Studies on the Plasma Albumin and Globulin Contents of the African Catfish (*Heterobranchus bidorsalis*) Adult Injected with Graded Concentrations of Bonny-Light Crude Oil

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ABSTRACT

The plasma albumin (ALB) and globulin (GLB) concentration of the plasma of *Heterobranchus bidorsalis* adults (mean weight, 138.32 ± 0.36 g) injected with graded concentrations of Bonny-light crude oil (BLCO) were studied. Two study periods viz: the toxicity (4 days) and the recovery (42 days) periods were adopted. Significant increases ($P < 0.05$) in the ALB content (mg/100ml) of the plasma were recorded with fish injected with 10.00 – 50.00 microlitre per gram ($\mu\text{L.g}^{-1}$) BLCO. Conversely, significant decreases ($P < 0.05$) in the GLB content (mg/100ml) of the plasma were also recorded. These results implied that increases in BLCO concentrations within the fish might have impacted for both ALB and GLB on the synthesis of these globular proteins and by extension on protein turn over in the Plasma. The 50.00 $\mu\text{L.g}^{-1}$ BLCO concentration in the fish gave the highest value of ALB, during both study period. The 10.00 $\mu\text{L.g}^{-1}$ BLCO concentration recorded the least values of GLB in the fish. This result also suggested that negative responses of ALB and GLB proteins to the presence of the crude oil compound were utmost at these BLCO concentrations. However, increases in ALB and GLB values during the recovery period attested to the apparent relief of the fish from the toxic and lethal effects of the oil pollutant. Fish mortality recorded within both study periods is consistent with the report of other workers that pollutants induced stress and perturbations in the functional integrity of the physiological processes in fishes, culminating in mortality.

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INTRODUCTION

The low survival rates of the giant African catfish (*Heterobranchus bidorsalis*, Geoffroy St. Hilaire, 1809) juveniles in the Nigeria Aquaculture system is a disturbing phenomenon. Part of the reasons for the low survivals include cannibalism (Ugwu *et al.*, 2006), nutritional problem (Faturoti *et al.*, 1986; Nwosu *et al.*, 2009, Okeke *et al.*, 2016; Okeke *et al.*, 2016 and Nwosu, 2017) and the environment (Ozmen *et al.*, 2005). Freshwater fish are used as 96 - hour bioassay test 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, fingerling and eggs are quite sensitive bioassay test organisms. *H. bidorsalis* in the Nigeria waters is a highly esteemed hardy fish due to the possession of accessory air-

breathing organs which, enable it tolerate diverse aquatic conditions (Reed *et al.*, 1967). *H. bidorsalis* adults and juveniles nevertheless may be very sensitive to aquatic pollutants including crude oil and its products.

The effect of xenobiotic contamination in an aquatic ecosystem can be estimated through analysis of biochemical changes in the organisms inhabiting that region (Tuvikene *et al.*, 1996; Norris *et al.*, 2001; Brewer *et al.*, 2001). Some deleterious effects of exposing fish species to crude oil have been determined to include: alteration of the immune response mechanisms, changes in liver metabolism, haemorrhage and even death (Neff and Anderson, 1981). The aquatic ecosystem, like the terrestrial environment, is continuously subjected to changes in quality following the introduction of substances of diverse characteristic arising from man's cultural activities (Oluah, 2001). This author stated that alteration in water quality usually predispose the

fish to stress and disease, and elicit quick responses in the physiology of the fish, especially the haematological parameters.

The need to determine the effect of crude oil infiltration into the blood stream of fish species has been expressed and the procedure of bio-chemical analyses has been elected as the best option (Tuvikene *et al.*, 1996). It has also been suggested that the uptake and translocation of crude oil compounds in fish may be through the gills, the gut and the intestinal walls (Roubal *et al.*, 1977). The parent compounds of crude oil were found to solubilize in the cell membranes and were carried via the erythrocytes to the general circulation of the blood. However, the specific individual metabolites that result from the uptake and metabolic conversion of petroleum aromatic compounds (ACs) (Oriji and Zakka, 2014). In fish have not been well characterized (Khan *et al.*, 1992 & 1995; Origi and Zakka, 2014). Crude oil exposures of adult marine fish species have been reported to increase the mortality rate and changes in the haemoglobin content of blood (Tatem *et al.*, 1979).

The damage done to the physiological processes in fish by crude oil compounds is likely to commence in the blood. Since the translocation of crude oil compounds goes via the erythrocytes to the general circulation of the blood (Roubal *et al.*, 1977), the tendency that the blood components would be depleted is certain. The albumin and globulin components of the plasma are globular proteins that occur in the blood of animals, including fish (McDonald *et al.*, 1995). These protein especially the albumin, are water soluble and heat-coagulable and therefore could be adversely affected by crude oil compounds. Against this background, this study was designed to investigate the effect of injecting *H. bidorsalis* adults with graded concentrations of Bonny-light crude oil (BLCO) on the albumin and globulin contents of the blood. The essence was to ascertain the degree of physiological damage done to these fish blood proteins consequent upon the infiltration of crude oil compounds into the general circulation of the blood.

MATERIALS AND METHODS

Eighteen (18) plastic containers (25-litres capacity) were randomly stocked with 360 adults of *H. bidorsalis* [mean weight $\pm$ standard error of mean (s.e.m), $138.22 \pm 0.36g$] at 20 fish per container. The experiment was designed to have 18 plastic containers (6 groups each having 3 containers) with 15 litres dechlorinated tap water and fish. The fish were injected with graded concentrations of BLCO at 10.00, 20.00, 30.00, 40.00, and 50.00 $\mu g l^{-1}$. Three (3) containers had fish not injected with crude oil served as the controls (0.00 $\mu g l^{-1}$). The injection of fish was done with 2.50ml disposable hydrodermic syringes, just below the dorsal fin.

Two study periods were adopted for the research viz: the toxicity and the recovery periods. Four (4) days was adopted as the toxicity period of the injected BLCO concentration in the fish since freshwater fish are used as 96 - hours bioassay test organisms for the determination of crude oil toxicity (Kopperdaul, 1976). The recovery period lasted for 42days and was monitored fortnightly (14 days interval). At the end of the toxicity period, the surviving fish and plastic containers

were washed and replenished with 15 litres dechlorinated tap water. A previously formulated fish diet (38% crude protein) (Table 2) was fed to the fish at 5% body weight per day (bwd-1) during the toxicity period and at 5% bwd-1 during the recovery period. The proximate composition of the diet (Table 3) was carried out as described by AOAC (1990).

Records of the water temperature ($27 \pm 0.12^{\circ}C$) and pH (6.76 $\pm$ 0.4) were taken with the aid of a maximum and minimum mercury-in-glass thermometer and a pH meter (Model pH-L 201-L) respectively. The percent mortality (PM) and the percent survival (PS) of the fish were estimated during the toxicity and recovery periods of the study. Blood samples of each triplicate treatment of BLCO and the control were collected during each study period. Ethylenediaminetetraacetic acid (EDTA) fluid was used to condition the syringes, needles and microfuge tubes before blood collection. Analysis of the albumin and globulin protein of the blood was carried out within 12 hours at Bronilla Diagnostic Laboratory, Abakpa Nike, Enugu, Nigeria. Here, the blood was centrifuged for 15 minutes at 1000rpm to obtain the plasma.

The albumin content of the blood was determined by the addition of 3.30ml disodium tetraoxosulphate (Na_2SO_4) to 0.40ml blood serum in a centrifuge tube with stopper. The serum and reagent were thoroughly mixed by inversion. 3.00ml of this mixture was transferred to a microfuge tube and further mixed with 1.00ml of ether analar. This was also mixed by inverting the tube 40 times. The tube was capped and centrifuged for 15 minutes at 1000rpm. The tube was then sloped in order to transfer 1.00ml of the lower clear aqueous phase to another tube marked A (Albumin). The upper portion of this mixture was introduced into another tube marked G (Globulin). This constituted the globulin component of the blood serum. Globulin is normally separated by disodium tetraoxo sulphate and is estimated by difference. For the blank test, 1.00ml of Na_2SO_4 was placed in a 300ml tube and marked B (Blank).

To each tube A and B, 4.00ml of biuret reagent comprising: 45.00g Rochelle salt, 2N NaOH and 15.00g $CuSO_4$ were introduced, mixed thoroughly, and allowed to stand for 30 minutes at room temperature. The final mixtures in tubes A and B were read in a colorimeter at 540 nanometer (nm). The protein standard assay of the blood serum was carried out without the biuret reagent and this was also read in a colorimeter at 540nm. The colour produced by the serum albumin protein with the biuret reagent was estimated and compared with the protein solution of known strength. Estimation of the serum albumin in each triplicate sample was carried out as follows:

$$\frac{gAlbumin}{100ml} = \frac{Albumin\ value}{Standard\ value} \times \frac{Concentration\ of\ standard}{1}$$

Where: the Albumin value was the colorimeter reading when the blood serum, Na_2SO_4 biuret reagent and ether analar were used. While the standard value was when the blood serum, Na_2SO_4 and distilled water (as blank) were used.

The statistical Programme was SPSS. All the data obtained were subjected to Analysis of Variance (ANOVA), least significant difference (LSD) and testing at 1% and 5% level

of significance to determine statistical differences between treatment means (Steel and Torrie, 1990). The simple percentages were also used to explain the analyzed data where appropriate.

RESULTS AND DISCUSSION

The physio-chemical properties of Bonny-light crude oil are present in Table 1. The gross and proximate compositions of the experimental diet fed to *H. bidorsalis* adults during the study period are shown in Tables 2 and 3 respectively. Tables 4 and 5 show the albumin (ALB) and globulin (GLB) contents of the blood respectively for fish injected with 10.00 - 50.00 μg^{-1} BLCO and the control (0.00 μg^{-1}). Table 6 shows the percentage mortality and survival of the fish.

The control fish recorded significantly ($P < 0.01$) higher values of ALB in the blood than in those injected with the different BLCO concentrations (Table 4). This situation was prevalent both at the toxicity and the recovery periods of the study. ALB values also increased significantly ($P < 0.05$) with increasing BLCO concentration in the fish during both study periods (Table 2). The recovery period of the fish (14 - 42 days) provided comparatively higher values of ALB in fish than what were obtained during the toxicity period. Generally, improvements in ALB values during the recovery period indicated that ALB values increased by 5% at day 14, 10% at day 28 and 18% at day 42. Nevertheless, the highest ALB value ($5.32 \pm 0.03 \text{ mg}/100\text{ml}$) recorded on day 42 with the fish recovering from 50.00 μg^{-1} BLCO injection was lower than the corresponding ALB value of the control fish ($5.99 \pm 0.03 \text{ mg}/100\text{ml}$) (Table 4).

The result of the globulin content of fish blood recorded in this study was apparently at variance with the values recorded for the albumin content. Whereas the ALB values increased with increasing BLCO concentrations, the globulin (GLB) values decreased (Table 5). Significant decreases ($P < 0.05$) in GLB values were therefore recorded and the range values of GLB in the control fish (2.53 ± 0.04 - $3.46 \pm 0.04 \text{ mg}/100\text{ml}$) were higher than those injected with BLCO concentrations (Table 5). Improvements in the values of GLB in the fish blood followed the pattern shown by the ALB values. Hence, as the recovery period extended from days 14 and 28 to day 42 the GLB values increased by 5, 10 and 18% respectively.

The percent mortality and survival of the fish (Table 6) indicated that those injected with 40.00 μg^{-1} and 50.00 μg^{-1} BLCO recorded the highest fish mortality and the lowest fish survivals. In contrast, those injected with 10.00 μg^{-1}

BLCO recorded the least percent mortality and the highest percent survival (Table 6).

Improvements in the values of albumin (ALB) and globulin (GLB) contents of the blood serum of *H. bidorsalis* adults during the recovery period of this study implied that the toxic effects of the injected BLCO was being reduced with time. It could be that the percent increases in ALB and GLB values at day 14 (5%), day 28 (10%) and day 42 (18%) were evidences of improved enzymatic synthesis due to protein accretion. Kaushik *et al.* (1995) reported that protein in the diet has an obligatory role of replacing lost body proteins as well as losses due to amino acid oxidation and utilization, for purposes other than protein synthesis and protein turn over. The improved feeding rate of the fishes during the recovery period must have provided the required amino acids for the synthesis of the lost globulins and albumins during the toxicity period.

The lower ALB values recorded in the injected fish at day 42 relative to the control implied that improvement in ALB values starting from day 14 up to day 42 did not guarantee any complete recovery of the fish from the toxic effect of BLCO. This result is consistent with the report of Khan *et al.* (1995), who recorded absorbed hydrocarbons (HCs) in minnows after 14 days of their transfer to uncontaminated sea water. Kennicutt and Sweet (1992) also detected spill-related contamination in intertidal limpet, *Nacella concinna* two years after the release of crude oil into Arthur Harbour in Antarctic Peninsula. It is deduced from this work that the low values of ALB in the fish blood relative to the control must be due to the deleterious effects caused by the presence of BLCO in the blood stream of the fish.

The decreasing GLB values due to the increasing BLCO concentration in the fish agrees with the report of Olua and Njoku (2001). The workers posited that the exposure of fishes to even sublethal concentrations of pollutants induces stress and perturbations in the functional integrity of their physiological processes. The exposure of life forms (including fish) to hazardous chemicals (e.g. pesticides, crude oil and agro-chemicals) causes cell injury and death of even non-target organisms (Sotheiton, 1991; Moreby *et al.*, 2001). The high fish mortality and low fish survival recorded with fish injected with 4.00 - 50.00 μg^{-1} BLCO are consistent with the observations of the above authors and attest to the lethal effects of the oil pollutants on the fish. The percent mortality of the fish nonetheless decreased with low concentrations of the injected BLCO.

Table 1: Physico-chemical properties of Niger Delta crude oil

Crude oil brand	Specific gravity	API	Viscosity	Sulfur content	Pour point	Wax cont.	Asph vol.)	(% Acidity
Bonny light	0.8	8.4	4.09	0.14	23	3.8	0.05	0.65
Bonny medium	0.9	9	16.44	0.28	28	2.1	0.29	0.44
Faroados Blend	0.087	8.7	5.4	0.2	24	4.1	0.32	0.21
Excravos Blend	0.77	7.7	3.67	0.12	25	5.3	0.14	0.67
Brass Blend	0.75	0.75	2.24	0.1	20	6.8	0.14	0.45

Source: Oriji and Zakka (2014).

Table 2: Gross Composition of Experiment Diet

Ingredient	% composition
Yellow maize	9.29
Soyabean meal	54.84
Fishmeal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix ¹	0.60
Mineral mix ²	2.40
Total	100.00

¹Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): thiamin, 10; riboflavin, acid, 40; choline chloride, 3000; niacin, 150; vitamin B₁₂, 0.06; retinyl acetate (500,000 IU/g), 6; menadione-Na-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alpha tocopherol, 50; cholecalciferol (1000,000 IU/g).

²Contained as g/kg of premix: FeSO₄·7H₂O, 5; MgSO₄·7H₂O, 132; K₂SO₄, 329.90; KI, 0.15; MnSO₄·H₂O, 0.7; and cellulose, 380.97.

Table 3: Proximate Composition of Experimental Diets

Ingredient	% composition
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen free extract	36.46
Total	100.00

Table 4: Albumin Content (mg/100ml) of the Blood of *H. bidorsalis* Adults Injected with Different Concentration of Bonny-Light Crude Oil

Study period	Duration (days)	BLCO ¹ Concentration (μl.g ⁻¹)					Control 0.00	Overall mean $\bar{x}$
		10.00	20.00	30.00	40.00	50.00		
Toxicity period	4	1.26±0.02 ^a	1.51±0.02 ^a	2.71±0.04 ^b	3.25±0.03 ^c	3.90±0.04 ^c	4.40±0.03 ^d	2.84±0.02
Recovery period	14	1.32±0.01 ^a	1.59±0.02 ^a	2.85±0.02 ^b	3.39±0.04 ^c	4.10±0.04 ^d	4.62±0.02 ^d	2.98±0.03
	28	1.45±0.03 ^a	1.75±0.03 ^a	3.14±0.02 ^b	3.73±0.03 ^b	4.51±0.03 ^c	5.08±0.04 ^d	3.28±0.03
	42	1.71±0.01 ^a	2.07±0.02 ^b	3.71±0.04 ^c	4.40±0.06 ^d	5.32±0.03 ^e	5.99±0.03 ^e	3.53±0.04

Values in the same row with similar superscripts are not significantly different (p>0.05).

Table 5: Globulin Content (mg/100ml) of the blood of *H. bidorsalis* Adults Injected with Different Concentration of Bonny-Light Crude Oil

Study period	Duration (days)	BLCO ¹ Concentration (μl.g ⁻¹)					Control 0.00	Overall mean $\bar{x}$
		10.00	20.00	30.00	40.00	50.00		
Toxicity period	4	0.63±0.02 ^a	0.41±0.01 ^a	0.27±0.01 ^b	0.18±0.01 ^c	0.01±0.00 ^d	2.53±0.04 ^e	0.67±0.01
Recovery period	14	0.66±0.02 ^a	0.43±0.02 ^a	0.28±0.02 ^b	0.10±0.01 ^c	0.01±0.00 ^d	2.66±0.03 ^e	0.71±0.02
	28	0.73±0.02 ^a	0.47±0.01 ^a	0.31±0.01 ^b	0.21±0.01 ^c	0.01±0.00 ^d	2.93±0.03 ^e	0.78±0.01
	42	0.86±0.03 ^a	0.47±0.01 ^b	0.37±0.02 ^c	0.25±0.01 ^d	0.01±0.00 ^e	3.46±0.04 ^f	0.90±0.02

Values in the same row with similar superscripts are not significantly different (p>0.05).

Table 6: Percent Mortality and Percent Survival of *H. bidorsalis* Adults Injected with Different Concentration of Bonny-Light Crude Oil

Study period	Duration (days)	% Mortality											
		BLCO ¹ Concentration (μl.g ⁻¹)					Control 0.00	BLCO ¹ Concentration (μl.g ⁻¹)					Control 0.00
		10.00	20.00	30.00	40.00	50.00		10.00	20.00	30.00	40.00	50.00	
Toxicity period	4	2.00	5.00	5.00	40.00	50.00	0.00	98.00	95.00	95.00	60.00	50.00	100.00
Recovery period	14	2.00	3.00	4.00	32.00	40.00	0.00	98.00	97.00	96.00	68.00	60.00	100.00
	28	1.00	2.00	2.00	36.00	36.00	0.00	99.00	98.00	98.00	76.00	64.00	100.00
	42	0.00	1.00	16.0	26.00	26.00	0.00	100.00	99.00	99.00	84.00	74.00	100.00

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