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Full-Length Research Paper

Haematological changes in *Clarias Gariepenus* exposed to rubber effluent

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ABSTRACT: The toxicological effects of rubber effluent on *Clarias gariepinus* (catfish) was studied utilizing haematological end points. The study found that exposing *Clarias gariepinus* to 50% and 100% concentrations of rubber effluent for 14 and 28 days caused alterations in haematological parameters such as White Blood Cell (WBC) and lymphocyte counts, Red Blood Cell (RBC) count, and RBC differentials. The severity of the changes in these assessment end points was shown to be dependent on the effluent concentration (50 and 100 percent) and the period (14 and 28 days) of exposure to the effluent, highlighting the toxicity of untreated rubber effluent to *Clarias gariepinus*. The rubber effluent's physicochemical characterization revealed that it had a high organic load but a somewhat low inorganic load.

Keywords: Rubber effluent, haematology, *Clarias gariepinus,* effluent concentration, exposure duration

INTRODUCTION

During the rubber processing, a large amount of waste water (effluent) is produced, which contains uncoagulated latex washings and a serum containing significant amounts of proteins, sugars, lipids, carotenoid, inorganic and organic salts, as well as plenty of process water (Kulkarni, 1972; Kulkani et al., 1973). This sewage is frequently released untreated into surrounding streams, rivers, ponds, and even farmlands (RRIM 1974; Ogiebor et al., 2000; Shing et al., 2003). The amount of effluent produced is usually determined by the size and capacity of the factory. Because untreated effluent is toxic, it has a negative impact on the flora and fauna (e.g., fish) in receiving environments such as streams, rivers, ponds, and even farm areas (RRIM 1974; Ogiebor et al., 2000; Shing et al., 2003). Untreated industrial waste water (effluent) has been shown to have negative effects on both the aquatic and terrestrial environments (Yeow and Ahmed, 1983; UNEP, 1991; Sharpley et al., 1998; Shing et al., 2003; Olajire and Ayodele, 2003). The rating of chemical substances in terms of their possible hazardous effects (toxicity) on wild life, aquatic flora and fauna, as well as higher species including people, is one of the current concerns in environmental sciences and toxicology. Bioassay tests are used to assess the severity of a toxicity problem (Odokuma and Kindzeka, 2003). Aquatic bioassays are required in water pollution control to evaluate whether a suspected toxicant is hazardous to aquatic life and, if so, to determine the relationship between toxicant concentration and aquatic animal effect (Olaifa et al., 2003). Fish have long been acknowledged to be the most popular bioassay test organisms. This is because they are thought to be the best understood species in the aquatic environment, as well as the most commercially valuable (Odokuma and Kindzeka, 2003). Toxic effects of some contaminants, such as heavy metals, on fish may affect physiological functions, individual growth rates, reproduction, and death. These metals can enter fish bodies through three routes: the

body surface, the gills, or the digestive tract (Javed, 2005). The composition and concentration of the contaminant, as well as the length of exposure, are all elements that contribute to the cytotoxic effects on aquatic species (fish) in the aquatic environment. The more severe the effects, the higher the concentration and the longer the length of exposure (Bervoet et al., 1996; Olomukoro and Ezemonye, 1998; Ezemonye et al.,and Olomukoro, 2001).

Hassan et al. (2011) investigated Cd and Pb levels in the tissues of two dried fish species, *Clarias gariepinus* and Oreochromisniloticus. The findings demonstrated that both metals were bio-accumulated at varying amounts in diverse tissues, including bone, gills, and flesh. *Clarias gariepinus*

Acute toxicity investigation on the impacts of industrial effluents from two petroleum tanks on fresh and brackish water fish Tilapia guineesis by Ezemonye and Olumukoro, (2001) indicated that the acute toxicity unit (TUa) and mortality values of the two effluents on the fish differed. The longer the exposure, the higher the mortality rate. Several studies, notably Blaxhall (1972) and Duthie and Tort ((1985), have reported on the use of haematological as a measure of fish health in the assessment of physiological changes following exposure to various stress conditions such as pollution, illness, metals, and hypoxia haematological. Larson et al. (1980) observed increased PVC and Hb values with a modest decrease in lymphocyte population after exposing flounders, *Platichthysflexus*, to two concentrations of industrial effluents.

Fish living in water polluted with bleached kraft mill effluents have higher (Hb) and (PVC) levels, as well as a lower white blood cell count (Oikari et al., 1985 and Andersson et al., 1988).

Sparusauratus and *Solea vulgaris* exposed to industrial effluents showed hemolytic anemia accompanied by leukocytosis (Wahbi, 1998). Mourad (1995) recorded an increase in (Hb) content of *Tilapia zilli* exposed to copper works effluent for 14 days. Because morphological and quantitative differences in blood parameters can be generated by pollutants and other environmental variables, the study of distinct biochemical and cellular elements in blood is critical in the physiological evaluation of animals (Juneja and Mahajan, 1983; Ranzani-Paiva et al., 1997). The analysis of the haematological image is frequently used for the identification of physiopathological alterations in different stress circumstances such as exposure to heavy metals, according to Nussey et al., (1995). As a result, this study was done to assess the impact of the quality of effluent waste from rubber factories on several hematological qualities arising from the exposure of the freshwater fish *Clarias gariepinus* to various effluent concentrations and exposure times. *Clarias gariepinus.*

MATERIALS AND METHODS

Collection of Experimental Fish

A total of thirty (30) female broodstock of *Clarias gariepinus* with initial mean weight of 160±3.5g and average length of 34±3cm were obtained from a commercial fish farm and transferred to the Laboratory where they were acclimatized for fourteen (14) days in 5 glass aquaria tanks measuring 60cm x 30cm x 30cm containing dechlorinated water and properly fed.

Exposure of test specimen

After acclimatization, the fishes were randomly separated into three (3) experimental aquaria tanks labeled A (control), B and C, with ten (10) fishes to each tank. Fishes in tank A were exposed to normal dechlorinated water, while those in tanks B and C were exposed to 50% concentration of rubber effluent (1:1 ratio of normal dechlorinated water and rubber waste water effluent) and 100% concentration of rubber effluent respectively in a 28 days static bioassay. Blood and tissue samples were collected from fishes in each experimental tank after 14 and 28 days of experimentation for haematological.

Collection of blood and tissue samples

3 ml blood samples were collected from fish in each experimental tank by the caudal peduncle lateral venipuncture using a 2ml syringe. Aspirated blood was then transferred into sterile bottles containing Potassium EDTA anticoagulant for haematological analysis. The bottles were then kept in wet ice prior to the analysis.

Collection of rubber wastewater effluent

Rubber effluent was collected regularly from a rubber processing factory within Benin City in thoroughly washed and rinsed with clean water 50 liter plastic cans. The effluent sample to be used for DO and BOD determination was collected in dark DO bottles in which reagents were added to fix the dissolved oxygen.

Haematological analysis

The whole blood samples were analyzed for red blood cell (RBC) count, white blood cell (WBC) count, haematocrit, haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular

haemoglobin automated machine (Sysmex Corporation, Kobe-Japan) following the manufacturer's instructions.

RESULTS AND DISCUSION

The result of the physicochemical characterization of rubber effluent is shown in (Table 1). It reveals high BOD, TSS and TDS which indicate high bio-loads and high degree of organic pollution of the effluent which may be lethal to aquatic lives. The low DO of 3.8mg/l can also partially attributed to increase in organic load which encourages aerobic biodegradation resulting to oxygen depletion of aquatic environment (Camp and Messerve, 1974) and cannot be suitable to aquatic life (Alabaster and Loyd, 1980). The presence of heavy metals though not relatively high, but continuous discharge of the effluent could lead to bioassmilation and bioaccumulation in the tissues, bones, gills etc of aquatic lives such as fish. Results of the haematological response of the fishes on exposure to the various concentrations of effluents are shown in (Tables 2 and 3).

Table 1: Physicochemical characteristics of Rubber effluent.

Parameters (mg/l)	Values
pH	6.58
Conductivity (us/cm)	160
Turbidity (NTU)	67.5
Total Suspended Solids	310
Total Dissolved Solids	92.5
Dissolved Oxygen	3.8
Biochemical Oxygen Demand	455
Organic Carbon	0.78
Alkalinity	2.2
Acidity	3.98
Phosphate	0.77
Sulphate	0.30
Chloride	15.8
Nitrate	1.75
Calcium	0.72
Magnesium	0.43
Potassium	2.29
Sodium	0.32
Iron	1.70
Lead	0.24
Copper	0.37
Chromium	0.20
Zinc	0.32
Nickel	0.51
Mercury	-
Cadmium	-
Total viable bacterial count	4.4×10^5

White Blood Cell (WBC) count (cells/mm^2)

Mean values of WBC ranged from 9546±114 to 14678±146 cells/mm^2. All values were significantly higher than the control value ($p<0.05$). Mean WBC values after 14 days of exposure were 10245±235 cells/mm^2 and 13221±211 cells/mm^2 in groups A (50% effluent) and B (100% effluent) while a significant increase was seen in these values after 28 days of exposure in group A (10995± 4.5 cells/mm^2) and B (14678± 146 cells/mm^2). Group A- 50% effluent; B- 100% effluent.

Red Blood Cell (RBC) count (cells/mm^2)

Mean values of RBC count were 2.8±0.02 cells/mm^2 and 2.65± 0.01 cells/mm^2 after 14 days of exposure and 2.8± 0.02 cells/mm^2 and 1.83± 0.01 cells/mm^2 after 28 days of exposure. These values varied significantly from the control group (5.51± 0.02 cells/mm^2) ($p<0.05$).

Haemoglobin Concentration (Hgb) (%)

Mean values of haemoglobin concentration were 10± 0.1% in group A and 9.65± 0.15 % in group B after 14 days of exposure and 9.8± 0.2 % and 7.1± 0.3% in groups A and B after 28 days of exposure. All recorded values were lower than the control value with significantly lower values ($p<0.05$) recorded after 28 days of exposure.

Haematocrit (Hct) (%)

All mean values of haematocrit were lower than the control group. Hct values after 14 days were 20.1± 0.3% and 13.25± 0.05% in groups A and B respectively and were significantly different from the control group at $p<0.05$. Further reduction in haematocrit count was noted in groups A and B with increase in exposure duration to 28 days (16± 0.3% and 11.3± 0.1%).

Mean corpuscular volume (MCV) (fL)

Values of mean corpuscular volume after 14 days of exposure were 7.85± 0.05fL and 5.2± 0.1fL in groups A and B and showed significant reduction at $p<0.05$ when compared with the control group (10.32 ±0.01fL). MCV values after 28 days of exposure were 7.25± 0.05fL and 5.7± 0.6fL in groups A and B. These values were significantly lower than the control value and MCV values after 14 days of exposure ($p<0.05$).

Mean corpuscular haemoglobin (MCH) (pg)

Values of MCH were generally lower than the control group. MCH values after 14 days of exposure were 2.315 ±0.085pg and 1.635± 0.035pg in groups A and B. These values were significantly lower than the control group

Table 2: Haematological data summary of fish after 14 days of exposure to three concentrations of rubber effluent.

Parameter (Unit)	Control (Mean ± S.E) 0%	A (Mean ± S.E) 50%	B (Mean ± S.E) 100%
WBC (/mm^2)	9546±114	10245±235	13221±211
RBC (/mm^2)	5.51± 0.02	2.8±0.02	2.65± 0.01
HGB (%)	10.1 ±0,1	10± 0.1	9.65± 0.15
HCT (%)	30.15 ±0.15	20.1± 0.3	13.25± 0.05
MCV (fl)	10.32 ±0.01	7.85± 0.05	5.2± 0.1
MCH (pg)	3.3± 0.1	2.315 ±0.085	1.635± 0.035
MCHC (%)	29.35± 0.45	30.5± 0.15	33.35± 0.25
LYM (%)	30.45 ±0.25	32.2± 0.1	33.95 ±0.15

Table 3: Haematological data summary of fish after 28 days of exposure to three concentrations of rubber effluent.

Parameter (Unit)	Control 0% (Mean±*SE*)	A (Mean±*SE*) 50%	B (Mean±*SE*) 100%
WBC (/mm^2)	9702.5 ±42.5	10995± 4.5	14678± 146
RBC (/mm^2)	3.51± 0.02	2.8± 0.02	1.83± 0.01
HGB (%)	10.1± 0.1	9.8± 0.2	7.1± 0.3
HCT (%)	30.05± 0.25	16± 0.3	11.3± 0.1
MCV (fl)	10.11± 0.21	7.25± 0.05	5.7± 0.6
MCH (pg)	3.1± 0.1	2.24± 0.06	0.96± 0.14
MCHC (%)	29.9± 0.1	28± 0.1	26.75± 0.35
LYM (%)	30.9± 0.2	32.3± 0.4	38.45± 0.15

($p<0.05$). 7.25± 0.05 pg and 5.7± 0.6 pg were recorded in groups A and B after 28 days of exposure.

Mean corpuscular haemoglobin concentration (MCHC) (%)

Values of MCHC were significantly elevated ($p<0.05$) in groups A and B (30.5± 0.15% and 33.35± 0.25%) when compared with the control group (29.35± 0.45%) after 14 days exposure but however showed a reduction after 28 days of exposure in groups A and B (28± 0.1 % and 26.75± 0.35%) respectively.

Mean corpuscular haemoglobin (MCH) (pg)

Values of MCH were generally lower than the control group. MCH values after 14 days of exposure were 2.315 ±0.085pg and 1.635± 0.035pg in groups A and B. These values were significantly lower than the control group ($p<0.05$). 7.25± 0.05 pg and 5.7± 0.6 pg were recorded in groups A and B after 28 days of exposure.

Mean corpuscular haemoglobin concentration (MCHC) (%)

Values of MCHC were significantly elevated ($p<0.05$) in groups A and B (30.5± 0.15% and 33.35± 0.25%) when compared with the control group (29.35± 0.45%) after 14 days exposure but however showed a reduction after 28 days of exposure in groups A and B (28± 0.1 % and 26.75± 0.35%) respectively.

Lymphocyte Count (%)

Mean values of lymphocyte count after 14 days of exposure were 32.2± 0.1% and 33.95 ±0.15% in groups A and B respectively. Lymphocyte count after 28 days were 32.3± 0.4 % and 38.45± 0.15% in groups A and B representing a significant elevation at $p=0.05$ when compared with the control. The toxicological assessment of various concentration of rubber effluent on *Clarias gariepinus* catfish) carried out in this project showed that aquatic toxicity bioassays are necessary in water pollution control and monitoring to determine whether a potential toxicant is dangerous to aquatic life or not and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa *et al.*, 2003). On the other hand, the use of haematology as an index of fish health in the assessment of physiological changes following exposure to different stress conditions such as pollutants, diseases, metals and hypoxia has been reported by several scientists including Duthie and Tort, (1985). According to Van Vuren, (1986) and Adeyemo, (2005), water quality is one of the major factors responsible for individual variation in fish haematology since water quality induced physiological changes are also reflected in changes in the values of

one or more haematological parameters. This may possibly explain the significant elevation in white blood cell count and lymphocyte count in rubber effluent exposed fishes of groups A and B in relation to the control group and the significantly high WBC count observed in group B fishes exposed to 100% of the effluent when compared with group A fishes exposed to 50% concentration of the effluent. Elevations in white blood cell count and lymphocyte count may also be attributed to humoral immune responses in the form of increased leucocytosis and specific immune defense, antibody production and cellular immune responses (Raven and Johnson, 1999) in response to exposure to rubber effluent.

Significant reduction in red blood cell counts observed in exposed fishes of group A and B at 14 and 28 days of exposure is consistent with decreased erythrocytosis and excessive haemolysis associated with exposure to exogenous substances reported by Adeyemo (2005); Hillman *et al*. (2005) and Oghenekevwe and Prekeyi, (2011).

Significant reduction in mean corpuscular volume (MCV) seen with increase in exposure duration to rubber effluent may be indicative of microcysticanaemia. This in addition to decreases in haematocrit (mass of red blood cells present per sample of blood) as well as significantly low haemoglobin content confirms the haemotoxic effect of rubber effluent to *Clarias gariepinus* .On the other hand, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) both indicate haemoglobin content per red blood cell and aid in the diagnosis of anaemia (Zuckerman, 2007). Decrease in MCV seen in both group A and B at 14 and 28 days of exposure denote low haemoglobin content and possible anaemia. Similar reduction in MCHC may be evidence of red blood cell swelling and/or decreased haemoglobin biosynthesis which according to Adeyemo, (2005) would be deleterious to oxygen transport and erythrocyte regeneration.

Conclusion

The results of the study indicated that exposure of *Clarias gariepinus* (catfish) to 50% and 100% concentrations of rubber effluent for 14 and 28 days induced changes in the haematological parameters of white blood cell (WBC) and lymphocyte counts, red blood cell (RBC) count and differentials of RBC and the severity of the changes in these assessment end points were observed to be dependent on the concentration of the effluent and duration of exposure to the effluent. This further highlights the toxicity effects of rubber effluent to *Clarias gariepinus* indicting that if rubber effluent is not properly treated prior to its discharge, it can be deleterious to the animals inhabiting in the receiving environment.

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