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Full Length Research Paper

Comparative effects of crude oil on juveniles *Clarias gariepinus* and *Clarias anguillaris*

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This study was based on biochemical assessment of the effect of crude oil spillage on marine life. Hence, life sustaining indices such as pH, dissolved oxygen (DO), and free CO_2 were analyzed in aquaria. However, a 96 h static acute toxicity test was also investigated on the juveniles *Clarias gariepinus* (African catfish) and *Clarias anguillaris* (mudfish) on exposure to different concentrations of crude oil polluted water. Over the period of the experiment there was an overall decrease in pH of the test vessel from 8.64 ± 0.03 to 5.50 ± 0.08. Decrease trend was also found in dissolved oxygen from 6.28 ± 0.07 to 3.01 ± 0.06 and free CO_2 levels from 8.35 ± 0.55 to 11.25 ± 0.17. However as the concentration of crude oil increases the haematological parameters such as red blood cell (RBC), packed cell volume (PCV), and white blood cell (WBC) reduced significantly ($p \leq 0.05$) in both fish species. After 96 h, probit analysis showed the LC_{50} of crude oil for *C. anguillaris* to be 1.22 mg/L while that of *C. garieinus* was 2.19 mg/L. Overall, less insults was observed from the crude oil on *C. gariepinus* compare to *C. anguillaris* that tend to elicit a low tolerance to the crude oil.

Key words: *Clarias gariepinus, Clarias anguillaris, crude-oil, LC_{50}*, and static-acute.

INTRODUCTION

The activities of man have been responsible for introducing contaminants into the environment in the last few decades. The resulting pollution has led to serious consequences on aquatic life (Clair et al., 2003). Apart from the presence of industrial wastes that are sources of constant threat to marine life, the ecological disasters caused by the oil industry while exploring crude oil and accidental discharge of crude oil on high seas is a serious cause for concern (Speight, 1999). The main threat posed to living resources by the persistent residues of spilled oils and water-in-oil emulsions ("mousse") is one of physical smothering that may consequently lead to depletion of biodiversity in the aquatic environment if adequate remediation is not provided (Cooney et al., 2001). The animals and plants most at risk are those that could come into contact with a contaminated sea surface (Longhurt, 1961). These include aquatic mammals, reptiles and birds that feed by diving or form flocks on the sea as well as aquatic lives on shorelines (Suchanek, 1993).

Juvenile *Clarias anguillaris* and *Clarias gariepinus* were selected as the aquatic organisms for this experiment due to the fact that their response and sensitivity to toxicants is high (Sunmonu and Oloyede, 2006). Both are species of the family *Clariidae*, the air breathing catfishes, found throughout Africa and the Middle East. They live in freshwater lakes, rivers, swamps, as well as human-made reservoirs, such as ponds or even urban sewer systems (Teugels, 1986). *C. gariepinus* commonly refer to as African Catfish is a large, eel-like usually of dark gray or black colouration on the back, fading to a white belly. It has a slender body, a flat bony head and abroad terminal mouth with four pairs of barbell (Andrews and Matsuda, 1975). However, *C. anguillaris* otherwise known as Mudfish have an oval-shaped to rectangular in dorsal outline with a broadly rounded snout (Teugels, 1986). Previous studies have shown that crude oil can have both lethal and sub-lethal effects on a wide range of organisms. These include the observation reported by in

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juvenile pink salmon (*Oncorhyncusgorbuscha)* (Wang et al., 1994); while drastic changes in liver enzyme activities of catfish (*C. gariepinus)* were reported following exposure to crude oil (Sunmonu and Oloyede, 2006). Against the foregoing this work was set to carried out comparative study on the effect of crude oil exposure on some physical parameters, haematological parameters and lethality in *C. gariepinus* and *C. anguillaris* under experimental conditions.

MATERIALS AND METHODS

Collection of crude oil

The crude oil was obtained from Chevron depot site, Lagos Nigeria.

Collection of test organism

Juvenile *C. anguillaris* and *C. gariepinus* were collected from a fresh water fish farm in Iperu Remo and Ilishan Remo respectively. The acute toxicity test was carried out at the Department of chemical and environmental Sciences Animal Laboratory, Babcock University Nigeria.

Experimental methods

Modified method of American Public Health Association (APHA, 1992) was used to carry out the static bioassay procedure. This involved carefully controlled environmental conditions to define the response of the test organism to crude oil.

Twelve rectangular glass tanks of the dimension 50 × 30 × 45 cm and capacity of 100.5 L were used as aquarium. The test organisms were brought into the laboratory and the weight of the fishes was taken. This ranged between 1.6 and 1.87 g and the length of the fishes ranged between 5.5 to 7.3 cm. The length and weight of the biggest was not more than twice that of the smallest, to avoid errors due to size. The stock populations were held for one week in the laboratory, inside these aquaria, filled with 30 litres of unchlorinated borehole water. The animals were held at a temperature range of 26 to 28°C. The water in the tanks was changed once every three days in order to avoid the accumulation of toxic metabolites and wastes secreted by the fishes. Juvenile *C. anguillaris* and *C. gariepinus* of fairly equal length and weight were taken randomly from the stock population using a hand net and carefully transferred to the glass test vessel containing the test solution.

Static acute toxicity study

Preliminary studies carried out using several concentrations provided the basis for the spread of test concentrations along pre determined toxicity range of test samples. Reference chemical used as a negative control is Sodium Dodecyl Sulphate (Gulley and West, 1996). Fish tanks filled with only freshwater were used as positive control. Concentrations of crude oil prepared as 0.6, 1.0, 1.4 and 1.8 mg/L were added to the *C. anguillaris* labelled aquarium while 1.2, 2.0, 2.8 and 3.6 mg/L were added to the *C. gariepinus* labelled aquarium for two hours to allow aeration. Thereafter, a total number of twenty of the fish species were added to each test vessel and covered with wire mesh to prevent the organisms from jumping out of the tanks. Mortalities of the test organisms were recorded at 24, 48, 72 and 96 h according to OSPAR offshore Industries Committee (OIC, 2002). The experimental animals were taken as dead when there were no opercula and other forms of body movements even on prodding with a glass rod.

Determination of physico- chemical parameters

Samples of the test solutions were taken from each vessel for the determination of physico-chemical parameters. The following parameters were measured after the placement of each test solutions in each tank: pH, dissolved oxygen and temperature using the HORIBA U10 and the dissolved oxygen meter. The level of carbon dioxide in each aquarium was determined by the titrimetric method (Maissonneuvve and Larose, 1988).

Determination of hematological parameters

The method of Dacie and Lewis (1975) as modified by Alexandra and Griffiths (1993) was used in the determination of the haematological parameters. After harvesting from the aquaria, the fishes were allowed to stay in a dissecting tray for about ten minutes to reduce the slime on their bodies. They were thereafter dissected and blood sample was collected with a disposable syringe and needle and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA) embedded vials for haematological analysis. The Automated Haematologic Analyzer (Sysmex KX – 21, UK) was used to analyze the haematological parameters namely packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC).

RESULTS AND DISCUSSION

One of the major problems of the inhabitants of the Niger Delta region of Nigeria is contamination of water and aquatic lives by crude oil spillage (Sunmonu and Oloyede, 2008).

The severity or degree of the problems in the inhabitants of the area is dependent upon the point of contact with the polluted water (Omoregie, 1998). This observation is reflected in the physico- chemical parameters of this study. There were overall decreases in pH of all the test solutions after a 96 h period of the experiment (Tables 1 and 2). The decrease in pH which poses a lethal effect on the marine life is indicated to be from the organic pollution from the fishes (Swingle, 1961; GodsWill, 1989). However, similar trends were also observed in the values of Dissolved oxygen (DO) in all the aquaria. A significant decrease ($P \leq 0.05$) in dissolved oxygen over 96 h period of the study was recorded. It is known that low DO causes anaerobic decomposition of organic matter in water, forming noxious and toxic substances such as hydrogen sulphide and methane which ultimately would have deleterious effect on the aquatic life (Mason, 1991; DPRM, 2000). There was a corresponding increase in free CO_2 in the aquaria containing *C. anguillaris* from 8.35 ± 0.23 to 11.25 ± 0.17 over a period of 96 h. and 8.69 ± 0.33 to 11.48 ± 0.15 for aquaria containing *C. gariepinus.* This observation can be attributed to the oil film formation that reduces the

Table 1. Physico- chemical parameters determined in test solutions with crude oil sample containing *Clarias anguillaris* (mudfish) 24 to 96 h into the experiment.

Concentration (mg/L)	Duration (h)	Positive control	Negative control	1.2 0.6	2.0 1.0	2.8 1.4	3.6 1.8
pH	24	7.86 ± 0.02[a]	7.25 ± 0.04[b]	7.96 ± 0.03[c]	8.20 ± 0.02[d]	8.43 ± 0.05[e]	8.64 ± 0.03[f]
pH	96	6.23 ± 0.03[a]	6.89 ± 0.02[b]	5.68 ± 0.06[c]	5.50 ± 0.08[d]	5.63 ± 0.04[e]	6.42 ± 0.07[f]
Dissolved oxygen	24	6.17 ± 0.1[a]	6.28 ± 0.0[a]	6.18 ± 0.1[a]	6.21 ± 0.1[a]	6.28 ± 0.1[a]	5.96 ± 0.1[a]
Dissolved oxygen	96	3.27 ±0.04[a]	5.46 ± 0.05[b]	3.27 ± 0.07[c]	4.81 ± 0.04[d]	4.28 ± 0.06[e]	3.01 ± 0.06[f]
Carbon (iv) oxide	24	8.53 ± 0.1[a]	8.45 ± 0.1[a]	8.35 ± 0.2[a]	8.46 ± 0.3[a]	8.67 ± 0.5[a]	8.35 ± 0.5[a]
Carbon (iv) oxide	96	11.00 ± 0.14[a]	9.25 ± 0.15[b]	10.86 ± 0.22[c]	10.00 ± 0.25[d]	9.69 ± 0.37[e]	11.25 ± 0.17[f]

Values are means ± SEM for 20 catfish. [a,b,c,d,e,f] column values with different superscript are significantly different (P ≤ 0.05).

Table 2. Physico- chemical parameters determined in test solutions with Crude oil sample containing *Clarias gariepinus* (African catfish) 24 to 96 h into the experiment.

Concentration (mg/L)	Duration (h)	Positive control	Negative control	1.2 0.6	2.0 1.0	2.8 1.4	3.6 1.8
pH	24	7.34 ± 0.05[a]	7.34 ± 0.07[a]	9.50 ± 0.02[b]	9.44 ± 0.05[c]	9.68 ± 0.02[d]	9.70 ± 0.08[d]
pH	96	5.43 ± 0.08[a]	6.34 ± 0.06[b]	6.28 ± 0.06[c]	5.87 ± 0.08[d]	6.43 ± 0.02[e]	6.67 ± 0.04[f]
Dissolved oxygen	24	6.21 ± 0.28[a]	6.16 ± 0.24[a]	6.20 ± 0.13[a]	5.96 ± 0.13[a]	6.00 ± 0.23[a]	6.36 ± 0.33[a]
Dissolved oxygen	96	3.59 ± 0.15[a]	4.06 ± 0.14[b]	3.29 ± 0.23[c]	3.76 ± 0.10[d]	3.64 ± 0.09[e]	3.36 ± 0.12[c]
Carbon(iv) oxide	24	8.36 ± 0.34[a]	8.27 ± 0.23[a]	8.69 ± 0.33[a]	8.45 ± 0.25[a]	8.56 ± 0.23[a]	8.76 ± 0.25[a]
Carbon(iv) oxide	96	11.45 ± 0.12[a]	10.67 ± 0.14[b]	11.50 ± 0.09[a]	11.23 ± 0.10[c]	11.36 ± 0.12[c]	11.48 ± 0.15[a]

Values are means±SEM for 20 fishes. [a,b,c,d,e,f] column values with different superscript are significantly different (P≤0.05).

Table 3. Hematological parameters determined from *Clarias gariepinus* and *Clarias anguillaris* exposed to vary concentrations of crude oil.

Concentration (mg/L)	Positive control	1.2 0.6	2.0 1.0	2.8 1.4	3.6 1.8
PCV(%)catfish	39.0 ± 1.45[a]	36.0 ± 1.23 [a]	34.0 ± 1.46 [a]	30.0 ± 1.45 [a]	27.0 ± 1.26 [a]
PCV(%)mudfish	38.0 ± 2.18 [a]	34 ± 1.98 [b]	31 ± 1.46 [b]	27 ± 1.50 [b]	17 ± 1.31 [b]
WBC(10^9/L)catfish	290.5 ± 2.78 [a]	285.0 ± 1.56 [a]	266.0 ± 2.25 [a]	250.0 ± 2.30 [a]	233.0 ± 2.56 [a]
WBC(10^9/L)mudfish	286. ± 2.50 [a]	282.0 ± 2.15 [b]	256.0 ± 1.98 [b]	230.0 ± 3.50 [b]	222.0 ± 2.75 [b]
RBC(10^{12}/L)catfish	2.11 ± 0.05 [a]	2.08 ± 0.02 [a]	2.00 ± 0.02 [a]	1.66 ± 0.05 [a]	1.00±0.03 [a]
RBC(10^{12}/L)mudfish	2.11 ± 0.05 [a]	2.11 ± 0.07 [b]	1.85 ± 0.02 [b]	1.06 ± 0.03 [b]	0.47±0.05 [b]

[a,b,] column values with different superscript are significantly different (P≤0.05).

dissolution of atmospheric oxygen that comes in contact with the test solutions (Basau, 1959; Mason, 1994), thereby leading to reduction in the dissolved oxygen in the aquarium.

Table 3 shows hematological parameters determined from the two fish species after 96 h exposure to different crude oil concentration. There was significant difference (P ≤ 0.05) in all the haematological parameters determined between the two fish species. As the concentration of crude oil increases the values of WBC (white blood cell) also increases. However, decline in the values of PCV (packed cell volume) and RBC (red blood cell) were observed after exposure to increasing levels of crude oil concentrations. There was a significant difference (P ≤ 0.05) in the effect of toxicant on the PCV of both species of fish after 96 hours of exposure to the toxicant. The PCV of the *C. anguillaris* appeared to be more reduced compared with *C. gariepinus* at double concentration of toxicant. The significant reduction (P ≤ 0.05) in RBC count may be attributed to cytotoxic effect and suppression of erythropoiesis caused by constituents of the crude oil (Suchanek, 1993). This would imply reduction in the level of oxygen that would be carried to the tissues and the level of carbon dioxide returned to the

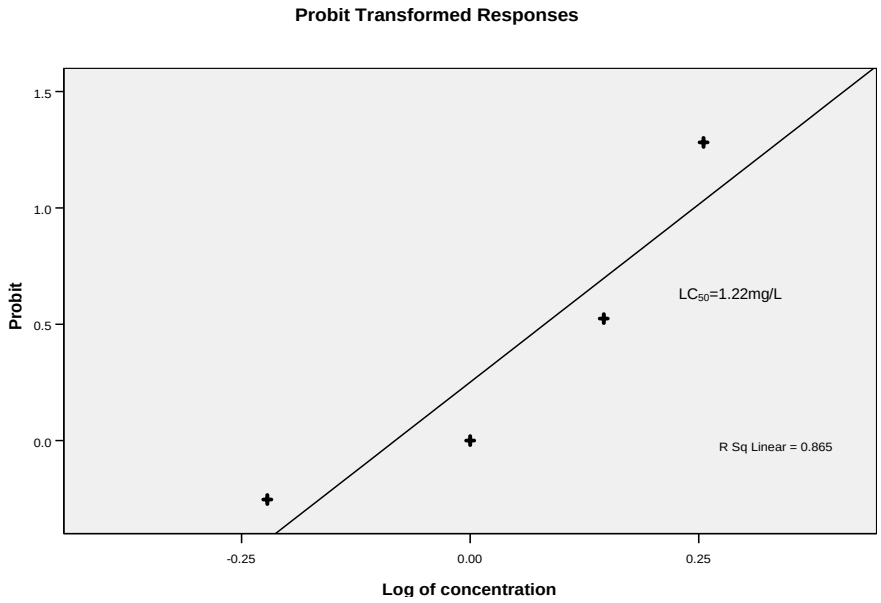

Figure 1. Probit transform responses of the 96 h acute toxicity of crude oil on *C. anguillaris.*

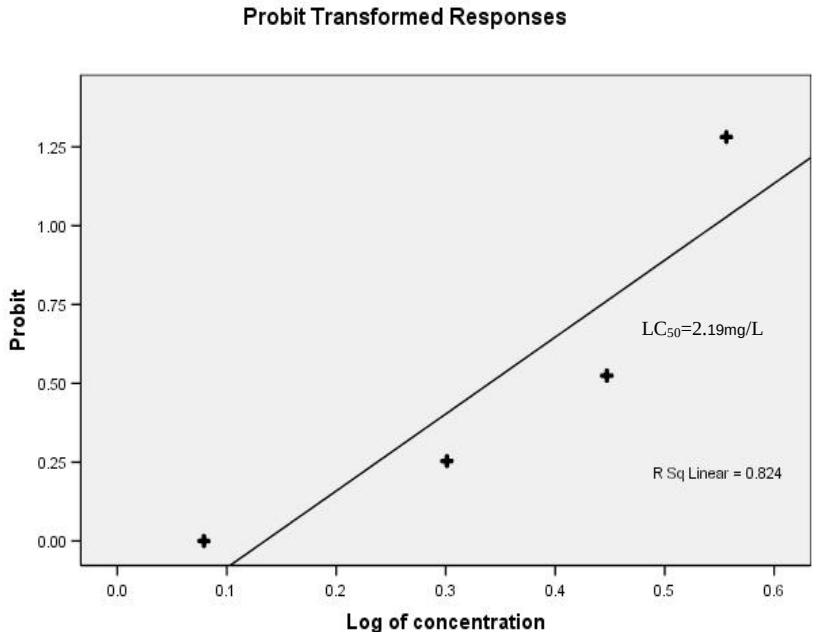

Figure 2. Probit transform responses of the 96hr acute toxicity of crude oil on *C. gariepinus.*

lungs would also be reduced (Sunmonu and Oloyede, 2008). The major functions of WBC are to fight infection, defend the body by phagocytosis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. A significant reduction ($P \leq 0.05$) in WBC count with increase in crude oil concentration may suggest that the species are exposed to high risk of infections with mudfish being most susceptible based on its comparative level of white blood cell count.

Figures 1 and 2 show probit transformed response of the 96 h acute toxicity of crude oil on *C. anguillaris* and

C. gariepinus respectively. From the probit analysis, the LC_{50} of *C. gariepinus* was 2.19 mg/L ($P \leq 0.05$) while the LC_{50} *C. anguillaris* (mudfish) was 1.22 mg/L ($P \leq 0.05$), suggesting that *C. gariepinus* showed better ability to resist adverse environmental condition than *C. anguillaris*.

Conclusion

Considering the observations on *C. gariepinus* in this study based on parameters selected. It is concluded that the specie is relatively more tolerant to high level of crude oil compared to *C. anguillaris.*

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