

TOXICITY, GROWTH AND SURVIVAL OF *Clarias gariepinus* JUVENILES EXPOSED TO DIFFERENT CONCENTRATIONS OF CRUDE OIL FRACTIONS-POLLUTED WATER

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ABSTRACT

Studies were carried out on the toxicity, growth and survival of Clarias gariepinus juveniles exposed to different concentrations of oil-polluted water. Thirty-nine aerated aquaria (60 × 30 × 30 cm³), arranged in a 4 × 3 Complete Randomized Block Design were used for the study. Three oil types: the Bonny light crude oil (BLCO), the premium motor spirit (PMS) and kerosene (DPK) at oil concentrations of 1.00, 1.50, 2.00 and 2.50 ml L⁻¹ were used in triplicates of 5 ml to contaminate 15 L of dechlorinated tap water and 20 fingerlings of Clarias gariepinus (22 ± 0.24 g) exposed to it. A control treatment (0.00 ml L⁻¹) of non-oil contamination was also used in triplicates. A 96-hour toxicity phase in the oil-polluted water preceded a 42 days recovery phase. 38% crude protein diet was fed to fish during exposure and recovery phases at 3% and 5% body weight per day, respectively. Water temperature, pH, fish mortality and normalized biomass index (NBI) of each aquaria were monitored. The total organic nitrogen, soluble organic nitrogen and colloidal organic nitrogen in addition to soluble and adsorbed ammonia in the aquaria water and sediments were analyzed using standard methods. Results showed that the water temperature was 26 ± 2.04° C, pH was 6.50 ± 0.30 and fortnightly feed intake of fish increased between days 14 and 42. This increase, which corresponded with the increase in the fortnightly weight gain, could be attributed to the reduction of stress caused during the 96-h toxicity phase. The increase in the soluble ammonium and the exchangeable ammonium concentrations of water correlated with the increase in the concentrations (1.50 – 2.50 ml L⁻¹) of BLCO, PMS and DPK. Percent mortality of fish reduced between days 14 and 42 irrespective of oil treatment while fish exposed to the control treatment had lower percent mortality than those exposed to the oil treatments. This trend was corroborated by the relatively higher NBI for the control during the exposure (-0.02) and recovery {0.08 (14 days), 0.08 (38 days) and 0.21 (42 days)} periods than those of oil treatments (-49.64 to -0.10).

Keywords: *Clarias gariepinus*, Toxicity, Soluble ammonium, Feed intake, Weight gain

INTRODUCTION

Akingbade (1991) recorded varying levels of petroleum hydrocarbons in the body organs of fishes, frog and snails resulting from over 3000 cases of oil spillages and release of about 2.4 million barrels of crude oil that had taken place in the Nigerian coastal environment. Concentrations of crude oil and fuel (0.05 - 10 ml L⁻¹) toxic to fish eggs and fingerlings have been studied (Lonning, 1977).

Freshwater fish are used as 96-hour bioassay organisms (Kopperdau, 1976) for the determination of crude oil toxicity. Many workers (Stobber *et al.*, 1978; Cardwell, 1979) have reported on the toxicity resulting from oil spills that occurred in aquatic environments near big oil industries and stated that fish larvae, fingerlings and eggs are quite sensitive bioassay test organisms. *Clarias gariepinus* Burchell, 1822 in the Nigerian waters is a highly esteemed hardy fish due to possession of accessory air-breathing organs which enable it to tolerate

diverse aquatic conditions (Reed *et al.*, 1967). *C. gariepinus* fry and fingerlings may be nonetheless very delicate and sensitive to aquatic pollutants including crude oil and its products.

Ammonia toxicity is reported to be one of the common causes of death of fish during rearing (Hampson, 1976). He also stated that nitrogen is generally excreted directly as ammonia without detoxicative metabolism, though some may be excreted as trimethylamine oxide, urea, uric acid or creatine. Ammonia concentrations in water depend on equilibrium between rate of production, exchange in water by flow-through in open or partially open systems and oxidative conversion to nitrite and nitrate by bacterial activity (Schulze-Weinhenbrauck, 1974). The mechanism of ammonia toxicity is by high ammonia concentrations in the blood resulting from failure of ammonia excretion or its uptake from the water at the surface membranes particularly at the gills (Hampson, 1975). The unionized ammonia (NH₃) is the form which can pass readily through cell

membranes (Hampson, 1976). This form is readily soluble in the lipid segments of the membrane and apparently needs no active transport, while the ionic forms occur as large hydrated and charged entities which cannot readily pass through charged micropores of the hydrophobic membrane components. The toxicity of ammonia is thus extremely dependent on conditions in the water which affect the equilibrium: $\text{NH}_4 + \text{H}_2\text{O} \leftrightarrow \text{NH}_3 + \text{H}_3\text{O}^+$. This equilibrium is drastically changed by variations in hydrogen ion concentration, temperature and ionic concentration (salinity) of the water.

Anderson *et al.* (1974) noted that an understanding of the effects of crude oil on ammonia toxicity, growth, feeding energetics and swimming activity of fingerlings is needed to assess the impact of oil pollution on fish production. Although the uptake of crude oil fractions and its components from water is very rapid and bioaccumulations if they are not metabolized do occur, much is not known about what happens to these compounds within the fish (Stageman and Sabo, 1976). Although fish has oxidative enzymes for metabolic detoxification of xenobiotics including aromatic petroleum hydrocarbons (Payne and Penrose, 1975), little is known about the metabolism of crude oil compounds in juveniles of *C. gariepinus*. In Nigeria, work has been done on the effect of different concentrations of Bonny-light crude oil on the mortality rate of *Heterobranchus bidorsalis* (Nwamba *et al.*, 2001). Also, working with *Oreochromis niloticus*, exposed to diesel, Dede and Kaglo (2001) suggested that death of the tilapia fingerlings might be related to decrease dissolved oxygen content in water due to presence of diesel.

Against this background, the toxicity, growth and survival of *C. gariepinus* juveniles in oil product-polluted water were studied. Criteria for assessment included: total organic nitrogen, soluble organic nitrogen, colloidal organic nitrogen, soluble ammonium, exchangeable ammonium, concentrations and their effects on feed intake, weight gain and normalized biomass index.

MATERIALS AND METHODS

Seven hundred and eighty (780) juveniles of *C. gariepinus* (22 ± 0.24 g) were purchased from a private fish hatchery at Otor-Oweh in Isoko North L. G. A., Delta State, Nigeria and conveyed to Ebonyi State University, Abakaliki. The movement of fish was done with five 25-litre plastic containers, while the water in the containers were aerated with a New Generation Bell portable aerator (Model PAT-NO49-83537), energized by a 6.0 volt motorcycle battery. To avoid undue stress arising from high temperatures, ice cubes were added at regular intervals to water containing fish during transportation. At the Fisheries Research Laboratory in Abakaliki, the fish were acclimatized for 14 days on a maintenance ration of chick starter diets fed at 3% body weight per day (bw d^{-1}). A 38% crude protein diet was formulated from locally available ingredients

(Table 1) and pelleted with a locally fabricated pelletizer. The feed was oven-dried at 60°C for 3 hours and preserved in a pest-free cupboard within the laboratory.

Table 1: Gross and Proximate Composition of Diets Fed to the African catfish (*Clarias gariepinus*) juveniles

Feed ingredient	Percent Composition
<i>Yellow maize</i>	9.81
<i>Soyabean meal</i>	54.76
<i>Fish meal</i>	16.43
<i>Blood meal</i>	10.95
<i>Palm oil</i>	5.00
<i>Salt</i>	0.25
<i>Vitamin mix</i> ¹	0.60
<i>Mineral mix</i> ²	0.40
Total	100.00
Nutrient	
<i>Crude protein (CP)</i>	34.88
<i>Ether extract (EE)</i>	4.44
<i>Ash (AS)</i>	11.08
<i>Dry matter (DM)</i>	8.23
<i>Nitrogen-free extract (NFE)</i>	41.87

¹Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): Thiamin, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; pantothenic acid, 40; choline chloride, 3000; niacin, 150; vitamin B₁₂, 0.06; retinyl acetate (500,000IU/g), 6; menadione-Na-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alpha tocopherol, 50; cholecalciferol (1,000,000 IU/g); ethoxyquin, 2.0.

²Contained as g/kg of premix: FeSO₄.7H₂O, 5; MgSO₄.7H₂O, 132; K₂SO₄, 329.90; KI, 0.15; Na₂Cl₂, 45; Na₂SO₄, 44.88; AlCl₃, 0.15; CoCl₂.6H₂O, 5; CuSO₄.5H₂O, 5; NaSeO₃, 0.11; MnSO₄.H₂O, 0.7, and cellulose, 380.97.

Thirty-nine aerator-equipped, transparent plastic aquaria (60 × 30 × 30 cm³) were arranged to accommodate 4 treatments (T₁, T₂, T₃ and T₄) each of Bonny-light crude oil (BLCO), premium motor spirit (PMS) and kerosene (DPK) at oil concentrations of 1.00, 1.50, 2.00 and 2.50 ml L⁻¹ per treatment in a 4 × 3 Complete Randomized Block Design (CRBD). A control experiment (T₅) had no oil treatment (0.00 ml L⁻¹). 5 ml of each of the oil concentrations (1.00 - 2.50 ml L⁻¹) were introduced in triplicates to 15 litres of water contained in each of the 36 aquaria while 3 aquaria (controls) had no oil treatments. Each of the 36 aquaria was randomly stocked with 20 juveniles of *C. gariepinus*. This fish were fed the formulated diet at 3% body weight per day (bw d^{-1}) for 96 hours during the toxicity phase of the study and later 5% bw d^{-1} for 42 days during the recovery phase. Fish mortality/survival was monitored during the toxicity phase of 96 hours at intervals of ½, 1, 2, 4, 8, 16, 32, 48 and 96 hours. The oil-treated water in all the 36 aquaria were discarded after 96 hours and the surviving fish rinsed several times in clean dechlorinated tap water. The recovery phase commenced immediately after the aquaria had been refilled with 15 litres of fresh water. Mortality/survival of fish was also monitored at fortnightly intervals within 42 days (i.e. 14, 28 and 42 days). The water temperature and pH were monitored with the aid of a maximum-minimum

thermometer and a pH meter (Model PH-1-201), respectively.

Laboratory analyses of nitrogen and ammonia in the water and sediments of each aquarium were carried out after 4 days and on a fortnightly basis. The soluble organic, colloidal organic and total organic nitrogen were determined by the method of Avnimelech and Lacher (1979). Soluble and absorbed ammonia extracted from water with potassium sulphate (K_2SO_4) solution were analyzed colorimetrically (Solorzano, 1969).

The four days and fortnightly fish survivals were estimated via the normalized biomass index (NBI) thus: $NBI = \{(W_F \times N_F)\} \{W_I \times N_I\} \times 1/100$ (Beck, 1979), where, W_F = final weight of fish, N_F = final number of fish, W_I = initial weight of fish, N_I = initial number of fish. The fortnightly feed intake (FFI) was determined from the equation: $FFI = \text{feed intake per day} \times 14$, while the fortnightly weight gain (FWG) was calculated thus: $FWG = \text{final fortnightly weight gain} - \text{initial fortnightly weight gain}$. The analysis of variance (ANOVA) was used to establish statistical differences between treatment means and Duncan's Multiple Range Test to partition the means (Steel and Torrie, 1990).

RESULTS

The water temperature was $26 \pm 2.04^\circ C$ and pH was 6.50 ± 0.30 throughout the study period. The results of the feed intake of *C. gariepinus* juveniles exposed to the crude oil and its products for 4 days and during recovery for 42 days are shown in Table 2. The fish consumed less feed as the concentrations of the three oil treatments increased from T_1 (1.00 ml L^{-1}) to T_4 (2.50 ml L^{-1}). Fish under the control treatment, T_5 (0.00 ml L^{-1}) consumed higher quantity of feed ($1.66 \pm 0.18 \text{ g}$) than those exposed to the crude oil fractions. The pattern of feed intake within the first 14 days of recovery indicated that less but not significantly different ($P > 0.05$) feed was consumed by fish exposed to BLCO concentrations of 1.00 ml L^{-1} (T_1), 2.00 ml L^{-1} (T_3) and 2.50 ml L^{-1} (T_4) than those exposed to the corresponding PMS and DPK concentrations (Table 2). Fish under the control treatment, 0.00 ml L^{-1} (T_5) consumed more diet than those exposed to the various concentrations of the three oil types (BLCO, PMS and DPK).

At day 28, the fish exposed to 2.00 ml L^{-1} and 2.50 ml L^{-1} BLCO concentrations still consumed comparatively less feed (3.84 ± 0.12 and $3.79 \pm 0.22 \text{ g}$ respectively) than those exposed to PMS (4.76 ± 0.06 and $4.79 \pm 0.05 \text{ g}$ respectively) and DPK (4.86 ± 0.13 and $5.60 \pm 0.174 \text{ g}$ respectively). The control fish at days 28 and 42 still consumed comparatively higher quantity of feed than those under oil treatments. At day 42, however, the fish exposed to DPK fed less ($5.99 \pm 0.21 \text{ g}$) (1.00 ml L^{-1}), ($4.87 \pm 0.20 \text{ g}$) (1.50 ml L^{-1}) and ($6.97 \pm 0.21 \text{ g}$) (2.00 ml L^{-1}) than those exposed to BLCO and PMS. Generally, the *C. gariepinus* juveniles exhibited significant differences ($P < 0.05$) in their responses to feed intake when recovering from BLCO, PMS and DPK exposures within day 14 and 28 recovery periods

and highly significant different ($P < 0.01$) responses within 42 days recovery period (Table 2).

Table 3 shows the weight gain of fish within 4 days oil exposure period and the fortnightly weight gain (FWG) within 42 days recovery period. At day 14, fishes exposed to 1.00 ml L^{-1} concentration of BLCO, PMS or DPK showed better weight gain (0.02 ± 0.001 , 0.03 ± 0.001 and $0.03 \pm 0.002 \text{ g}$, respectively) than those exposed to higher concentrations (1.50 - 2.50 ml L^{-1}) of the oil types. However, fishes under the control treatment (0.00 ml L^{-1}) had better weight ($0.04 \pm 0.001 \text{ g}$) than those exposed to the oil treatments. The FWG of fish within the first 14 days increased in accordance with the increasing concentrations of oil (1.00 - 2.50 ml L^{-1}) exposed to the fish. Hence, FWG decreased from T_1 (1.00 ml L^{-1}) i.e. $\{1.48 \pm 0.02 \text{ g}$ (BLCO), $1.45 \pm 0.02 \text{ g}$ (PMS) and $1.48 \pm 0.03 \text{ g}$ (DPK)}. The weight gain recorded with the control ($1.74 \pm 0.03 \text{ g}$) was also higher than those recorded with fish subjected to the oil treatments (Table 3). In addition, the fish had better weight while recovering from the DPK-treated water than from the PMS- or the BLCO-treated water (Table 3). The same trend in weight gain was demonstrated by the fish at 28 and 42 days recovery periods. There were significant differences ($P < 0.05$) in the FWG of *C. gariepinus* juveniles as they recovered from exposures to BLCO, PMS and DPK at days 14, 28 and 42 (Table 3).

Tables 4 show the nitrogen and ammonium concentrations of water in which *C. gariepinus* juveniles were exposed (4 days) and recovered from their exposures (42 days) to concentrations of BLCO, PMS and DPK. The total organic nitrogen (TON), the soluble organic nitrogen (SON), the colloidal organic nitrogen (CON), the soluble ammonium (SA) and the exchangeable ammonium (EA) in water (Table 4) increased in accordance with the increasing concentrations of crude oil fractions to which the fishes were exposed.

The control experiment had lower concentrations of TON, SON, CON, SA and EA than those of the oil-treated water (Tables 4a and 4b). The DPK-treated water had comparatively lower values TON, SON, CON, SA and EA irrespective of the exposed concentrations (Table 4).

Table 5 shows the percent mortality and the normalized biomass index (NBI) of fish within the toxicity (4 days) and the recovery (42 days) phases of the study. The NBI estimated the magnitude of growth and survival of fish within the study period. At day 4, fish percent mortality was least in the DPK-treated water (25.00%) than in the PMS-treated (27.00%) and the BLCO-treated (30.00%) waters. These results were corroborated by the lower NBI values recorded with fish exposed to BLCO (-49.64) and PMS (-49.40): relative to DPK (-13.31) (Table 5). Fish of the control experiment had a better NBI value (-0.02) than those subjected to the oil treatments. The trend exhibited by fish during the toxicity phase (4 days), with regard to percent mortality and NBI values, was reflected during the fortnightly recovery phase (42 days). At days 14, 28 and 42, fish recovering from exposure to the DPK-treated water

Table 2: Mean feed intake (g) of *Clarias gariepinus* juveniles within 4 days exposure to oil fractions and within 42 days recovery period recorded at fortnightly intervals¹

Experimental phase	Period (days)	Oil type	Treatment (ml L ⁻¹ crude oil fraction)				
			T ₁ (1.00)	T ₂ (1.50)	T ₃ (2.00)	T ₄ (2.50)	T ₅ (0.00)
Exposure	4	BLCO	1.56 ± 0.10 ^a	1.54 ± 0.10 ^a	1.50 ± 0.10 ^a	1.47 ± 0.10 ^a	4.42 ± 0.12 ^b
		PMS	1.58 ± 0.12 ^a	1.55 ± 0.08 ^a	1.52 ± 0.11 ^a	1.49 ± 0.13 ^a	
		DPK	1.61 ± 0.11 ^a	1.58 ± 0.10 ^a	1.56 ± 0.10 ^a	1.53 ± 0.11 ^a	
Recovery	14	BLCO	3.40 ± 0.12 ^a	3.74 ± 0.10 ^b	3.27 ± 0.11 ^a	3.31 ± 0.11 ^a	4.42 ± 0.20 ^d
		PMS	3.44 ± 0.13 ^a	3.10 ± 0.06 ^b	3.78 ± 0.10 ^c	3.51 ± 0.12 ^a	
		DPK	3.64 ± 0.14 ^a	3.06 ± 0.08 ^b	3.34 ± 0.11 ^{ab}	3.61 ± 0.12 ^d	
	28	BLCO	4.88 ± 0.21 ^a	4.03 ± 0.14 ^b	3.84 ± 0.12 ^b	3.79 ± 0.05 ^b	5.38 ± 0.11 ^e
		PMS	4.37 ± 0.04 ^a	4.44 ± 0.03 ^a	4.76 ± 0.06 ^b	4.79 ± 0.22 ^a	
		DPK	4.50 ± 0.14 ^a	3.62 ± 0.16 ^b	4.86 ± 0.13 ^c	5.60 ± 0.17 ^d	
	42	BLCO	8.35 ± 0.21 ^a	10.00 ± 0.13 ^b	5.21 ± 0.32 ^c	6.14 ± 0.22 ^d	12.14 ± 0.22 ^e
		PMS	6.77 ± 0.20 ^a	7.03 ± 0.14 ^b	19.44 ± 0.30 ^c	5.92 ± 0.32 ^d	
		DPK	5.99 ± 0.21 ^a	4.87 ± 0.20 ^b	6.97 ± 0.21 ^c	8.68 ± 0.30 ^d	

¹BLCO = Bonny light crude oil, PMS = Premium motor spirit, DPK = Kerosene, T₁ – T₅ = Treatments, T₅ = Control treatment. ²Means follow by the same superscript in the same row are not significantly different ($P > 0.05$).

Table 3: Mean weight gain (g) of *Clarias gariepinus* juveniles within 4 days exposure to oil fractions and within 42 days recovery period recorded at fortnightly intervals^{1,2}

Phase	Period (days)	Oil type	Treatment (ml L ⁻¹ crude oil or crude oil product)				
			T ₁ (1.00)	T ₂ (1.50)	T ₃ (2.00)	T ₄ (2.50)	T ₅ (0.00)
Exposure	4	BLCO	0.02 ± 0.001 ^a	0.01 ± 0.002 ^b	0.02 ± 0.002 ^a	0.01 ± 0.001 ^b	0.04 ± 0.001 ^d
		PMS	0.03 ± 0.001 ^a	0.02 ± 0.001 ^b	0.02 ± 0.001 ^b	0.02 ± 0.002 ^b	
		DPK	0.03 ± 0.002 ^a	0.01 ± 0.001 ^b	0.02 ± 0.00 ^c	0.02 ± 0.001 ^c	
Recovery	14	BLCO	1.48 ± 0.01 ^a	1.45 ± 0.02 ^a	1.41 ± 0.01 ^b	1.32 ± 0.02 ^c	1.74 ± 0.03 ^d
		PMS	1.60 ± 0.04 ^a	1.57 ± 0.03 ^a	1.50 ± 0.02 ^b	1.45 ± 0.02 ^b	
		DPK	1.64 ± 0.04 ^a	1.59 ± 0.03 ^{ab}	1.53 ± 0.04 ^{bc}	1.48 ± 0.03 ^c	
	28	BLCO	1.72 ± 0.02 ^a	1.67 ± 0.01 ^b	1.63 ± 0.03 ^b	1.56 ± 0.02 ^c	1.96 ± 0.03 ^d
		PMS	1.80 ± 0.04 ^a	1.76 ± 0.04 ^{ab}	1.71 ± 0.03 ^b	1.63 ± 0.02 ^c	
		DPK	1.81 ± 0.03 ^a	1.76 ± 0.04 ^b	1.69 ± 0.04 ^b	1.61 ± 0.03 ^c	
	42	BLCO	1.71 ± 0.03 ^a	1.67 ± 0.02 ^a	1.62 ± 0.03 ^{ab}	1.57 ± 0.02 ^b	1.86 ± 0.03 ^d
		PMS	1.76 ± 0.02 ^a	1.73 ± 0.03 ^a	1.67 ± 0.03 ^b	1.59 ± .02 ^c	
		DPK	1.48 ± 0.14 ^a	1.41 ± 0.04 ^{ab}	1.35 ± 0.04 ^b	1.28 ± 0.00 ^c	

¹BLCO = Bonny light crude oil, PMS = Premium motor spirit, DPK = Kerosene, T₁ – T₅ = Treatments, T₅ = Control treatment. ²Means followed by different superscript in the same row are significantly different ($P < 0.05$).

Table 4: Nitrogen and ammonium concentration of crude oil- and crude oil product-treated water stocked with *Clarias gariepinus* juveniles within 4 days exposure and 42 recovery period^{1, 2, 3}

Water parameter ²	Oil type	Treatment (ml L ⁻¹ crude oil or crude oil product)				
		T ₁ (1.00)	T ₂ (1.50)	T ₃ (2.00)	T ₄ (2.50)	T ₅ (0.00)
4 days exposure period						
Total organic nitrogen (TON) mgN L ⁻¹	BLCO	0.42 ± 0.03 ^a	0.46 ± 0.02 ^{ab}	0.52 ± 0.03 ^b	0.61 ± 0.05 ^{ac}	0.20 ± 0.03 ^d
	PMS	0.44 ± 0.04 ^a	0.51 ± 0.03 ^{ab}	0.58 ± 0.04 ^b	0.66 ± 0.06 ^c	
	DPK	0.20 ± 0.01 ^d	0.24 ± 0.01 ^a	0.35 ± 0.01 ^b	0.43 ± 0.0 ^c	
Soluble organic nitrogen (SON) µgN L ⁻¹	BLCO	5.53 ± 0.21 ^a	5.62 ± 0.21 ^a	5.83 ± 0.22 ^a	5.92 ± 0.31 ^a	0.26 ± 0.02 ^b
	PMS	6.62 ± 0.63 ^b	6.71 ± 0.56 ^b	8.75 ± 0.63 ^b	9.12 ± 0.72 ^b	
	DPK	0.26 ± 0.03 ^b	0.31 ± 0.02 ^a	0.37 ± 0.04 ^a	0.46 ± 0.01 ^a	
Colloidal organic nitrogen (CON) µgN L ⁻¹	BLCO	83.52 ± 4.10 ^a	91.60 ± 4.02 ^b	94.21 ± 4.17 ^b	96.74 ± 2.10 ^b	8.77 ± 4.11 ^c
	PMS	76.13 ± 3.22 ^a	78.54 ± 3.33 ^a	70.71 ± 3.13 ^a	74.61 ± 4.32 ^a	
	DPK	8.42 ± 0.72 ^c	9.62 ± 0.03 ^a	10.12 ± 0.14 ^b	11.27 ± 0.52 ^c	
Soluble ammonia (SA) µgNH ₄ L ⁻¹	BLCO	15.08 ± 1.15 ^a	15.06 ± 1.44 ^a	16.06 ± 1.21 ^a	16.36 ± 1.13 ^a	3.09 ± 0.22 ^c
	PMS	17.03 ± 2.21 ^a	17.08 ± 2.10 ^a	17.23 ± 2.20 ^a	17.28 ± 1.24 ^b	
	DPK	4.06 ± 0.03 ^a	4.02 ± 0.01 ^a	4.06 ± 0.31 ^a	5.23 ± 0.10 ^b	
Exchangeable ammonia (EA) µgNH ₄ L ⁻¹	BLCO	44.62 ± 1.40 ^a	45.61 ± 2.41 ^a	46.72 ± 2.22 ^a	48.46 ± 3.12 ^a	4.61 ± 0.06 ^d
	PMS	50.43 ± 1.32 ^a	53.47 ± 2.62 ^{ab}	55.24 ± 2.36 ^{bc}	58.13 ± 2.29 ^c	
	DPK	5.03 ± 0.35 ^a	5.69 ± 0.24 ^{ab}	6.77 ± 0.42 ^{bc}	6.27 ± 0.32 ^c	
42 days recovery period						
Total organic nitrogen (TON) mgN L ⁻¹	BLCO	3.10 ± 0.63 ^a	3.70 ± 0.31 ^{ab}	4.40 ± 0.30 ^b	5.10 ± 0.41 ^{bc}	1.01 ± 0.22 ^d
	PMS	3.40 ± 0.62 ^a	4.01 ± 0.53 ^{ab}	4.43 ± 0.40 ^b	5.24 ± 0.42 ^c	
	DPK	1.11 ± 0.20 ^d	1.71 ± 0.15 ^a	2.43 ± 0.25 ^b	3.13 ± 0.20 ^c	
Soluble organic nitrogen (SON) µgN L ⁻¹	BLCO	46.20 ± 2.01 ^a	46.80 ± 0.21 ^{ab}	47.50 ± 1.12 ^b	48.22 ± 1.12 ^{bc}	1.06 ± 0.11 ^d
	PMS	77.15 ± 8.10 ^a	77.70 ± 6.20 ^a	78.40 ± 7.02 ^a	79.11 ± 6.11 ^a	
	DPK	1.35 ± 0.20 ^d	2.05 ± 0.22 ^a	2.80 ± 0.13 ^b	3.52 ± 0.21 ^c	
Colloidal organic nitrogen (CON) µgN L ⁻¹	BLCO	925.42 ± 40.04 ^a	926.02 ± 41.03 ^a	926.72 ± 30.16 ^a	927.42 ± 21.04 ^a	75.57 ± 31.01 ^c
	PMS	671.31 ± 22.02 ^a	673.01 ± 23.03 ^b	673.81 ± 20.02 ^a	674.53 ± 11.14 ^b	
	DPK	94.72 ± 1.10 ^a	95.32 ± 0.81 ^a	96.21 ± 0.51 ^a	97.11 ± 1.23 ^a	
Soluble ammonia (SA) µgNH ₄ L ⁻¹	BLCO	149.18 ± 40.16 ^a	149.88 ± 44.05 ^a	150.58 ± 42.22 ^a	151.28 ± 43.02 ^a	29.14 ± 11.10 ^b
	PMS	163.22 ± 10.20 ^a	163.90 ± 11.12 ^a	164.63 ± 10.30 ^a	163.34 ± 11.14 ^a	
	DPK	34.53 ± 12.01 ^b	35.13 ± 9.11 ^b	35.83 ± 11.01 ^b	36.53 ± 10.11 ^b	
Exchangeable ammonia (EA) µgNH ₄ L ⁻¹	BLCO	345.23 ± 13.00 ^a	346.14 ± 11.01 ^a	346.94 ± 10.20 ^a	347.64 ± 12.12 ^a	32.50 ± 14.07 ^b
	PMS	441.11 ± 14.07 ^b	441.73 ± 15.06 ^b	443.43 ± 16.08 ^b	444.13 ± 13.08 ^b	
	DPK	48.57 ± 0.70 ^a	49.17 ± 0.52 ^a	49.87 ± 0.61 ^a	50.57 ± 0.60 ^a	

Table 5: Percent mortality and normalized biomass index of *Clarias gariepinus* juveniles during 4 days of exposure to crude oil fractions and 42 days of recovery

Experimental period	Duration (days)	Oil type ¹	Initial number of juveniles ^{3, 4}	Number of dead juveniles	Number of survivors	Percent mortality	Normalized biomass index
Exposure period ⁵	4.00	BLCO	240.00	72.00	168.00	30.00	-49.64
		PMS	240.00	65.00	175.00	27.00	-49.40
		DPK	240.00	60.00	180.00	25.00	-13.31
		Control	60.00	1.00	59.00	2.00	-0.02
Recovery period of 42 days ⁶	14.00	BLCO	168.00	31.00	137.00	18.11	-0.52
		PMS	175.0	26.00	149.00	15.00	-0.33
		DPK	180.00	23.00	157.00	13.00	-0.27
		Control	59.00	1.00	58.00	1.00	0.08
	28.00	BLCO	137.00	22.00	115.00	16.00	-0.35
		PMS	149.00	18.00	121.00	12.00	-0.57
		DPK	157.00	14.00	143.00	9.00	-0.27
		Control	58.00	1.00	57.00	1.00	0.08
	42.00	BLCO	115.00	14.00	101.00	12.00	-0.17
		PMS	121.00	10.00	111.00	8.00	-0.10
		DPK	143.00	9.00	134.00	6.00	-0.04
		Control	57.00	0.00	57.00	0.00	0.21

¹BLCO = Bony light crude oil, PMS = Premium motor spirit, DPK = Kerosene. ²Concentrations of oil types used = 1.00, 1.50, 2.00 and 2.50 ml L⁻¹; ³Initial number of juveniles derived from four treatments x 3 replicates x 20 juveniles = 240; ⁴Initial number of juveniles in the control is derived from 3 replicates x 20 juveniles = 60 juveniles; ⁵Four-day summations of fish mortality/survivals in the oil types and Control were adopted; ⁶Fortnightly summations of fish mortality/survivals were adopted.

had lower percent mortality and higher NBI values than those recovering from BLCO and PMS exposures (Table 5). Fish of the control experiment expectedly died less and survived better than those exposed to the oil treatments.

DISCUSSION

The decrease in the feed consumption by fish in the first 4 days (toxicity phase) with increasing concentrations of BLCO, PMS and DPK (Table 2), indicated that feed intake was affected by oil concentrations in water. Irrespective of the BLCO concentrations (1.00 - 2.50 ml L⁻¹), a similar trend of increase in the fortnightly feed intake (FFI) of fish (Table 2) existed between days 14 and 42. This result indicated that fish response to feed intake improved with time. This improvement was also exemplified by the fish exposed to the various concentrations of PMS and DPK (Table 2). The quantity of feed consumed by the fish during the recovery phase (42 days) was generally higher than that consumed during the toxicity phase (4 days) (Table 2). This implied that the presence of crude oil fractions in water affected the quantity of food consumed by the fish. In addition, there were consistent increases in the quantity of feed consumed by the fish as the recovery period progressed from day 14 to day 42. Lagler *et al.* (1977) reported that the increased food consumption relative to increasing size and age may be due to the interaction of factors affecting internal motivation or drive for feeding, such as: season, temperature, time and nature of last feeding, food stimuli perceived by the senses, lateral line system, hunger, curiosity and gluttony. The increase in the sizes of fish (mean weight gain)

between days 14 and 28 of the recovery period (Table 3) might have been accompanied by the development of more senses to perceive food stimuli in accordance with the report of Lagler *et al.* (1977).

Fish under the control treatment, T₅ (0.00 ml L⁻¹) apparently fed better on forth nightly basis than those subjected to oil pollution (Table 2). This result further indicated that the contamination of water with crude oil fractions affected food consumption by fish. This result was consistent with the report of Hunt and Linn (1990) who reported a reduced feed intake by *Clarias* sp. exposed to 1.50 ml L⁻¹ of an agro-chemical, carbynl (1-naphthyl methyl carbonate) solution.

The values of the weight gain for fish treated with the various concentrations of BLCO, PMS and DPK (Table 3) followed the pattern exhibited by the feed intake of fish. This result implied that there was a concomitant increase in weight with time (days 14 to 42) as the fish recovered. Jauncey (1982) reported a similar weight increase with time for juvenile *Sarotherodon mossambicus* fed eight isoenergetic diets with protein levels ranging from 0% to 56%.

The increases in the values of the soluble ammonium (SA) and the exchangeable ammonium (EA) of water with increasing concentrations of BLCO, PMS and DPK during the toxicity phase (Table 4), corresponded with the increases in SA and EA values recorded during the recovery phase (Table 4). The comparatively higher values of SA and EA recorded during the recovery phase (42 days) than those recorded during the toxicity phase (4 days) may be due to copious excretion of SA and EA into water by *C. gariepinus* juveniles. The values of SA and EA in the control treatment were comparatively lower than those of the oil-polluted treatment (Table 4).

This result was in agreement with Avnimelech and Lacher (1979).

The higher percent mortality (30.00%) and lower NBI (-49.64) values of fish when exposed to BLCO at day 4 than when recovering from this exposure at day 14 (18.00%; -0.52), day 28 (16.00%; -0.35) and day 42 (12.00%; -0.17) (Table 5) indicated that fish mortality and the propensity to survive increased with the period the fish was allowed to recover from oil pollution. The range values of SA ($149.18 \pm 40.16 - 151.28 \pm 43.02 \mu\text{g NH}_4 \text{L}^{-1}$) and EA ($345.23 \pm 13.00 - 347.64 \pm 12.12 \mu\text{g NH}_4 \text{L}^{-1}$) for BLCO treated water alone (Table 4) implied that these range values of SA and EA could cause fish mortality of between 12.00% and 18.00% within 42 days (Table 5). The range values of the growth and survival index (NBI) of fish recovering from the BLCO exposure (-0.52 to - 0.17) (Table 5) corroborated the range values of fish mortality (12.00-18.00%) already indicated. Hampson (1976) had earlier reported that ammonia toxicity was one of the common causes of fish mortality during rearing. The stress factor occasioned by oil pollution must have retarded the incorporation of nitrogen in the fish into fish flesh and facilitated its conversion to ammonia. In his study, ammonia must have entered the water through the metabolic and excretory mechanism of fish or by bacterial action in the water (Hampson, 1976).

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