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Full Length Research Paper

Effect of Goldcrew and Corexit on selected blood parameter of the African cat-fish *Clarias gariepinus* following sublethal exposures

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Sublethal effect of the dispersants Goldcrew and Corexit on selected blood parameters (White blood cell count- WBCC; Red blood cell count- RBCC; Platelet; Haemoglobin-Hb; and Packed Cell Volume - PCV) in *Clarias gariepinus* (Burchell) were studied. This was done in other to gauge the usefulness of blood parameters as good indicators of pollution and simulate the possible effect of clean-up operations on fish. Fish were exposed for four consecutive weeks in a static renewal condition and blood parameters measured weekly for the entire period at different levels of inclusion of dispersants. Results from the investigation revealed that dispersant effect on all blood parameters were not significantly different for the two dispersants (P > 0.05). Concentration of dispersants had a marked significance on blood parameters (P < 0.05); RBCC, PCV, Hb, platelet counts all decreased with increased concentration, while WBCC increased with increase in dispersant concentration. Duration of exposure had a marked significance on all blood parameters (P < 0.05) except in WBCC which was not significant at all exposure times. This study inferred that the two dispersants (Goldcrew and Corexit) are equally deleterious to fish health as they impact negatively on fish blood on the same scale.

Key words: *Clarias gariepinus*, corexit, blood, goldcrew, sublethal.

INTRODUCTION

Clarias gariepinus are outstanding members of the family claridae. They contribute greatly to the commercial catch of fishermen in coastal and fresh waters of Nigeria and an important source of protein in these times when beef availability has continued to show signs of steady decline (Borode, 1998). Regrettably, fresh water fishes are often subjected to the problem of pollution especially near industrial or populated area (Annune et al., 1994). The presence of crude oil in the aquatic environment through industrial accidents is one of such perennial problems in Nigeria (Alagoa, 2005).

Crude oil spills are sometimes allowed to disperse and degrade naturally, but clean up efforts are initiated when biological and economically important areas are threatened (GECL, 1991). The use of dispersants is one of the classical options for dealing with crude oil spills (Oyewo, 1986). However the addition of these chemicals to floating oils for the purpose of dispersing the oil can modify the effects of the oil as the dispersants themselves have some toxic properties that could independently affect the ecosystem (Oyewo, 1986; Akintonwa and Ebere, 1990).

The use of haematological values as indices of the state of fish health is receiving research efforts (Blaxhall, 1972; Musa and Omoregie, 1990). Studies of fish blood might reveal conditions within the fish long before there is any outward manifestation of disease (Sampath et al, 1993).

The objective of this investigation is to determine haematological responses of *C. gariepinus* to the dispersants Goldcrew and Corexit in a bid to simulate their possible effects on fish and the implication within the ecosystem. This will serve useful purpose for the management of the environment and protection of our fisheries.

MATERIALS AND METHODS

Goldcrew and Corexit were obtained from the Shell Petroleum Development Company of Nigeria Limited (SPDC) Forcados Terminal Pollution Control Store in Warri, Delta State, Nigeria. Live Juveniles of *C. gariepinus* average length 15.60 ± 0.2 cm were obtained from Ellah Lakes Obrikom in Ogba Egbema Ndoni Local

Table 1. Mean blood parameters of C. gariepinus exposed to different concentrations of Goldcrew and Corexit over four weeks.

Concentration (ml/l)/dispersant	WBCC ($\times 10^{9}/_{L}$)	Platelet ($\times 10^{9}$)	RBCC ($\times 10^{2}/_{L}$)	PCV (%)	Hb (2/dl)
Control	**5.70 ± 0.24**	**395.25 ± 4.11**	**5.18 ± 0.28**	**42.03 ± 1.38**	**15.80 ± 1.07***
0.0625					
G	6.90 ± 0.14	324.00 ± 57.90	4.88 ± 0.70	38.95 ± 1.40	14.78 ± 0.90
C	6.22 ± 0.56	329.50 ± 52.80	4.90 ± 0.53	38.74 ± 0.95	15.01 ± 0.92
0.125					
G	7.12 ± 0.15	327.75 ± 37.77	4.80 ± 0.82	38.07 ± 1.91	14.05 ± 0.42
C	6.75 ± 0.53	374.25 ± 17.74	4.60 ± 0.37	38.68 ± 2.75	14.30 ± 0.42
0.1875					
G	6.70 ± 0.48	333.50 ± 53.20	4.35 ± 0.72	38.36 ± 3.93	13.85 ± 0.87
C	6.15 ± 0.64	276.25 ± 26.89	4.42 ± 0.27	38.07 ± 3.40	13.75 ± 0.42
0.25					
G	6.85 ± 1.07	227.25 ± 102.55	4.00 ± 0.59	35.52 ± 5.64	13.28 ± 1.45
C	7.12 ± 0.22	222.25 ± 28.76	4.27 ± 0.19	36.98 ± 3.60	12.92 ± 0.31

G - Goldcrew
C - Corexit
*Standard Deviation (SD).

Government Area of Rivers State Nigeria. Acclimatization of fish to experimental conditions was done in plastic basins containing borehole water in groups of six (6) for seven (7) days in twenty seven (27) plastic basins. A total of 162 fish were acclimatized. The mean weekly water characteristic and standard deviations were temperature 16.8 ± 0.60℃, pH 7.12 ± 0.02 and dissolved oxygen 6.8 ± 0.22, alkalinity 22.7 ± 2.0 mg/l.

The fish were fed *ad-libitum* twice daily during and after acclimation using African Regional Centre (ARAC) compounded experimental feed. A hunzu(R) air pump was used to provide aeration during acclimation. Mortality during this period was 2.5% of total fish population. Therefore fish were considered healthy for experimentation.

Test concentrations of 0.0625, 0.125, 0.1875 and 0.25 m/l of Goldcrew and Corexit in dilution water were prepared by dissolving 0.1, 0.2, 0.3 and 0.4 ml of Goldcrew and Corexit respectively in 16 L of water. Homogenous mixing was achieved by measuring pre-determined volumes of the dispersants into a bottle to which dilution water was measured and added into the bottle from the plastic basin to be prepared. The bottle was corked then vigorous shaking carried out to give a homogenous mixture before being poured into the plastic basin. The process was repeated severally for each test concentration and each replicate basin using one dispersant at a time. These ranges were obtained after conducting a range finder test to establish safe sublethal limits. This was done by exposing test fish to 0.025, 0.05 and 0.075 m/l of Goldcrew and Corexit respectively and measuring mortality of fish over 96 h. Test concentrations of 0.05 and 0.075 m/l resulted in total mortality after only 48 h. Test concentration of 0.025 m/l did not lead to mortality after 96 h for the two dispersants. Test concentrations and water in plastic basins were renewed daily by siphoning using rubber hoses.

The control basins (0 m/l) had no dispersants and were prepared simply by adding 16 L of water into the pre-washed plastic basins. Introduction of fish into plastic basins containing various treatment levels of the dispersants and control was done randomly. Each plastic basin contained 5 fish. All treatment levels and control had 3 replicates. There was no sexual consideration.

A 21 “gauge hypodermic needles and syringes (Innoson shanchuan®) were used to collect blood from only one fish at a time in each plastic basin weekly for four consecutive weeks. Each sampled fish was removed from the test medium after sampling. The method of cardiac puncture using physical restraint was employed because of its relative ease, and the probability of collecting ‘frank’ blood is highest (Snieszko and Axelrod, 1989). The site chosen for the puncture was about half an inch behind the apex of the ‘V’ formed by the gill cover and isthmus as described by Klontz and Smith (1968).

Collected blood samples were put in previously tagged anticoagulant bottles containing potassium salt of ethylene diamine tetra acetic acid (EDTA) and sent for laboratory analysis at the University of Port Harcourt Teaching Hospital, Port Harcourt. Cyanment-haemoglobin method was used to obtain haemoglobin values (Larsen and Sniezko, 1961). Packed Cell Volume (PCV) was determined by the method of Snieszko (1960).

In this method micro-haematocrit tubes were ¾ filled with the blood samples and then the end of the tubes sealed. The capillary tubes were centrifuged at a speed of 12,000 rev/min. for 10 min. The centrifuged tubes were then placed on a PCV reader and PCV values read for each of the blood samples. Red blood cell count was determined by the use of formal citrate, formal-dehyde and a counting chamber. White blood cell count (WBCC), using Turks solution and an improved Neubauer counting chamber. Platelet was determined by using 1% ammonium oxalate solution and a counting chamber.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) at the 95% probability level. Duncan multiple range tests were employed to compare means according to standard procedures using the general linear model (GLM) of statistical analysis system software (SAS, 1999). Pearson correlation coefficient was employed to determine the relationship between the two dispersant effects,

Table 2. Mean weekly blood parameters of *C. gariepinus* to cumulative treatment levels of Goldcrew and Corexit and control.

Weeks	Dispersant	WBCC ($\times 10^{9}/_{L}$)	Platelet ($\times 10^{9}$)	RBCC ($\times 10^{2}/_{L}$)	PCV (%)	Hb (g/dl)
	G	6.22 ± 0.69	363.60 ± 47.63	5.50 ± 0.46	42.34 ± 1.53	15.40 ± 1.09
	C	6.62 ± 0.83	340.20 ± 67.75	5.02 ± 0.53	42.07 ± 1.24	14.96 ± 1.68
	Control	6.00 ± 1.56	390.00 ± 36.55	5.50 ± 1.13	43.53 ± 1.28	17.00 ± 2.64
	G	6.90 ± 0.67	335.40 ± 83.23	4.62 ± 0.54	38.87 ± 1.99	14.62 ± 1.33
	C	6.60 ± 0.58	327.20 ± 69.83	4.82 ± 0.37	38.90 ± 1.96	14.75 ± 1.24
	Control	5.80 ± 0.20	396.00 ± 2.64	5.30 ± 0.26	42.30 ± 0.81	16.40 ± 0.52
	G	6.88 ± 0.79	304.20 ± 81.51	4.28 ± 0.43	36.75 ± 3.01	13.80 ± 1.04
	C	6.02 ± 0.57	301.60 ± 85.27	4.48 ± 0.29	36.82 ± 2.63	14.10 ± 0.74
	Control	5.50 ± 0.10	395.00 ± 2.60	4.90 ± 0.30	40.46 ± 0.25	15.00 ± 1.73
	G	6.62 ± 0.64	283.00 ± 87.15	4.16 ± 0.61	36.38 ± 4.38	13.58 ± 0.95
	C	6.32 ± 0.63	309.00 ± 76.03	4.38 ± 0.38	37.71 ± 2.70	13.62 ± 0.86
	Control	5.50 ± 0.55	400.00 ± 32.78	5.00 ± 1.32	42.20 ± 1.85	14.80 ± 0.72

Table 3. Chemical properties of the dispersants.

Chemicals	Lead (Pb) (mg/l)	Copper (Cu) (mg/l)	Zinc (Zn) (mg/l)	Iron (Fe) (mg/l)	Cadmium (Cd) mg/l	Nickel (Ni) (mg/l)	Cyanide	Arsenic (ppm)	Chlorinated hydrocarbon	Chromium (ppm)
Goldcrew	0.089	0.149	0.096	0.092	0.003	0.055	ND	<1.0	ND	<1.0
Corexit	0.011	0.204	0.062	0.128	0.005	0.039	*ND	0.16	ND	<1.0

Adapted: Global environmental consultant limited, (1991)
ND = Not detected.

blood parameters and other variables.

RESULTS

The mean physico-chemical parameters of the experimental water and the mean lengths of the experimental fish are highlighted in the introduction. The results of the investigation are presented in Tables 1 - 5. Table 1 shows the mean blood parameters of *C. gariepinus* with varying concentrations of dispersants (Goldcrew and Corexit) and control over a four week period. The result reveals a significant difference ($p < 0.05$) in all blood parameters measured with concentration changes and control over the four week period. Apart from the white blood cell count (WBCC) which increased from control to higher treatment levels; all other measured blood parameters declined in value from control to higher treatment levels.

Table 2 shows the mean weekly changes of blood parameters to summative treatment levels of dispersant and control. The result shows a significant weekly reduction in all measured blood parameters except in WBCC which did not exhibit any significant reduction in weekly values.

Table 3 shows the chemical composition of the dispersants (Goldcrew and Corexit). The Table shows little but negligible variations in the chemical constituents of the two dispersants.

Table 4 shows the analysis of variance (ANOVA) revealing degrees of significance of measured blood parameters to sources of variability. It shows that there are no significant differences in toxicant effects on blood parameters of the two dispersants. The Table also reveals that treatment levels of the dispersants have a significant effect on all measured blood parameters. Except for WBCC which was not significant at all exposure times, duration of exposure has a marked effect on all blood parameters. The interaction of toxicant and concentration reveal that apart from platelet counts, no other blood parameter showed any form of significance.

The interaction of Toxicant and Exposure period reveal that only platelet counts was significant ($p < 0.005$) of all blood parameters. Also, the complex interactions of concentration and duration of exposure (weeks) and the interaction of toxicant, concentration and duration of exposure (weeks) reveal that only PCV and platelet counts to be highly significant.

Table 5 shows the Pearson's correlation coefficient

Table 4. Analysis of variance showing levels of significance of measured blood parameters to sources of variability.

Source of variation	Df	White blood cell count	Red blood cell count	Packed cell volume	Haemoglobin	Platelet count
*Toxciant	1	1.98^{ns}	0.55^{ns}	5.47^{ns}	0.0009^{ns}	104.53^{ns}
Concentration	4	6.51***	2.91***	107.55***	25.82***	9475.14***
Duration of exposure (week)	3	0.69^{ns}	5.04***	208.20***	15.3***	19897.0***
Toxicant × concentration	4	0.90^{ns}	0.61^{ns}	4.23^{ns}	0.38^{ns}	7439.1***
Toxicant × week	3	1.90^{ns}	1.55^{ns}	2.91^{ns}	0.75^{ns}	3124.60*
Concentration × week	12	0.77^{ns}	0.38^{ns}	15.82***	0.72^{ns}	8113.1***
Toxicant × concentration × week	12	0.47^{ns}	0.37^{ns}	4.90^{ns}	1.16^{ns}	4051.16***
Error	80	0.96	0.67	4.79	2.31	1118.38
R^2		0.42	0.45	0.78	0.49	0.87
CV (%)		14.99	17.45	5.64	10.58	10.44

ns = not significant
* p = 0.05
** p = 0.01
*** p = 0.001

Table 5. Pearson correlation coefficients showing the level of inter-relationship between all variables.

	Treat	Conc	Week	WBCC	Platelet	RBCC	PCV	HB
Treat	1.00000	0.00000 1.0000	0.00000 1.0000	0.12287 0.1812	0.01211 0.8956	- 0.06593 0.4743	- 0.04273 0.6431	- 0.00159 0.9863
Conc	0.00000 1.0000	1.00000	0.00000 1.0000	0.32892 0.0002	- 0.67238 <.0001	- 0.34013 0.0001	- 0.47430 <.0001	- 0.53091 <.0001
Week	0.00000 1.0000	0.00000 1.0000	1.00000	- 0.01499 0.8709	- 0.28276 0.0018	- 0.35008 <.0001	- 0.53596 <.0001	- 0.35424 <.001
WBCC	0.12287 0.8956	- 0.67238 <.0001	- 0.28276 0.0018	- 0.34294 0.0001	0.0001	0.31648 0.0004	0.57687 <.0001	0.54781 <.0001
Platelet	0.01211 0.8956	- 0.67238 <.0001	- 0.28276 0.0018	- 0.34294 0.0001	0.0001	0.31648 0.0004	0.57687 <.0001	0.54781 <.0001
RBCC	- 0.06593 0.4743	- 0.34013 0.0001	- 0.35008 <.0001	- 0.19360 0.0341	0.31648 0.0004	1.00000	0.42785 <.0001	0.30794 0.0006
PCV	- 0.04273 0.4731	- 0.47430 <.0001	- 0.53596 <.0001	- 0.38912 <.0001	0.57687 <.0001	0.42785 <.0001	1.00000	0.46736 <.0001
HB	- 0.00159 0.9863	- 0.53091 <.0001	- 0.35424 <.0001	- 0.34400 0.0001	0.54781 <.0001	0.30794 0.0006	0.46736 <.0001	1.00000

revealing the inter-relationship between variables.

DISCUSSION

The mean values of the physico-chemical parameters of the bore-hole water used for the analysis revealed that the water parameters measured were within the limits recommended by the United States Environmental Protection Agency (1973) for optional health of warm and cold water fishes. This implied that there was no water quality mediated stress and therefore any stress factor observed in the study was due only to the addition of the dispersants at various concentration levels.

Haematological parameters relating to oxygen transport, red blood cell count [RBCC], haemoglobin (Hb), packed cell volume (PCV) and platelet declined in values with increases in concentration of the dispersants over a four week period (Table 1). This reduction is in agreement with Sharma and Gupta (1984) whom noticed a dose dependent reduction of erythrocytes following the exposure of *C. batracus* to carbon-tetrachloride. Similar reductions in haemoglobin, packed cell volume and erythrocytes of fish have been reported by Omoregie et al, (1994). The decline in RBCC, Hb, platelet and PCV may be due to the swelling of red blood cells, haemo-dilution or the distortion of cellular components of fish by invading toxin (Musa and Omoregie, 1990).

The decrease in Hb, PCV, platelets and RBCC with increasing concentration of dispersants may also suggest a progressive macrocytic anaemia. This is an indication of bone marrow erythropoietin response to an ensuing anaemia resulting from possible metabolic dysfunction in organs like the liver and kidney (Olukunle et al., 2002). This occurs if the diet is deficient in iron, vitamin B_{12} and folic acid the digestive system will be unable to absorb them, the marrow therefore fails to produce enough red blood cells or produces defective ones.

It may also be a signal to the unset of a hemolytic anaemia. An anaemia of this type results when if red blood cells are produced in adequate numbers but are destroyed faster than new ones can be produced. Anaemia of this type is produced by severe infectious diseases such as scarlet fever, malaria and puerperal fever. This can also be caused by poisons and certain drugs especially the sulfonamides.

In contrast, the parameter relating to defense mechanism (white blood cell count, (WBCC) showed a gradual but fluctuant pattern of increase with increase concentration of dispersants (Table1). This is an indication of increased production of leucocytes into peripheral circulation from marginated pool, the bone marrow or spleen probably in response to bacterial infection, invading toxin in the already compromised and anaemic fish (Olukunle et al., 2002). This may also be due to the stress-mediated release of WBCC as noted by Sampath, et al (1993). Who reported increase in lymphocytes of the Nile tilapia *Oreochromis niloticus* exposed to toxic condition. However WBCC did not show any significant decline during the four weeks of exposure (Table 2). This may be due to a time related homeostasis of WBC or a remote indication of actualization of threshold concentrations of dispersants as suggested by Davids et al. (2002). They noticed a time related stability in white blood cell counts in the fishes *Sarotherodon melanotheron* and *Tilapia guiniensis* exposed to varying treatment levels of industrial effluents.

The dispersants Goldcrew and Corexit contain similar contents of heavy metals and hydrocarbons (Table 3). This may be due to the fact that both dispersants are sulfactants of the same group and are used routinely together by multi-national oil company's operating in Nigeria.

Goldcrew and Corexit dispersants respectively did not exhibit any significant difference in measured blood parameter presentation between the two dispersant as shown in the analysis of variance (ANOVA) Table 4. This may be due to the fact that both dispersants presented similar blood parameter presentations.

The findings in this study indicate that all haematological parameters assessed except WBCC were affected by concentrations of dispersants and exposure periods. This agreed with the findings of Annune et al. (1994) that the most susceptible parameters PCV, Hb, WBCC, RBCC and platelet counts of fresh water fish were affected by the stress agents.

Although Goldcrew and Corexit are routinely used oil spill dispersants in Nigeria, this study indicate their deleterious effect to our environment and our fisheries.

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