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The Effect of Crude Oil on Hematological Indices of the Frog, Ptychadena Mascariensis in the Niger Delta, Nigeria.

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Abstract: *Amphibian population declines have been reported and many causes have been implicated which include habitat alteration, environmental change and pollution. Unfortunately, not much is known of the effects of crude oil pollution on the species. This study therefore investigated the haematological changes associated with exposure of the frog, Ptychadena mascariensis to sub-lethal concentrations of the water-soluble fractions of crude oil using static renewal bioassay system for 12 weeks. Water parameters were also monitored throughout the duration of the experiment. Water quality parameters remained within the ranges for water bodies in the Niger Delta with no significant differences between the control and treatment groups. The results also revealed a dose-dependent reduction in RBC count, PCV, MCV and haemoglobin levels. As the concentration of crude oil increased, there was a corresponding significant ($p<0.05$) decrease in the haematology indices. This showed that the crude oil had a negative effect on the haematological indices of the frogs.*

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I. Introduction

Oil pollution, an environmental consequence of crude oil exploration and exploitation activities in the Niger Delta have resulted in marked reduction in the number of both freshwater and marine animals[1] and aqua-toxicological effects, which are deleterious to aquatic life[2]. A variety of pollutants including crude oil and its products are known to induce stress conditions, which impair the health of fish[3]. Water soluble fractions constitute of dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions[4].

Amphibian populations are in decline in many areas of the world and numerous physical and chemical causes have been implicated. They include habitat alteration and habitat destruction, predation, competition from exotic non indigenous species, parasites, diseases, climate change[5]. The frog, *Ptychadena mascariensis* are endemic to West Africa. They are typical representatives of amphibians of the Niger Delta ecological zone. It was chosen for this study because they are edible, highly prolific[6] and easy to handle and maintain under laboratory conditions. Ecologically, they occupy important positions in the food chain as they serve as food for both aquatic and terrestrial predators. Frogs are excellent indicators of pollution due to the sensitivity of their skin and eggs to both aquatic and terrestrial pollutants as they can readily absorb substances from their environment. For this reason, their aquatic larval stages are increasingly viewed as bio-indicators of health of aquatic systems[7].

The use of changes in the haematological parameters as indices for assessing organism health status and thus the degree of pollution present in the aquatic environment is widely applied in fisheries and aquaculture[8]. Changes in haematological parameters can be used to effectively monitor the response of organisms to stress and thus the health status and also assists fish biologists interpret physiological responses to stress[9]. Red blood cell count is a process which determines the number of red blood cells (RBC), erythrocytes, in the blood. Red blood cells serve as a carrier of haemoglobin. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates and has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals[10]. Packed Cell Volume (PCV) also known as haematocrit (Ht or Hct), is the percentage (%) of red blood cells in blood. According to[10] Packed Cell Volume is involved in the transport of oxygen and absorbed nutrients. Increased Packed Cell Volume is an indication of better transportation and thus results in an increased primary and secondary polycythemia. Mean

corpuscular volume (MCV) measures the average red blood cell volume (size) and is used to tell the difference between types (causes) of anemia.

The aim of this study was to identify the changes in the values of various haematological parameters under the effect of crude oil toxicants.

II. Materials and Method

Frogs collection, acclimatization and feeding were carried out following the method of[11]. A static renewal bioassay was carried out with triplicates for 12 weeks. Treatments consisted of six different concentrations (0mg/L, 0.3mg/L, 0.75mg/L, 1.5mg/L, 2.25mg/L and 3.0mg/L) of water-soluble fractions of crude oil. The toxicant used was Bonny light crude oil and the water soluble fractions was prepared following the method of[12] by adding 1 part of crude oil to 9 parts of fresh water.

The water samples were analyzed as described by[13].

Blood collection was carried out by the process as described by[11]. Blood was collected from the adult frogs by cardiac puncture with $1cm^3$ sterile heparinised plastic disposable syringe by piercing ventrally about half inch into the heart. The collected blood was taken to the laboratory were haematology analysis was carried out.

According to[14], the haematological techniques used for mammals are also applicable for fishes; hence all the haematological parameters were determined by using the standard techniques as described by[15].

Red blood cell counts were carried out with a Haemocytometer method.

Haemoglobin concentration (Hb) was measured by Sahlis method.

Packed cell volume (PCV) was determined by means of a Haematocrit method.

Mean Corpuscular Volume (MCV) was calculated mathematically according to formula by[16] by dividing hematocrit per liter of blood by total RBC count.

Statistical Analysis

Data on haematology and physico-chemical parameters of the water were analysed by one-way analysis of variance (Anova) and their correlation coefficient values determined. Observed differences in treatment mean values were separated by using the Duncan's Multiple Range Test (DMRT). Statistical significance was considered at $p<0.05$ level of significance.

III. Results

The water quality parameters (Dissolved oxygen, pH, salinity, total alkalinity, and total hardness) were monitored during the exposure periods for the sub lethal assay and were not significantly different between various concentrations of the WSF of crude oil and the control groups ($p > 0.05$). The parameters showed consistent variation with increase in crude oil concentration and time however, these water quality parameters were within the FEPA (1999) recommended limits for aquatic organisms. The results of the water parameters are given in Table 1. These variations remained within tolerable ranges throughout the bioassay period.

Table 1: Results of physic-chemical parameters

Conc.	Temp.	DO	pH	Alkalinity	Salinity	Total Hardness
0 mg/L	27.6 ± 0.3	4.48 ± 0.37	7.33 ± 0.07	54.24 ± 0. 85	6.20 ± 0.49	1657 + 13.87
0.3mg/L	27.6 ± 0.3	4.48 ± 0.42	7.33 ± 0.07	54.18 ± 0.90	6.20 ± 0.37	1670 + 20.00
0.75mg/L	27.6 ± 0.3	4.48 ± 0.45	7.33 ± 0. 07	54.18 ± 0.90	6.20 ± 0.60	1670 + 21.37
1.5mg/L	27.6 ± 0.3	4.48 ± 0.43	7.33 ± 0.07	54.10 ± 0.90	6.20 ± 0.50	1675 + 22.84
2.25mg/L	27.6 ± 0.3	4.40 ± 0.44	7.30 ± 0. 07	54.10 ± 0.90	6.20 ± 0.37	1680 + 20.82
3.0mg/L	27.6 ± 0.13	4.20 ± 0.40	7.30 ± 0.07	53.78 ±0.90	6.00 + 0.44	1680 + 26.48

The results of the analysis carried out on the haematological parameters showed a significant decrease ($p<0.05$) in crude oil treated frogs. The values of red blood cell (RBC) count, haemoglobin content, packed cell volume, mean corpuscular volume (MCV) showed a reduction in values in the crude oil treated groups compared to the controls, in that as the concentration of WSF of crude oil increased there was a corresponding decrease in the values of the haematological indices which showed dose dependent relationship. The results obtained are shown on Table 2.

The mean RBC count of the control group was $1.07 \times 10^6 \pm 1.15 \times 10^4$, while results for the test groups ranged from $9.68 \times 10^5 \pm 9.95 \times 10^3$ to $7.54 \times 10^5 \pm 1.12 \times 10^3$. The mean Haemoglobin value for the control group was 6.0 ± .01 and was higher than the value recorded for the treatment groups. The value for the treatment groups ranged from 5.24 ± .02 to 3.3 ± .02. The values for mean PCV was 18.4 ± .15% for the control group while for the treatment groups, the values ranged from 15.8 ±.09% to 10.0 ±.04%. MCV value was $1.72 \times 10^{-4} \pm 2.2 \times 10^{-6}$ for the control group while for the treatment groups, the values ranged from $1.67 \times 10^{-4} \pm 1.7 \times 10^{-6}$ to $1.32 \times 10^{-4} \pm 1.9 \times 10^{-6}$.

Table 2: Haematological Parameters associated with exposure of *P. mascariensis* to WSF of crude oil

Conc.	RBC count (10^6/ml)	PCV (%)	MCV (fl)	Haemoglobin (g/dl)
0 mg/l	$1.07 \times 10^6 \pm 1.15 \times 10^4$	18.4 ± .15	$1.72 \times 10^{-4} \pm 2.19 \times 10^{-6}$	6.0 ±.02
0.3mg/l	$9.68 \times 10^5 \pm 9.95 \times 10^3$	15.8 ± .09	$1.67 \times 10^{-4} \pm 1.67 \times 10^{-6}$	5.24 ± .03
0.75mg/l	$8.60 \times 10^5 \pm 5.64 \times 10^3$	14.4 ± .11	$1.63 \times 10^{-4} \pm 5.81 \times 10^{-6}$	5.12 ±.03
1.5mg/l	$8.51 \times 10^5 \pm 5.84 \times 10^3$	13.37±.10	$1.62 \times 10^{-4} \pm 1.12 \times 10^{-6}$	4.92 ± .04
2.25mg/l	$8.00 \times 10^5 \pm 1.53 \times 10^3$	12.1 ± .10	$1.51 \times 10^{-4} \pm 1.08 \times 10^{-6}$	3.82 ± .02
3.0mg/l	$7.54 \times 10^5 \pm 1.12 \times 10^3$	10.0 ± .01	$1.32 \times 10^{-4} \pm 1.97 \times 10^{-6}$	3.3 ±.01

IV. Discussion

Blood is a pathological reflector of the whole body. Consequently, changes in its parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant[17] and provide information on the physiological response of fish to environmental stressors[18]. Crude oil caused a significant reduction in RBC count, PCV, MCV and HGB and were dose dependent. These reductions suggested an anaemic condition in the crude oil treated frogs. This finding is in agreement with the studies of[19, 20]. The reduction in all haematological parameters analysed for is an indication that crude oil adversely affected the frogs.

The significant drop in RBC count as the concentration of crude oil increased implied a reduction in the level of oxygen that would be carried to the tissues and the level of carbon dioxide returned to the lungs would also be reduced. This reduction may be due to increased rate of breakdown of red blood cells and/or reduction in the rate of formation of red blood cells [21]. This in agreement with the results of[22] who reported a significant decrease in RBCs of fresh water fish exposed to toxicants. The lowering of total RBC count may also be due to destructive action of pollutants on erythrocytes after sub-lethal exposure and as a result the viability of the cells may be affected[23].

The physiological function of haemoglobin is to transport oxygen to the tissues and it is therefore crucial to the survival of frog, being directly related to the oxygen binding capacity of blood. This process depends on the ability of ferrous form (Hb^{2+}) to reversibly bind molecular oxygen. However, in the presence of toxins, oxyhaemoglobin (Oxy-Hb) is turned to methaemoglobin (met-Hb) (the HB^{3+} form) which is unable to transport oxygen. This is a stress response to the influx to toxins into the body of the frogs. The reducing Hb levels recorded may also be as the result of the presence hydrocarbons which altered the properties of haemoglobin by decreasing their affinity towards oxygen i.e. their binding capacity rendering the erythrocytes more fragile and permeable. This position is also supported by the results in the work of[24].

The data on MCV values indicated erythrocyte shrinkage due to crude oil exposure and may possibly be due to impaired water balance as a result of the crude oil or stress. Since mature erythrocytes are large in size, the decreased MCV values further indicate erythrocytosis resulting in the production of immature cells enhance extraction of oxygen from the environment. Similar decrease in MCV was recorded by[25] when *Clarias albopunctatus* was exposed to actellic. Reduction in PCV values as the concentration of crude oil increased as observed in the present study, is an indication of anaemia, which was also reported in previous studies[26].

The degree of ecosystem contamination by toxic organic chemicals can be estimated by the analysis of haematological changes. From the results of this study, it is suggested that crude oil is an environmental stressor which causes damage to haematological parameters in frogs.

V. Conclusion

Hematological parameters in *P. mascariensis* observed in this study confirmed other researchers' viewpoints that these parameters can be used as biomarkers for ecological monitoring. Hence, the present investigation confirmed that exposure of the frogs to crude oil does create hematological disturbances. Given the frequent cases of oils spills in the Niger Delta which expose frogs to persistent levels of crude oil in the environment, there are possible long term effects on the physiological well-being of the frogs as demonstrated by the results of this study. It is therefore plausible that the observed effects are contributing to the decline of frog populations in the area.

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