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Acute Toxicity of Water Soluble Fractions of Crude Oil to the Nile Tilapia, *Oreochromis niloticus* (L.)

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One of the most important environmental factors acting, as "stressor" to fish is pollution. There are many potential pollutants whose occurrence causes a reduction in the quality of the aquatic environment (Awachie, 1980). Contamination of the aquatic environment by petroleum and petroleum effluent, whether as a consequence of acute or chronic events constitutes an additional source of stress for aquatic organisms as noted by Sindermann (1982). Considerable data are available on the acute toxicity of various components of aqueous fractions of the crude oil on fish, however, only very few are available on the acute toxicity of the crude on fish. Crude oil from different parts of the world have been reported to vary in the various constituents, hence as reported by Eisler and Kissil (1975), have varying toxicological effects on fish.

Several toxic components of crude oil have been identified. According to Cote (1976), these include; saturated non-cyclic hydrocarbons (paraffins), saturated cyclic hydrocarbons (cycloalkanes), alkenes, aromatic hydrocarbons and heavy metals such as copper, chromium, cadmium, nickel, lead, zinc, etc. Individual components of petroleum has been reported by different authors to have varying toxicological effects on fish, death of fish have also been reported at various acute concentrations. Baker (1979) observed that the toxicity of refinery effluent is further complicated by the presence of hydrocarbon breakdown products, unidentified components and interactions among various components.

Contamination of the aquatic environment associated with normal shipping operations and with accidental spills is becoming on the increase world wide, hence the importance of this investigation. Specifically, we sought to test the acute toxicity of the water soluble fractions (WSF) of the Bonny Light crude oil on the Nile tilapia, Oreochromis niloticus. The Bonny Light crude oil is actively exported from Nigeria to various European countries. The Nile tilapia is a common aquatic fauna in the tropics hence its choice in this investigation.

MATERIALS AND METHODS

The soluble fractions of the crude oil were prepared in ten clean 50-litre glass reservoir. Methods of the preparation of soluble water fractions for toxicological

bioassays as described by Smith and Cameron (1979) and Omoregie and Ufodike (2000) were employed in this investigation. Each reservoir contained 45 litres of clean tap water upon, which was poured 500 ml of the crude oil to make a film about 0.5 cm thick. These solutions were mixed vigorously with stainless steel stirring rod for 24 hours and allowed to stand an additional 12 hours for the oil droplets to rise to the surface. These reservoirs remained tightly capped during the entire experimental period to minimize evaporation.

Static bioassays were employed during which periods the set-ups were continuously aerated. Five different concentrations of the water soluble fractions were prepared, each concentration had a replicate. Preliminary experimental test were carried out to determine suitable concentration of the water soluble fractions of the crude oil that will not result in an instant mortality of fish on exposure. The various concentrations used were in the following order: 10.00, 5.00, 2.50, 1.25, 0.625 and 0.00 (control) mL/L. In order to minimize evaporation during the exposure period, fresh preparations were introduced into the experimental media on a daily basis. The water physio-chemical parameters of the various experimental media were monitored at 24-hour intervals using methods described by APHA *et al.* (1980).

Ten fingerlings of O. niloticus (mean weight 9.84 ± 0.67 g and mean length 7.56 ± 0.41 cm) were introduced into each of the aquaria. The fish had previously been acclimated to laboratory conditions for a period of two weeks. During the acclimation period, fish were fed 5% of their body weight with pelleted diets once daily.

Methods of conducting acute bioassays as described by Sprague (1973) were employed for this investigation. The exposure period lasted for duration of 96 hours (4 days) during which period the fish were not fed. The fish were observed at 2-hour intervals for the higher concentrations until complete mortality occurred. At lower concentrations they were observed every 6 hours. Dead fish were immediately removed from the experimental set-up. Raw mortality data were analysed by probit analysis (Barr et al. 1979) to determine the concentration of the water soluble fraction of the crude oil that will result in 50% mortality of the fish. The 96-hour LC_{50} was determined as a graphical summary of the percentage mortality data. The opercular ventilation rate and tail fin beat frequencies per minute were read at the start of the exposure period and every 24 hours thereafter. The mean of three readings was used to plot histograms. In the case of the highest concentration where 100% mortality occurred before the 96 hours, these readings were not taken. Results obtained during the investigation were subjected to statistical analysis using the Duncan's multiple range tests at 95% level of significance.

RESULTS AND DISCUSSION

The mean values of mortality rate of the exposed fish are presented in Table 1. It was observed that during the exposure period, fish in the control experimental media survived the 96-hour exposure period. No mortality was observed in the group of

fish exposed to 0.625 mL/L within the first 48 hours. The mean 96-h LC50 of the fish was calculated to be 2.43 mL/L with upper and lower limits of 2.98 and 1.85 mL/L respectively. Results indicated that mortality rate of the exposed fish and the concentrations of the pollutant are positively related, in other words, mortality rates of the fish increased with increased in concentrations of the pollutant.

During the exposure period, the fish exhibited various behavioural patterns before death occurred. Restlessness, loss of balance (i.e. over turning), air gulping and convulsion were frequently observed. However, such behavioural responses were minimal in the groups exposed to 1.25 and 0.625 mL/L of the water soluble fractions, and were not observed in the control groups of fish.

Results of the opercular ventilation rates per minute of the exposed fish are presented in Table 2. Statistical analysis showed that the water soluble fractions led to an initial significant increase ($P < 0.05$) in the opercular ventilation rate of the fish exposed to 10.00, 5.00, 2.50 and 1.25 mL/L compared to the groups of fish exposed to 0.625 and 0.00 mL/L. The increase was observed to be directly proportional to the pollutant concentrations. By the 48th hour of exposure, the rate of opercular ventilation was reduced to the status quo in all the concentrations. By the 96th hour, the opercular ventilation rates of the groups of fish exposed to 10.00, 5.00, 2.50 and 1.25 mL/L had decreased significantly ($P < 0.05$) below those of the fish exposed to 0.625 and 0.00 mL/L. The opercular ventilation rates of the control groups of fish were statistically the same ($P > 0.05$) throughout the exposure period.

Results of the tail fin beat frequencies per minute of the exposed fish are presented in Table 3. Statistical analysis showed that the water soluble fractions led to an initial significant increase ($P < 0.05$) in the tail fin beat frequencies of the fish exposed to 10.00, 5.00 and 2.50 mL/L compared to the groups of fish exposed to 1.25, 0.625 and 0.00 mL/L. The increase was observed to be directly proportional to the pollutant concentrations. By the 24th hour of exposure, the initial increase had reduced to the status quo. By this time, there was no significant difference ($P > 0.05$) in the tail fin beat frequencies of the exposed fish. By the 96th hour, the tail fin beat frequencies of the groups of fish exposed to 10.00, 5.00, 2.50 and 1.25 mL/L had decreased significantly ($P < 0.05$) below those of the fish exposed to 0.625 and 0.00 mL/L. The tail fin beat frequencies of the control groups of fish were statistically the same ($P > 0.05$) throughout the exposure period.

The free carbon dioxide, dissolved oxygen, total alkalinity, temperature and pH of the experimental media were observed to be maintained within the following ranges: 4.60 – 4.87 mg/L, 5.95 – 7.52 mg/L, 22.93 – 23.90 mg/L, 16.01 – 16.40^{O}C and 6.02 – 6.97 respectively.

It is generally acknowledged that the lethal properties of petroleum are modified or lost with time under aerobic conditions (Eisler and Kissil, 1975), however, the exact nature of the toxicological mechanism in crude oil is imperfectly understood. It is known however, that low boiling and high boiling point saturated and aromatic

hydrocarbons occur in every crude oil, and though their numbers may approach the thousands, individual members of the series have similar chemical and biological properties (Blumer, 1972). It is generally agreed that the water soluble phenolic compounds are the most toxic, closely followed by the volatile aromatic compounds. Cote (1976) noted that the non-hydrocarbon compounds of crude oil closely resemble the corresponding aromatic compounds in their effects on survival and metabolism of aquatic animals.

The LC_{50} value of 2.43 mL/L reported in this investigation is somewhat similar to what was reported by Eisler and Kissil (1975) for the juvenile rabbit fish, Siganus rivulatus when exposed to the Iranian crude oil. These authors reported a value of 2.10 mL/L for this fish, though they also reported LC_{50} value of 17.5 mL/L for the same fish when exposed to the Sinai crude oil. La-Roche et al. (1970) reported LC_{50} value of 8.20 and 16.50 mL/L for the mummichog fish, Fundulu heteroclitus exposed to the West Texas crude oil and the Langunillas crude oil respectively. The differences between the LC_{50} values reported in this investigation and those of these other workers could be attributed to the different species of fish used and the natural composition of the various crude. UNEP (1990) noted that the water soluble fractions of various crude oil varied depending on their natural composition.

Restlessness, loss of balance, air gulping and convulsion observed in this investigation are in agreement with earlier reports of Wise et al. (1987), De-Silva and Ranasinghe (1989), Ufodike and Omoregie (1990) and Okwuosa and Omoregie (1995) when they exposed fish to concentrations of various toxicants. These behavioural responses are indications of death due to nervous disorders and insufficient gaseous exchange across the gill epithelia.

Table 1. Mortality of Oreochromis niloticus exposed to various concentrations of water soluble fractions of the Bonny light crude oil for 96 hours.

Conc. (mL/L)	Log Conc. (mL/L)	Mean mortality in 2 replicates				Mean Mortality (%)	Mean Probit Mortality
		24 Hours	48 Hours	72 Hours	96 Hours		
10.00	1.000	3.5	2.0	2.0	1.0	85	6.036
5.00	0.699	2.0	1.5	1.0	1.0	55	5.126
2.50	0.399	1.5	1.0	2.0	1.0	55	5.126
1.25	0.097	0.5	1.0	2.0	-	35	4.615
0.625	-0.204	-	-	0.5	2.0	25	4.323
0.00	0.000	-	-	-	-	-	0.000

The initial hyperventilation of the opercular and increase in tail fin beat frequencies in this investigation suggest that O. niloticus in an environment polluted by crude oil tends to exhibit avoidance syndrome as earlier reported by Ufodike and Omoregie (1990) and Okwuosa and Omoregie (1995). As the fish swam faster so as to escape from the toxic area, the tail fin beat increased. The

operculum also beat faster as the fish needed more oxygen for metabolism. However, as the exposure period increased, the fish became fatigued, hence the subsequent drop in the opercular ventilation and tail fin beat. The combined effects of this fatigue and the direct effects of the crude oil on the exposed fish led to subsequent death. The role played by fatigue is being reported here to be very important in acute toxicity test of aquatic organisms exposed to crude oil. The opercular ventilation rates and tail fin beat frequencies are therefore important physiological parameters in the measurement of stress in fish, which could be used to evaluate the sensitivity of fish species to oil pollution.

Table 2. Opercular ventilation rates per minute* of Oreochromis niloticus exposed to various concentrations of water soluble fractions of the Bonny light crude oil for 96 hours.

Conc.	Opercular ventilation rates per minute in 2 replicates				
(mL/L)	Start	24 Hours	48 Hours	72 Hours	96 Hours
10.00	132 ± 10.25^{a}	105 ± 5.21^{b}	85 ± 5.20^{c}	72 ± 6.02^{d}	65 ± 5.02^{e}
5.00	126 ± 9.05^{a}	106 ± 6.24^{b}	81 ± 10.00^{c}	75 ± 8.05^{cd}	68 ± 4.36^{d}
2.50	108 ± 10.00^{a}	98 ± 3.20^{b}	80 ± 5.64^{c}	75 ± 5.28^{cd}	70 ± 10.00^{d}
1.25	100 ± 11.62^{a}	95 ± 8.10^{b}	80 ± 5.55^{c}	78 ± 4.10^{c}	75 ± 5.55^{c}
0.625	92 ± 8.05^{a}	86 ± 8.05^{ab}	78 ± 8.00^{b}	79 ± 10.05^{b}	80 ± 8.02^{b}
0.00	88 ± 2.00^{a}	84 ± 6.21^{a}	82 ± 2.12^{a}	80 ± 4.21^{a}	81 ± 3.02^{a}

*Values in the same row with same superscripts are significantly the same ($P > 0.05$)

Table 3: Opercular ventilation rates per minute* of Oreochromis niloticus exposed to various concentrations of water soluble fractions of the Bonny light crude oil for 96 hours.

Conc.	Opercular ventilation rates per minute in 2 replicates				
(mL/L)	Start	24 Hours	48 Hours	72 Hours	96 Hours
10.00	121 ± 5.25^{a}	70 ± 4.20^{b}	69 ± 2.50^{b}	58 ± 6.12^{c}	56 ± 1.51^{c}
5.00	115 ± 5.15^{a}	73 ± 2.22^{b}	71 ± 2.04^{b}	59 ± 4.25^{c}	56 ± 2.16^{c}
2.50	108 ± 6.00^{a}	74 ± 1.80^{b}	72 ± 3.14^{b}	60 ± 3.28^{c}	59 ± 1.09^{c}
1.25	85 ± 1.60^{a}	71 ± 3.19^{b}	72 ± 4.51^{b}	63 ± 3.15^{b}	60 ± 2.50^{c}
0.625	71 ± 5.05^{a}	70 ± 2.05^{a}	70 ± 3.05^{b}	62 ± 1.02^{b}	62 ± 2.04^{b}
0.00	69 ± 2.05^{a}	71 ± 1.21^{a}	69 ± 1.12^{a}	68 ± 0.23^{a}	69 ± 1.04^{a}

*Values in the same row with same superscripts are significantly the same ($P > 0.05$)

The importance of crude oil in the world economy can not be over emphasized. However, contamination of the aquatic environment with crude oil and its products should be prevented at all cost, the clean up exercise of those already contaminated should be taken very seriously by those concerned. The major fisheries of the world are concentrated in areas of high biological productivity such as the waters. In some parts of the world, the continental shelf is shared with offshore petroleum industries, a situation already becoming widespread as interest in offshore petroleum production activity increases.

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