemical elements, Crude oil also consists of polycyclic aromatic hydrocarbons (PAHs) which are considered the most toxic component of oil (Snyder et al., 2015). This might have accounted for the significant changes in the oxygen transport vehicles (Hb, RBC, MCH, MCHC) of *Clarias gariepinus* exposed to crude oil.

Observed behavioural changes, such as erratic swimming, gasping for air, sudden quick movement and summersaulting are similar to changes linked to the toxicity of xenobiotics in fish and has been reported in previous studies (Sarikaya and Yilmaz, 2003). The observed repeated attempts to jump out of the holding containers will be described as adaptation behavioural responses to escape from the toxic environment. For example, at higher concentration of crude oil (1%), it was observed that most of the juvenile were projecting their heads out of water to gasp for fresh air, indicating that they were apparently having dissolved oxygen problems, however, such behavior could also be attributed to damage to the gills, which impairs oxygen uptake and leads to toxicity induced hypoxia, this is in line with the observation of Barbieri (2008) in a recent study. The damage induced on the gill of the test fish was found to be concentration and exposure period dependent.

Conclusion

The effects of crude oil varied in the blood of the exposed juveniles compared to the control group. However, such measurable changes depend upon the biological status of exposed fish as well as upon the type and duration of their exposure to toxicants within that aquatic

environment. Stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic response. If severe or long lasting, the response then becomes mal-adaptive and threatens the fish health and wellbeing. Even though individual concentrations of any toxicant might be low, the combined concentrations could be fatal to aquatic health. These toxicants no matter how little the concentration could be biomagnified in a water body, with the resultant effect being gradual accumulation of the toxicants in water, which in turn becomes toxic to aquatic organism. Although, *Clarias gariepinus* have a high adaptive ability, higher concentration of crude oil may be detrimental to their health as shown in this study. Consumption of *C. gariepinus* cultivated in contaminated water may pose threat to public health.

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