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Haematological Response of *Clarias gariepinus* to Rubber Processing Effluent

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOD performed the physico-chemical and statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Author USO designed the study and wrote the protocol. Both authors read and approved the final manuscript.

Research Article

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ABSTRACT

Industrialization has led to huge waste generation over the last decades, the absence of adequate facilities for treating such wastes in most developing nations has led to the discharge of effluents into the environment without proper treatment. Toxicological effects of effluents from rubber processing plant (collected during the period of low rivertide i.e. between October 2012 and February 2013) were carried out in this study. Lethal concentration (96-h LC_{50}) was evaluated using 0.25mg/L, 0.30mg/L, 0.35mg/L and 0.40mg/L while sub-lethal effects (42 days) was carried out on haematological parameters like Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) using 0.064mg/L, 0.048mg/L, 0.032mg/L and 0.016mg/L which are the 20%, 15%, 10% and 5% of the 96-h LC_{50} value. Mortality increased as the concentrations of the effluent increases and 0.32mg/L was obtained as LC_{50}. In comparison with the control, the mean value obtained for PCV, HB and RBC showed significant differences ($P<0.05$) most especially at highest concentration while there was no significant difference in all values obtained for WBC, MCV, MCH and MCHC. It was concluded that the rubber processing effluent had some negative effect on the haematology of *Clarias gariepinus*. Therefore, it is recommended that the effluent should be properly treated before discharge into the environment.

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1. INTRODUCTION

Water pollution may be defined as any impairment in its native characteristics by addition of anthropogenic contaminants to the extent that it either cannot serve humans for drinking purposes and/or to support the biotic communities [1]. A change in the quality of water by the presence of toxins/contaminants, makes it potentially harmful to life forms, instead of sustaining them [1]. The entry of toxicants into aquatic media may affect the water quality parameters which in turn leads to changes in the haematological variables of fish and other aquatic lives due to close association with the external environment [2,3].

Organic pollution of inland water systems in Africa is alarming, in contrast to the situation in developed countries of the world and it is often the result of extreme poverty, economic and social under-development [4]. Biological monitoring techniques like haematological and biochemical variables have become attractive and useful for monitoring environmental quality, water pollution, and the health conditions of aquatic organisms [5,6,7]. Haematological indices are usually considered important parameters for the assessment of fish physiological state and their changes depend on the fish species, age, the cycle of sexual maturity and general health status [8,9,10,11,12,13].

Metal contamination of aquatic ecosystems has long been recognised as a serious pollution problem [14]. Chemical additives effluent at different concentrations was found to impair the swimming pattern, skin colouration, feeding rate and general behaviour of fish which suggests that fish can tolerate low concentrations of pollutants with reduced mortality [15]. Also, in our previous research, synthetic resin effluent has been found to induce behavioural changes as well causing death of *Clarias gariepinus* especially at higher concentrations with reduced mortality at lower concentrations [16]. Saffa and Mohsen [17] observed that commercial petroleum fuel had a negative impact on the growth performance and survival of Nile Tilapia. The eco-physiological effects of crude oil on *Macharium lunatus* has also been reported [18]. Abdel-Hadi et al. [19] observed that oxytetracycline induced significant mortality in experimental Tilapia. Many laboratory studies have shown the toxicity of plant extract to fish and changes in haematological and biochemical profiles leading to death of fish [7,20]. The toxic effects of *Moringa oleifera* seed powder observed in guppies (*Poecillia reticulata*), protozoa (*Tetrahymens pyriformis*), bacteria (*Escherichia coli*) and *Oreochromis niloticus* [21]. *Moringa oleifera* seed extract was found to be toxic to *Cyprinus carpio* at higher concentrations [22]. Kumar et al. [23] reported that aqueous extracts of *Euphorbia tirucalli* latex was toxic to *Heteropneustes fossilis.* Adequate management of our environment requires the correct tools which allows us to accurately predict the fate and effects of contaminants within the environment [24]. The rubber processing effluent used in this study was selected because as at the time of this research, there is paucity of information on its usage for toxicological studies and also due to our concern about its large volume being channeled into the nearby river where the residents rely on such waters for domestic activities and fishing on a daily basis. The objective of this study therefore is to investigate the possibility of using haematology as biomarker of toxicity for *Clarias gariepinus* exposed to rubber processing effluent. The African catfish, *Clarias gariepinus* was chosen for this work because of its availability as the most cultured fish in Nigeria and Sub Saharan Africa and also due to its ability to adapt and yield easily to laboratory and scientific experiments.

2. METHODOLOGY

The effluent used for the toxicity test was collected from a company that processes raw rubber for other industries in Sango-Ota, Ogun-State, Nigeria. Samples were collected in batches from four different discharge/release points from where the effluent run into the nearby river and this was done during the period of low rainfall i.e. between October 2012 and February 2013) and were immediately kept in the refrigerator to avoid further activities of microorganisms before commencement of experiments. During the test, all the samples were pooled together to avoid variability in concentration.

2.1 Test Organisms

The test organism, *Clarias gariepinus* of about four months old, (weight 0.5±0.2kg; lenght 22±3cm) were purchased from an Agricultural farm and were transported to the laboratory in well ventilated containers. The test solution was aerated during the 42 days exposure period. The test organisms were kept in a large plastic container that has already been washed and rinsed with 5% potassium trioxonitrate to remove any adhered metals and thereafter acclimatized for a period of fourteen days. During this period of acclimatization, renewal bioassay was employed and fish were fed twice daily (12 hourly) with formulated fish feed having 37% crude protein content.

2.2 Physicochemical Parameters Determination

Prior to the laboratory experiment, the physicochemical analysis of the effluent was carried out to quantify the concentrations of the metals and other parameters. The APHA/AWWA/WEF, [25] Standard methods for examination of water and wastewater was used.

2.3 Acute Toxicity Test

After the acclimatization period, range finding test following the method of [26] was carried out to determine the definitive concentrations to be used for the acute evaluation test. Five plastic bowls were labeled A – E in two replicates. The test solution (effluent) was thoroughly mixed by pouring into a big plastic bowl before exposing the test organisms in the labeled plastic bowl. The varying concentrations used were A = 0.25mg/L, B = 0.30mg/L, C = 0.35mg/L, D = 0.40mg/L and E = ordinary water, serving as the control experiment. Ten fishes were exposed to each concentration including the replicates. The experiment was monitored for 96 hours during which the following parameters were evaluated:

a. Behavioural Responses
b. Total number of death (mortality) after 96 hours
c. The percentage mortality at 96 hours
d. Calculation of the LC_{50} which is the concentration at which half or 50% of the test organism died on exposure.

Since the organisms were exposed for 96 hours, the 96-h LC_{50} was determined from the graph of percentage mortality against concentration.

2.4 Chronic Toxicity Test

After determining the LC_{50} in acute test, five plastic containers were then labeled A-E in two replicates. Sixteen test organisms were placed into each of the plastic bowl with test solution. The varying concentrations used were 20%, 15%, 10% and 5% of the 96-h LC_{50} value obtained which are 0.064mg/L, 0.048mg/L, 0.032mg/L and 0.016mg/L respectively. This experiment was carried out for 42 days with a change of test solution every 48 hours.

2.5 Haematological Analysis

In doing this, 0.5ml of blood was sampled from 3 fish in each concentration. The specimens were anesthetized with 40mg/L of Fish Calmer (active ingredients: acetone, dimethylketone, alpha methyl quinoline, Jungle Laboratories, Cibolo, TX, USA), and the peripheral blood was collected by puncture of the caudal vein with a heparin-coated 25 gauge x 0.5 in. needle, attached to a 1ml syringe. After sampling, fish were placed in separate tanks of freshwater for necessary recovery. The routine method of fish haematology designed by [27] was employed. The RBC count (RBCc, $x10^6$ µl) was determined by counting the erythrocyte from 5 small squares of Neubaner hemocytometer using Vulpian dillution solution. The hematocrit (PCV, %) was determined by duplicate using heparised cappilary tubes centrifuged for 4 minutes at 13000 rpm in a micro hematocrit centrifuge. The photometrical cyanohemoglobin method was used for determining the hemoglobin concentration (Hb, g/dl) using standard formular [28]. The White blood cell count (WBC) was evaluated according to the routine clinical methods [29].

Mean Corpuscular Volume (MCV):- This is the average volume of a single RBC count which is calculated from the data obtained for RBCc and PCV using standard formular by [30].

$$MCV = PCV \times 10/RBC\ (10^6)\ fl\ (fentrolitres)$$

Mean Corpuscular Haemoglobin (MCH):- This is the quantity or amount of haemoglobin present in one RBC. It is the amount of Hb expressed in relation to the volume of one RBC and is calculated from the data obtained for Hb and RBCc using standard formular by [30].

$$MCH = Hb \times 10/RBC\ (10^6)\ pg\ (pictograms)$$

Mean Corpuscular Haemoglobin Concentration (MCHC):- This is the concentration of Hb in one RBC. It is the amount of Hb expressed in relation to the volume of one RBC and also calculated using the formular propounded by [30].

$$MCHC = Hb \times 100/PCV\ g/dl\ (decilitres).$$

2.6 Statistical Analysis

The statistical analysis of the haematological parameters was done using [31]. The values obtained were confirmed using one-way ANOVA at 0.05 level of significance. Further test on those found to be significant was done using Duncan Multiple Range tests (DMRT).

3. RESULTS

3.1 Physicochemical Characteristics of Rubber Processing Effluent

This is shown in Table 1. The data obtained have some of its values conforming to [32] specifications for maximum limits allowed for effluent discharge into water bodies while the values for lead, cyanide, total hardness, total dissolved solids, calcium, oil and grease and alkalinity do not conform to the standard.

Table 1. Physicochemical characteristics of rubber processing effluent

Parameters	Experimental values	FEPA 1991 specification
pH	6.80	6.50-8.50
DO (mg/L)	2.70	5.00
BOD (mg/L)	0.60	50.00
TSS (mg/L)	68.00	30.00
Iron (µg/L)	0.58	<1.00
Cadmium (mg/L)	ND	<1.00
Chromium (µg/L)	0.06	<1.00
Sulphide (µg/L)	0.26	0.20
Nitrate	4.10	10.00
Cyanide (µg/L)	16.00	5.00
Lead (µg/L)	10.12	<1.00
Copper (µg/L)	0.08	5.80-6.00
Zinc (µg/L)	0.42	<1.00
Total hardness	64.10	ND
Ca^{2+}	21.20	ND
Mg^{2+}	0.67	ND
TDS (mg/L)	3700.60	2000.00
TS (mg/L)	3.89	ND
Oil and grease	13.40	10.00
Alkalinity	70.00	ND
Manganese (µg/L)	0.03	100.00

Note: DO = Dissolved Oxygen, BOD= Biochemical Oxygen Demand, TSS= Total Suspended Solids, TDS= Total Dissolved Solids, TS= Total Solids, ND = Not Detected. All values are in milligram per litre except where otherwise stated.

3.2 Behavioural Responses of Test Organisms

Table 2 show the behavioural responses of the test organisms during the acute toxicity test, *Clarias gariepinus* exhibited distress behavioural responses due to the effects of the rubber processing effluent. These were noticed by the sudden change in the organism's response to the environment such as erratic swimming, occassional gasping for breath and frequent surfacing which increases as the concentration increases. All these are indications that the concentrations have become hypoxic and have induced brain dysfunction in the test organisms due to low oxygen supply. As the experiment progressed, some of the test organisms were seen to get weaker evident by reduction in movement, their ventral surfaces were subsequently turned upward while those that couldn't tolerate the concentrations any longer went into a state of motionlessness. Normal behaviour were however observed in the control.

Table 2. Behavioral responses of C*. gariepinus* during exposure to acute concentrations of rubber processing effluent

Behaviour	Concentrations				
	0.00	0.064	0.048	0.032	0.016
Erratic swimming	-	-	+	+	+
Gasping for breath	-	+	+	+	+
Loss of reflex	-	+	+	+	+
Frequent surfacing	-	-	+	+	+
Motionlessness	-	+	+	+	+
Hyperventilation	-	-	+	+	+
Discolouration	-	-	-	+	+

Absent (-), Present (+)

3.2.1 Observed mortality

The result of the acute toxicity shows minimal mortality rate in the lower concentrations while maximum mortality was observed in the highest concentration.

Fig. 1 shows the arithmetic graph of percentage mortality against concentration for the acute evaluation. The 96-h LC_{50} was calculated to be 0.32mg/L.

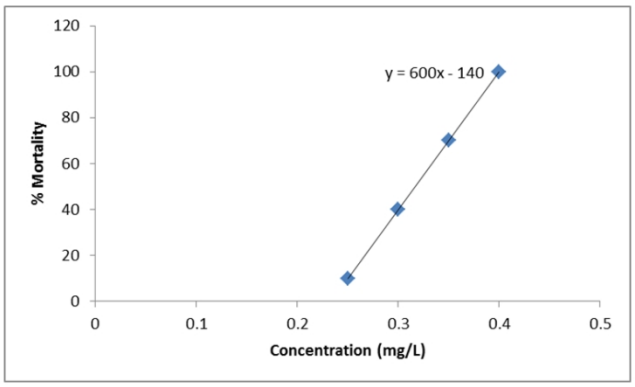

Fig. 1. LC_{50} Determination for *Clarias gariepinus* exposed to lethal concentrations of rubber processing effluent

3.2.2 Effects of rubber processing effluent on the haematological indices of Clarias gariepinus

Table 3 shows the result of the haematological parameters of the test organisms after exposure to sub-lethal concentrations of the synthetic resin effluent for 42 days. These are the WBC, RBC, Hb, MCV, PCV, MCH and MCHC. In comparison with the control, all the parameters showed an increase in values obtained as the concentration increased for the same parameter except for WBC and MCH which showed decrease in values as concentration increases. The PCV, Hb and RBC values for the treated organisms all showed a level of significant difference ($P<0.05$) from the control at the highest concentrations while all the values obtained for MCV, MCH, MCHC and WBC showed no significant difference from the control stocks. The RBC's were also discovered to have undergone lysis during the exposure period.

Table 3. Mean and Standard Deviations for haematological parameters of *Clarias gariepinus* exposed to different concentrations of rubber processing effluent for 42 days

Conc. mg/L	Parameters						
	PCV (%)	Hb (g/dl)	RBC (10^6µl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC (µl)
0.064	32.0000[b] ±1.7300	10.4667[b] ±0.2906	3.1667[b] ±0.1764	101.0760[b] ±1.0700	33.8160[b] ±1.2300	32.8200[b] ±0.8900	1062400.0000[b] ±90735.6600
0.048	28.3300[b] ±2.4000	10.5333[b] ±0.4842	2.9667[b] ±0.8192	98.8160[b] ±4.7800	35.4930[b] ±0.9900	35.9930[b] ±0.7500	920800.0000[b] ±129326.4600
0.032	32.6600[b] ±1.7600	11.5667[b] ±0.7446	3.4333[b] ±0.3930	96.3860[b] ±5.4300	34.0560[b] ±1.5800	35.3700[b] ±0.3700	800000.0000[b] ±80531.5600
0.016	41.6600[a] ±3.4800	14.1000[a] ±0.6000	4.6667[a] ±0.2728	89.0460[b] ±2.6800	30.2700[b] ±0.5500	34.0660[b] ±1.3600	1066666.6000[b] ±112885.3800
Control	28.6600[b] ±3.1700	10.3667[b] ±1.2197	3.1667[b] ±0.4177	90.9830[b] ±1.7400	32.8460[b] ±0.4200	36.1130[b] ±0.2300	736033.3300[b] ±151219.0500

Note: Mean values with the same alphabet for same parameter are not significantly different (P<0.05). Values are average from three test organisms. (g/dl)= gramme/decilitre, fl= fentrolitre, pg= pictogram, (µl)= microlitre, mg/l= milligramme/litre

4. DISCCUSSION

The work shows the rubber processing effluent to be high in total suspended solid (TSS), total dissolved solids (TDS), lead and cyanide, low biochemical oxygen demand (BOD), lower dissolved oxygen (DO), and high alkalinity content which shows the effluent to be toxic for discharge into our immediate environment. This corresponds to the findings of [33] that the observed characteristics features may have resulted from the organic loads in the wastewater. The abnormalities (gasping for breath and frequent surfacing) observed prior to mortality are indications of depleted oxygen content (hyposia) due to higher demand for oxygen. There was an observed positive correlation between concentration and response of the test organisms. From this study, it is obvious that the effluent inhibits activity of enzyme AChE, which is present in synaptic regions and mediates transmission of impulses by breaking acetylcholine into acetic acid and choline. The acetylcholine at neural and neuromotor regions upon accumulation causes “hyperexcitability,” which in turn might also influence behavioural pattern and may lead to death of fish [34]. Other research works supported this finding; Exposure to Cycloart-24-en-3-ol isolated from *Euphorbia royleana* latex caused significant behavioural changes such as suffocation, body irritation, increased mucus secretion and loss of body equilibrium of fish [35]. Similarly, Tiwari et al. [36] observed that the nature and rapidity of the onset of behavioural responses indicates that Cycloart-24-en-3-ol is active at the neuromuscular system of the exposed fish *Channa punctatus*. Similar behavioral changes were also observed in guppy fish *Poecilia reticulata*, after exposure to cypermethrin [37,38] and permethrin [39]. Das and Mukherjee [40] and Tiwari et al. [41] also reported that cypermethrin inhibits AChE activity in the brain of *L. rohita* fingerlings.

The investigation further show that fishes can tolerate low concentrations of pollutants with reduced mortality and this agrees with [42] that the abnormal behaviour observed in fish subjected to *Morinda lucida* increased with increasing concentration of the pollutant used. The 96-h LC_{50} value for the acute test was 0.36mg/L which mean that at this concentration of the effluent in the aquatic environment, half of the entire natural population will become dead and this corresponds to the report of [33] that at this concentration, the fitness of the natural population of an aquatic environment would be relatively impaired and as the concentration increases, the mortality rate also increases.

The high WBC count recorded could be due to attempt by the fishes to fight against the antigens (pollutants) and this led to the production of more antibodies (WBC) to improve the health status of the organism. This agrees with [43] that the increase in WBC during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defence mechanism of the fish to tolerate the toxicity. The increase in leucocyte count indicates the stimulatory effects of the toxicant on immune system and also depend on the toxicant stress. The gradual reduction in the values of WBC as the concentrations increased may be due to the breakdown of vital metabolic activities as a result of possible blockage in the metabolic pathway which then lowered the toxiproduction of WBC.

The observed reduction in haematocrit (PCV) percentage and haemoglobin concentration of the organism on exposure to the effluent could be a result of the bioaccumulation of the toxicant in the body. This decrease in the two indices was as a result of uncontrolled lysis of the RBC due to the toxicity level of the effluent; while the decrease in haematocrit compared to the haemoglobin standards was attributed to shrinkage of the erythrocytes. These are in agreement with [19,44,45] that the decrease in haemoglobin content during stress condition may indicate a decrease in the rate of haemoglobin synthesis which lead to impaired oxygen

supply to various tissues resulting in decrease in the number of RBC through hemolysis. The lysis of erythrocyte leads to a reduction in haematocrit value. The mean cell volume (MCV) showed an elevated trend in values in comparison with the control, but a depression was recorded at higher concentrations. The values obtained for the erythrocyte constant is in agreement with [46,47]. The decreasing trend in mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were evident in the organisms kept in different concentrations and this correlates with the findings of [19] that MCHC is an indicator of RBC swelling and the lowered MCHC during treatment might have resulted from release of young erythrocytes containing less haemoglobin into circulation.

5. CONCLUSION

It is evident from this study that increasing concentration of the rubber processing effluent when present in any water body could lead to changes in bahaviour and haematological dysfunction in fish health and general condition. There is therefore a need for preventive measures to be taken in order to prevent the indiscriminate discharge of this effluent into nearby streams and ponds. It is recommended that the application of appropriate effluent technology be adopted by the concerned industries and individuals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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