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The Effects of Crude Oil on the Blood Parameters and Serum Enzymes of the African Catfish *Clarias Gariepinus*

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ABSTRACT:

The effects of crude oil on the blood parameters and serum enzymes of the widely consumed catfish *Clarias gariepinus* were studied based on the results of the 96 hour acute toxicity tests carried out on *Clarias gariepinus* of mean weight 138 ± 12 g and mean total length 28 ± 1.5 cm. The LD50 of crude oil was 823.3ppm. The experiment lasted for 10 weeks after which blood samples were collected by cardiac puncture for Haemoglobin (Hb), Haematocrit (Ht), red blood cell (RBC) count, white blood cell (WBC) count, and WBC differential count. RBC indices; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular Haemoglobin concentration were calculated. Serum enzymes; alkaline phosphatase (ALP) Alanine transaminase (ALT) and aspartate transaminase (AST) were assayed. Haemoglobin concentrations decreased significantly ($P < 0.05$) between 7.50 ± 0.70 g/dl and 6.90 ± 0.64 g/dl in all sub-lethal concentrations compared to the control values of 8.58 ± 0.48 g/dl. Haematocrit were similarly significantly ($P < 0.05$) lowered from the control value of 26.8 ± 0.68 μ/L to 20.8 ± 1.78 μ/L and 23.8 ± 2.07 μ/L for 300ppm and 600ppm crude oil respectively. The RBC count significantly reduced ($P < 0.05$) from the control value of $4.38 \times 10^{12} \pm 0.26$ to between $3.46 \times 10^{12} \pm 0.31$ and $3.86 \times 10^{12} \pm 0.35$ in 300ppm and 600ppm crude oil respectively. The WBC count significantly ($P < 0.05$) increased from 20720.00 ± 307.77 in the control to 21220 ± 845.40 and 24160 ± 735.71 in the two sublethal levels respectively. The serum enzymes of the control 40.0 ± 4.07 iu/L (ALP), 34.8 ± 1.18 iu/L (ALT) and 119.8 ± 13.24 iu/L (AST) were significantly ($P < 0.05$) higher than the experimental group at 300ppm crude oil 38.2 ± 6.83 iu/L (ALP) 23.8 ± 3.67 iu/L (ALT) and 95.0 ± 14.82 iu/L (AST) while the values at 600ppm crude oil were 36.6 ± 5.13 , 29.6 ± 4.07 iu/L, and 105.6 ± 15.81 iu/L respectively. There were no significant differences ($P < 0.05$) in MCHC, MCH and MCV values between the control and the experimental groups. This study has sufficiently contributed to the basic research needs of aquatic toxicology and fish pathology.

KEY WORDS: *Clarias gariepinus*, Crude oil, blood parameters, Serum enzymes.

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INTRODUCTION:

1.0 INTRODUCTION

Aquatic toxicology has advanced from a descriptive approach which was necessary to explore those concentrations of single toxicants within water that were not corruptible with the life of individual fishes, to

considerations of sub-lethal concentrations that do no harm to the individual thus making it expend resources to survive in a state of altered equilibrium. Biomarkers of exposure, response and genetic susceptibility were derived from research and these helped to cut across questions of bioavailability as the emphasis shifted to host response. Biomarkers illustrate the multiple organ, tissues

and cellular sites of action and the spectrum of responses that were possible. Biomarkers and validated methods have been designed and are now in use to assess chemical exposures and effects or responses arising from various forms of chronic toxicity^[1].

Blood parameters are considered patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants^[2, 3]. Haematologic analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance^[4, 5]. A number of haematological indices such as red blood cells (RBC) count, Haemoglobin (Hb) concentration, Haematocrit (Ht) or packed cell volume (PCV) and so on are used to assess the functional status and oxygen carrying capacity of blood stream^[6].

Biochemical and physiological indicators such as enzymes could be used as biomarkers to identify possible environmental contaminations before the health of aquatic organisms is seriously affected, and to develop water quality indices. Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish^[7]

Transaminases are groups of enzymes that catalyze the interconversion of amino and keto acids through the transfer of amino groups. It has been shown that L-glutamic acid serves as the donor of amino groups in most transamination reactions^[8]. In clinical medicine, the assay of transaminases: Glutamic pyruvate transaminase (GPT) and Glutamic Oxaloacetic transaminase (GOT) are gainfully used in diagnosis of disease and tissue damage^[9]. In aquatic toxicology these transaminases have been utilized in chemical diagnosis^[10].

Crude oil spills during operations, accidental spill during shipping and leakages from underground pipes are becoming a common phenomenon on a global scale. These have over the years led to polluting the world aquatic ecosystems. Several toxic components of crude oil (saturated non-cyclic and cyclic hydrocarbon, alkenes, aromatic hydrocarbons and heavy metals such as copper, lead, chromium, nickel, zinc etc have been documented^[11].

The aim of this study is to investigate the effects of sublethal concentrations of crude oil on some blood parameters and serum enzymes of the African catfish *Clarias gariepinus*.

2.0 MATERIALS AND METHODS

Acute toxicity tests were performed according to Adeyemo *et al.*,^[12]. Six plastic tanks of 52.8 litres capacity measuring 32cm x 50cm x 33cm were used for the investigation. Sixty *Clarias gariepinus* juveniles were selected after acclimatization and divided into three groups, A, B, and C.

Group A consisted of 20 fish kept as control. Group B consisted of 20 fish treated with 300ppm Crude oil in replicate while Group C consisted of 20 fish treated with 600ppm Crude oil also in replicate. Experiment lasted for 70 days (10 weeks) after which blood samples were collected by cardiac puncture for haematologic and serologic studies. Haemoglobin (Hb), haematocrit (Ht), Red blood cell (RBC) count, white blood cell (WBC) count, WBC differential count were carried out using the methods of Blaxhall and Daisely^[13]. Red blood cell indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were determined. Haemoglobin concentration (MCHC) were determined according to Jain^[14].

Serum enzyme analyses were done using the test kits^[15] for Alanine Transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP).

Mean and standard error of the means of experimental fish were calculated for each treatment group and the control. Data were statistically analyzed using ANOVA^[16].

3.0 RESULTS:

The LD₅₀ of Crude oil was 823.3ppm.

3.1 HAEMATOLOGY:

The results of the haematological responses of *Clarias gariepinus* to sublethal concentrations of Crude oil after 70 days exposure are shown in Figures 1, 2, 3 & 4.

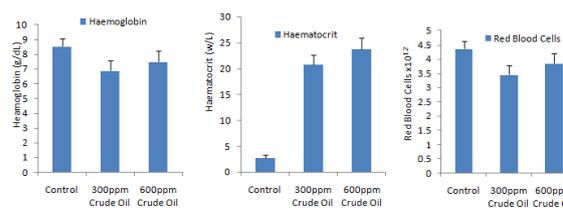


Figure 1: Haemoglobin, Haematocrit and Red blood cells (Mean \pm SEM) of *Clarias gariepinus* after 70 days exposure to crude oil.

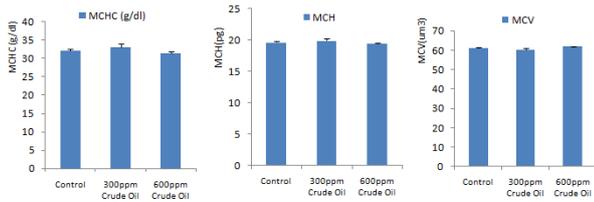


Figure 2: The Red blood cell indices (MCHC, MCH & MCV) of *Clarias gariepinus* after 70 days exposure to crude oil.

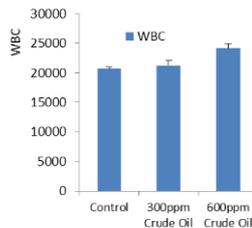


Figure 3: The White blood cells (Mean ± SEM) of *Clarias gariepinus* after 70 days exposure to crude oil.

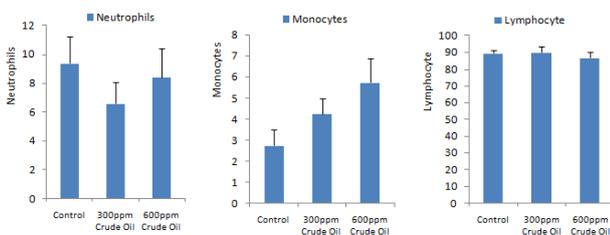


Figure 4: The WBC Differential counts of *Clarias gariepinus* after 70 days exposure to crude oil.

Significant differences ($P < 0.05$) between the blood parameters of the control and treated groups occurred in Hb, Ht, RBC and WBC. There were no significant differences ($P > 0.05$) between the control and treated groups in MCHC, MCH and MCV.

3.2 SERUM ENZYME ANALYSIS

The results of the enzyme analysis of *Clarias gariepinus* after 70 days exposure to crude oil are shown in Figure 5.

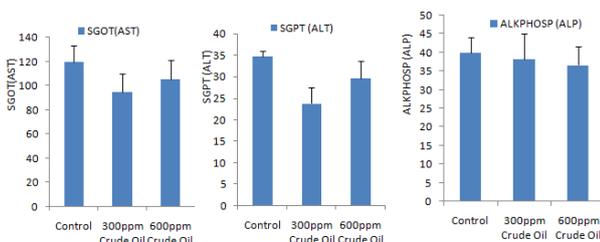


Figure 5: AST, ALT & ALP (Mean ± SEM) of *Clarias gariepinus* after 70 days exposure to crude oil.

Significant differences ($P < 0.05$) occurred between serum enzymes AST, ALT and ALP of the control and treated groups. The serum enzymes of the control were significantly higher than the treated groups.

4.0 DISCUSSION:

The physiological and pathological conditions of animals can be assessed by the evaluation of haematological and biochemical analyses of the blood [17, 18]. According to Van Vuren [19], studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematologic parameters. Thus water quality is one of the major factors responsible for individual variations in fish haematology since they are sensitive to slight fluctuations that may occur within their internal Milieu [20].

In the present study, the main haematological response of the Catfish *Clarias gariepinus* to the exposure of Crude oil was a significant decrease ($P < 0.05$) of red Blood cell count, Haemoglobin content and Haematocrit or packed cell volume compared to the control group. Prasad *et al.*, [21] discovered reduced Haemoglobin level as the major effect of crude oil on fresh water fish *heteropneustes fossils*. Gabriel *et al.*, [22] recorded decrease in the values of Red Blood Cell Count, Haemoglobin and Haematocrit in *Clarias gariepinus* exposed to kerosene. Similar trend was reported in *Clarias gariepinus* and *Oreochromis niloticus* exposed to crude oil [11, 23]. The decrease in the values of RBC, Hb, and Ht could be attributed to haemolysis, resulting in haemodilution, a mechanism for diluting the concentration of the toxicant in the circulatory system [24].

White blood cells (WBCs) are known to defend the body against toxic and foreign substances and antibody production [25]. As a result of crude oil toxicity, the fish body's cellular humoral response was triggered to produce more WBCs in the treated fishes, which migrated to the site to engulf or destroy the toxins. This explains the leucocytosis observed in this study. Leucocytosis was reported in *Clarias gariepinus* exposed to crude oil [11] and malachite green [26].

According to Udem and Asogwa [27], an initial step in detecting liver damage is a simple blood test to determine the presence of certain liver enzymes in the blood. The activity of these enzymes is normally used to evaluate liver function. Under normal circumstances, these enzymes reside within the cells of the liver, but when the liver is injured, these enzymes are spilled into the blood

stream. Among the most sensitive of these liver enzymes are the aminotransferases or the transaminases which include aspartate amino transferase (AST or SGOT) and alanine amino transferase (ALT or SGPT). AST is normally found in a diversity of tissues including liver, heart, muscles, kidney and brain. It is released into the serum when any of these tissues is damaged. ALT (SGPT) is by contrast normally found largely in the liver. It is released into the blood stream as a result of liver injury. It therefore serves as a fairly specific indicator of liver status. Alkaline phosphatase is a membrane associated enzyme located in a variety of tissues of which bone and hepatobiliary systems are of diagnostic importance^[28]. It is used extensively as a tumor marker. It is also present in bone injury, pregnancy, or skeletal tissues.

The results of the serum enzyme assay are presented in Figure 5. There were significant reduction ($P < 0.05$) in the levels of these enzymes in the experimental groups when compared with the control. This indicates that crude oil has toxic effects on the liver.

5.0 CONCLUSION:

This study revealed that exposure of fish to sublethal concentrations of crude oil produces significant changes in the physiology of *Clarias gariepinus* as manifested by changes in the blood parameters and disruptive changes in the liver which is a vital organ for metabolism.

It is highly recommended that relevant institutions should endeavour to create awareness of the inherent public health risks associated with consumption of contaminated fishery products so that private, government and corporate entities can willingly contribute to effective prevention and control of pollution of aquatic environments.

6.0 REFERENCES

- Guilio R. T. and Hinton, D. E. (2008). The toxicology of Fishes. 1st edition CRC Press, Taylor and Francis Gwup. Florida, 3-14.
- Adhikari, S. and Sarkar (2004). Effects of cypermethrin and carbofuran on certain haematological parameters and prediction of their recovery in fresh water teleost, *Labeo rohita* (Ham). Ecotoxicol. Environ. Safety 58:220-226.
- Maheswaran, R, Devapaul A, Velmurugan B, Ignacimuthu S (2008). Haematological studies of freshwater fish, *Clarias batrachus* (L.) exposed to mercuric chloride. Inter. J. Integr. Biol. 2(1): 49-54.
- Rehulka, J., Minarik B., Rehulkov A.E. (2004). Red Blood cell indices of rainbow trout, *Onchorhynchus mykiss* (Walbaum) in aquaculture. Aquac. Res. 35: 529-546.
- Tavares-Dias M, Barcellos JFM (2005). Peripheral blood cells of the armored catfish *Hoplosternum littorale* Hancock, 1828: a morphological and cytochemical study. Braz. J. Morphol. Sci. 22: 215-220.
- Shah, S.L., and Altinag, A. (2004). Haematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol. 73:911-918.
- Osman, Alaa, G.M., Abd-El-Baset M. Abd El Reheem, Khalid Y. Abuelfadl, Ali G. Gad El-Rab. (2010). Enzymatic and Histopathologic Biomarkers as indicators of aquatic pollution in fishes. Nature Science Vol. 2 No.11 1302-1311.
- Cohen P.P. and Sallach H.J. (1961) Nitrogen Metabolism of amino acids. In Metabolic Pathways (Greenberg, D.M. ed) Vol. 2 Academic Press New York
- Schmidt, E. and Schmidt F.W. (1976). The Importance enzymatic analysis in Medicine, diagnosis. Control of progress and therapy, in *Methods of enzymatic analysis* (Bergmeyer H.E. ed). P.14-30.
- Michael, M.I., Hilmy A.M., El-Domiatiy N.A. and Wershara K. (1987) Serum transaminase activity and histopathological changes in *Clarias lazera* Chronically exposed to nitrite *Comp. Biochem. Physiol.* 86:255-262.
- Omoriegbe, E., (1998): Changes in the Haematology of Nile Tilapia, *Oreochromis niloticus* under the effects of crude oil. *Acta Hydrobiol.*, 40:287-292.
- Adeyemo, O.K., Adedeji O.B., and Offor C.C., (2010): Blood Lead Level as biomarker of environmental Lead pollution in Feral and Cultured African Catfish *Clarias gariepinus*. *Nig. Vet. Journal* 31 (2), 48-56.
- Blaxhall, P.C, Daisley K.W (1973). Routine haematological methods for use with fish blood. *J. Fish Biol.* 5: 771-781.
- Jain, N. C. (1986). Schalm's Veterinary Haematology 4th edition (ed N.C. Jain) Lea and Febiger, Philadelphia USA 1221 pp.

15. Reitman, S. and Frankel, S. (1957). A Colorimetric method for the determination of serum glutamate-oxaloacetate and glutamate-pyruvate transaminases. *American Journal of Clinical Pathology*. 28:56.
16. MS Excel (2001). MS USA
17. Coles, E.H. (1986): *Veterinary clinical Pathology*. Saunders, Philadelphia Pp. 17-19.
18. Bush, B. M (1991): *Interpretation of Laboratory results for small animal*. Clinician Blackwell Scientific, London.
19. Van Vuren J.H.J (1986). The effects of toxicants on the haematology of *Labeo urubratu*s (Teleostei cyprinidae). *Comp Bioch Physiol* 83C:155-159.
20. Fernandez, M.N. and A.F. Mazon, (2003). 'Environmental Pollution and Fish Gill Morphology. In: Val, A.L. Kapoor B.G. (Eds.), *Fish Adaptations*. Science Publishers, Enfield, USA., Pp:203-231.
21. Prasad, M.S., M. Prasad and D Singh (1987). Some Haematological effects of crude oil on fresh water *heteropneustes fossilis*. *Acta Hydrochemhydrobiolo*, 15: 199-204.
22. Gabriel, U.U., Amakiri, E.U., and Ezeri, G.N.O. (2007). Haematology and Gill Pathology of *Clarias gariepinus* exposed to Refined Petroleum oil, Kerosene under Laboratory Conditions. *Journal of Animal and veterinary Advances* 6(3), 461-465.
23. Gabriel, U.U., Alagoa, J.K., and Allison, M.E., (2001). Effects of dispersed crude oil water dispersion on the haemoglobin and haematocrit of *Clarias gariepinus*. *J. Appl. Sci. Environ. Manage.* 5(2): 9-11.
24. Smith, G.R.W., Bortish T.M., Kubiak T.J. (1999). Assessing the exposure of fish to environmental contaminants. Information and technology Report Usas/BRO-1999 0007. US. Geological Surgery. Biological Resources Division, Columbia M.O. pp.547.
25. Oxford Medical Dictionary (2002). 3rd Edition Oxford University Press New York 754 pp.
26. Musa, S.O. and E. Omoregie, (1999). Haematological changes in *Clarias gariepinus* exposed to malachite green. *J. Aquat. Sci.*, 14:37-42.
27. Udem, S. C. and Asogwa, O. (2011): Effects on haematological and biochemical parameters in albino mice fed *ipomoea batatas* leaf aqueous extract. *Comp. Clinical Pathology* 20: 475-479.
28. Meyer, D.J., Codes, E.H. and Rich L.J., (1992). *Veterinary Laboratory Medicine Interpretation and Diagnosis*. W.B. Saunders Company, Philadelphia, Pp.55-70.

