Toxic Effect of Crude Oil on Hatchery Reared *Oreochromis niloticus* Fingerlings

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**Abstract**

The toxicity of crude oil on hatchery-reared *Oreochromis niloticus* fingerlings obtained from University of Calabar fish farm was studied in triplicates (A, B and C) using the water soluble fraction of crude oil for 96 h under laboratory conditions. Six concentrations ranging from 0, 10, 20, 30, 40 and 50 mg/L were prepared from the water soluble fraction (WSF) of crude oil for the toxicity test. The experimental animals showed different percentage mortalities with toxicant concentrations. The 96 h LC₅₀ for *O. niloticus* in the three batches (A, B and C) was 20 mg/L. The three batches of *O. niloticus* (P>0.05) had no significant difference in mortality indicating that the WSF of crude oil had same toxic effects on the test organisms. Toxicant exposure induced behavioral changes such as abnormal and uncoordinated swimming movement, restlessness, respiratory difficulties and attempt at jumping out. It was also observed that the WSF of crude oil had adverse effects on the test water causing alterations of the physico-chemical parameters. The results of the present study suggest that the water soluble fraction of crude oil had severe impacts on the test organism resulting in mortality.

**Keywords:** Toxicity, mortality, Qua Iboe light crude oil, *Oreochromis nilotius*, fingerlings.

**Introduction**

Crude oil is a naturally occurring substance derived from the decomposition of thousands of years of plant and animal organic matter under elevation, temperature and pressure. It is the remains of living matter buried in sediments that have decomposed to a state in which carbon and hydrogen are the principal elements (Owate and Okujagu, 1995). In appearance, crude oil ranges from mobile, volatile light colored liquids to dark, viscous tar-like materials with low vapor pressure. Petroleum oil provides ingredients for thousands of products that we use every day, but this involves potential problems. In recent years, oil pollution has become a global environmental issue, in that oceanic ecosystem and Inland aquatic breeding ecosystem are threatened greatly. The other components include toxic phenols and anilines (Traven, 1992). The content of the components differs depending on the area of oil drilling. All crude oils and many refined products in sufficient concentrations are poisonous to aquatic organisms (Traven, 1992). Direct killing of aquatic organisms can occur through coating with oil and by asphyxiation (Hoong et al., 2001).

Crude oil exploration has been going on in many parts of the world including Nigeria for several decades. To date, this and other related activities in the petroleum industry have sustained the Nigerian economy since 1970s. Unfortunately, these highly profitable activities have environmental and biological hazards as observed during incidents of oil spills. Oil and oil products concentrations of 0.01 mL/L are known to accelerate the death of fish in aquatic environments (Lee, 1975).

In addition, the incorporation of finely dispersed particles of oil and oil products into organisms can negatively affect the body organs and systems, either directly or as a consequence of bioaccumulation processes (Lockhert et al., 1996). On the other hand, there is a growing concern that several aquatic bodies in Nigeria are being affected by crude oil and hydrocarbons which are suggested to be of anthropogenic contribution. Also, several studies have shown that petroleum contaminants in Nigerian aquatic environment are detected in water, sediment and aquatic animals. Fish responses have been used as biomarkers of aquatic pollution (Ek et al., 2005). Adult fishes are more resistant to oil pollution since their bodies, mouth and gill chambers are coated with slimy mucus that resist wetting by oil. Quite unlike adult fish, which swim away from oil spill, many fish eggs and larvae float with the plankton at the water surface. The overall effect of crude oil on aquatic biota depends on a great number of factors acting individually or in combination. These range from biological to physio-chemical parameters as indicated by Klinhold (1980). The impacts include outright mortality, defoliation, growth inhibition, reduction in population size, susceptibility to predators and parasites interference with chemical communication and sub-lethal effects on reproduction and behavior. Azad (2005) observed that eggs and young stages (Fingerlings) of fishes are especially vulnerable to the toxic effects of crude oil and its refined products.
It is important to examine the toxic effect of crude oil on fish since they constitute an important link in the food chain. The Nile Tilapia Oreochromis niloticus is a widely distributed freshwater fish that can persist in a highly polluted habitats and it is possible to use it as a potential bio-indicator for aquatic environmental contaminants including crude oil. The Nile Tilapia has distinctive, regular, vertical stripes extending as far down the body as the bottom edge of the caudal fin, with variable coloration. The fish can live for up to 9 years and tolerates brackish water and survive temperature between 8-42°C (Jonathan, 1989). This species has been used extensively in laboratory studies and has been shown to be a suitable organism for monitoring the effects of xenobiotic. Considering the above facts, this study is aimed at assessing the effect of crude oil on the mortality rate of hatchery reared Oreochromis niloticus fingerlings under laboratory conditions.

Materials and methods

Crude oil: The fraction of Qua Iboe light crude oil was obtained from Mobil Producing Unlimited, Eket, Akwa Ibom State in an airtight plastic cans and transported to Research Laboratory of the Institute of Oceanography (IOC), University of Calabar for studies.

Test organism: One hundred and eighty Oreochromis niloticus fingerlings in the range of 2-4.5 cm in size procured from the University of Calabar Fish Farm, Cross River State located within the University of Calabar at latitude 04°5, 020'N and longitude 008°20' 450'E, respectively (Asuquo and Bassey, 1999; Akpan et al., 2002) were used for the studies and was transported to the Research Laboratory of the Institute of Oceanography (IOC), University of Calabar for acclimatization. The test organisms were acclimatize to laboratory conditions for 24 h in the glass tank and aerated with air stone connected to electrically powered aquarium pumps for the test organisms to get acquainted with the environment.

Preparation of toxicant solution: The preparation of toxicant solution (water soluble fraction) of Qua Iboe light crude oil was gotten by vigorously shaking crude oil with filtered habitat water in a separatory funnel to obtain an homogenous solution then allowed to settle for 6 h to effect complete phase separation, after which the lower aqueous layer containing the WSF was decanted to be used for the toxicity test.

Stocking of specimens: Oreochromis niloticus fingerlings were picked using a hand net in order to avoid stress on the test organisms into glass tanks measuring 25 x 10 x 15 cm from an acclimatized tank. The glass tank was filled with 2 L of dechlorinated water.

Monitoring of water quality parameters: Water quality parameters such as Dissolved Oxygen (DO), pH, temperature (°C), Nitrite (NO₂⁻) and Ammonia (NH₃) were monitored before the start of experiment and also on daily basis according to standard method (APHA, 1992).

Monitoring of specimen for mortality: The test animals were designated as dead when their bodies were observed ‘not moving’. They float or sink into bottom when probed gently with a glass rod. During assessment for mortality each fish was removed from a test medium with a pair of forceps, placed in a clean empty petri dish and recorded.

Definitive test: The concentration of WSF of Qua Iboe light crude oil ranging from 0, 10, 20, 30, 40 and 50 mg/L was chosen for the toxicity test on Oreochromis niloticus fingerlings. The duration of the experiment was carried out for 96 h and the LC₅₀ was calculated using a modified method (Finney 1971; Stephan, 1977). The fish were not fed in order to minimize waste production. The distress behavior and the deaths were closely monitored and recorded from start of the experiment in (6, 12, 24, 48, 72 and 96 h respectively). The initial water parameter and daily water parameter, dissolved oxygen, temperature; pH, nitrite and ammonia were monitored using mercury-in-glass thermometer and Lurton Do and pH meters. The battery operated meters were calibrated according to manufacturer’s instructions before being used for measurement (Boyd, 1989, 1990).

Statistical analysis: Exact numbers of dead organisms between control and experimental group were analyzed using ANOVA at (P<0.05) to test the significant difference in the organisms. Statistical analysis was powered by SPSS 18.0 (SPSS Inc; Chicago, USA).

Results

The test organism Oreochromis niloticus showed behavioral changes and mortalities. Behavioral changes observed were erratic swimming behaviour, restlessness, loss of balance, attempt at jumping out, respiratory difficulties and mortalities were observed in the WSF exposure groups as compared to the control groups. The means (± SD) water parameters of the test medium were 29.4 ± 2.2°C (temperature), 7.57 ± 0.79 (pH), 0.1 ± 0.0 mg/L (Nitrite), 4.1± 2.7 mg/L (DO) and 0.0 ± 0.0 mg/L (Ammonia) as shown in Table 1. The percentage mortality and survivors of Oreochromis niloticus fingerlings at the end of the test period in each of the concentration are computed in Table 2 for the three batches of the experiment. In these studies, 0 mg/L of toxicant showed no mortality throughout the test period. For 10 mg/L of toxicant, 20% mortality was recorded leaving 80% survivors; while in 20 mg/L of the toxicant, 50% mortality was recorded leaving 50% survivors. In the 30 mg/L of toxicant, 80% mortality and 20% survivors were recorded; in the 40 mg/L of the toxicant, 90% mortality and 10% survivors were recorded, and in 50 mg/L of the toxicant, all test organisms were observed dead, leaving 0% survivors in the three batches (Table 2).
The 96 h LC50 for *Oreochromis niloticus* is shown in Fig. 1 for the three batches. The 96 h LC50 is given at log concentration of 1.30, a point where 50% of the organism would be killed at the end of the 96 h, if toxicant finds its way to the habitat of the fish. The log transformation of the different concentration of the toxicant is shown in Table 3 for the three batches.

It was further observed that there was no significant difference (P > 0.05) in mortality between the three batches (A, B and C) of the test organism.

**Discussion**

In the present study, percentage mortalities were concentration-dependent. The higher the concentration, higher the percentage mortalities. Similar report was presented by Ogundiran et al. (2010) when investigating toxicological impacts of detergent effluent in fingerlings of African catfish *Clarias gariepinus*. Calta et al. (2004) when studying acute toxicity of the synthetic pyrethroid deltamethrin to young minnow cap, *Cyprinus carpio*, Ayotunde et al. (2011) when investigating in the toxicity of *Carica papaya* on adult *C. gariepinus*, Ayuba and Ofojekwu (2002) when investigating on acute toxicity of dazinon to African catfish *C. gariepinus*. *Oreochromis niloticus* generally are ecologically adapted to muddy environment in which temporary changes in water chemistry are more rapid and the contaminant concentration are usually higher (Koivisto, 1995; Ayotunde et al., 2011). This view may however not be supported by some contaminants or toxicant such as crude oil which produced 100% mortality of the fish in 96 h. The findings of this study also agrees with the work of Ayuba and Ofojekwu (2002) when investigating acute toxicity of Jimson’s weed, *Datura innoxia* to the African catfish *Clarias gariepinus* fingerlings.
The 96 h LC50 of any toxicant is the dose or concentration which will kill 50% of the stocked organisms at the end of the experimental period of 96 h (4 d) (Samabaswa and Rao, 1985; AKpan et al., 1999; Udo et al., 2006). The 96 h LC50 is known to vary from toxicant (Samabaswa and Rao, 1985; Ayotunde et al., 2010) and from concentration to concentration of the toxicant (Cagauan et al., 2004; Ayotunde et al., 2010). The 96 h LC50 was 20 mg/L representing log concentration of 1.30 mg/L for the three batches A, B and C. The 96 h LC50 are known to vary with toxicant (Adewoye, 2010; Ogundiran et al., 2010). Ogundiran et al. (2010) reported 96 h LC50 of 0.0166 mg/L and 0.0038 mg/L for batch A and B Claris garipeinus fingerlings under the toxicity effect of detergent effluent. A 96 h LC50 of 0.1 mg/L and 0.03 mg/L was reported by Adewoye (2010) when working on the effect of soap and detergent effluents in Clarias garipeinus. The varied 96 h LC50 values usually obtained from different toxicants and test organisms is again reported by Ekanem et al. (2011), when they reported a 96 h LC50 of 5.0 ± 1.76 and 4.0 ± 1.76 mg/L for Macrobrachium macrobrachion and M. vollenhovenii. In this study, the 96 h LC50 of 1.30 mg/L obtained for Batches A, B and C might have depended on the range of the toxicant after series of preliminary test which produced the concentration finally used for the test.

Conclusion
Conclusively, this study has deduced that Qua Iboe light crude oil is toxic and has adverse effect on Oreochromis niloticus at low concentrations. In view of the toxicity effect, the recommended level of this toxicant in aquatic environment should not exceed the 10% of their 96 h LC50. Therefore, as a result of high percentage mortalities of Oreochromis niloticus when exposed to the water soluble fraction of crude oil appropriate measures should be adopted for efficient effluent treatment technology which would ensure proper treatment of industrial spillage prior to their disposal into the aquatic environment. Since Oreochromis niloticus is an important aquaculture candidate in most parts of the world including Nigeria, there is need for researchers to emulate from such developed nations where environmental monitoring agencies are more effective and environmental laws and legislations are strictly adhered to.

References