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Acid Phosphate Concentration of the Blood of Adult *Heterobranchus bidorsalis* Injected with Graded Micro Concentrations of Bonny-Light Crude Oil

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Abstract: The Acid Phosphate Concentration (APC) of the blood serum of adult *Heterobranchus bidorsalis* (mean weight, 138.24±0.16 g) injected with graded concentrations of Bonny-Light Crude Oil (BLCO) was studied. Two study spanned through 2 periods, the chronic toxicity (4 days) and the recovery (42 days) periods. Significant decreases ($p<0.01$) in the acid phosphate concentration (g 100 mL^{-1}) were recorded in the blood serum as BLCO concentrations in the fish increased from 10.00 to 50.00 μL g^{-1}. Fish samples injected with 10 μL g^{-1} BLCO recorded highest values of serum acid phosphate than those injected with 20.00-50.00 μL g^{-1} BLCO. This situation was observed both at the chronic toxicity and the recovery periods. This result implies that an increase in BLCO infiltration into the fish blood might have impacted more negatively on the interaction of acid phosphate with the serum globular proteins (albumins and globulins) (enzymes) resulting in a reduction in APC values. Recorded increases in APC values during the recovery period suggested that the fish blood was apparently relieved of the toxic and lethal effects of the crude oil pollutant. The highest percent mortality recorded with the fish injected with 40.00 and 50.00 μL g^{-1} BLCO is consistent with the observations of other authors that pollutants induce stress and perturbations in the functional integrity of the physiological processes in fish, culminating in mortality.

Key words: *Heterobranchus bidorsalis*, acid phosphate, bonny-light crude oil, blood serum, toxicity

INTRODUCTION

Low survival rate in aquaculture systems of juveniles of the cherished African catfish has been attributed to nutritional problem (Faturoti *et al*., 1986) the environment (Ozmen *et al*., 2005) and cannibalism (Ugwu *et al*., 2006a). In addition, adults and juveniles of these hardy fish species have been noted to be sensitive to such aquatic pollutants as cadmium (Oluah, 2001), paraquat (Oluah and Njoku, 2001) and crude oil (Ugwu *et al*., 2006b). The chronic toxicity of crude oil compounds to fish eggs, larvae and fingerlings, especially near big oil industries where oil spills are prevalent, has been reported (Cardwell, 1979).

The aquatic ecosystem, like the terrestrial environment is continuously being subjected to changes in quality following the introductions of substances of diverse characteristics arising from man's cultural activities (Oluah, 2001). This author posited that alterations in water quality usually predispose the fish to stress and disease and elicit quick physiological responses especially on the haemotological parameters. The potential utility of biomarker for monitoring both environmental quality and health of organisms inhabiting polluted ecosystems has received increasing attention in recent times (Lopes *et al*., 2001; Gauthier *et al*., 2004). It has been reported that the uptake and translocation of crude oil compounds in fish might be through the gills, the gut or the intestinal walls (Roubal *et al*., 1977) where the parent compounds solublize in the cell membrane and are carried via the erythrocytes to the general circulation of the blood.

This study presents the results of injecting graded micro concentrations of Bonny-light crude oil into the blood stream of adult *Heterobranchus bidorsalis*. The essence was to investigate the effect of this oil pollutant on the inorganic acid phosphate concentration in the blood as an indication of the status of its protein content.

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MATERIALS AND METHODS

Eighteen plastic containers (25 L capacity) were filled to 22 L mark with dechlorinated tap water and randomly stocked with 360 adults of *H. bidorsalis* (138.24±0.16 g) (mean weight±standard error of mean) at 20 fish per container. Fish in 15 plastic (5×3) containers were injected with 10.00, 20.00, 30.00, 40.00 and 50.00 μL g^{-1} Bonny-Light Crude Oil (BLCO). The injection of fish was done with 2.50 mL disposable hypodermic syringes, just below the dorsal fin. Fish in three plastic containers were not injected with crude oil (0.00 μL g^{-1}) and these served as the controls.

Two study phases were adopted for the research, viz: 4-day chronic toxicity phase and 42-day recovery phase. Four days was adopted as the chronic toxicity period of the injected BLCO pollutant concentrations as 96 h LC_{50} is generally assessed within 4 days. The recovery period lasted for 42 days and the fish was monitored fortnightly for acid phosphate concentration in the blood. At the end of the chronic toxicity period, the surviving fish and plastic containers were washed and the container replenished with 22 L of dechlorinated tap water. A formulated 38% crude protein fish diet (Table 1) was fed to the fish at 3% body weight per day (bw d^{-1}) during the two periods. The proximate composition of the diet was carried out as described by Windham (1996). Records of the water temperature and pH 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 determined during the chronic toxicity and recovered periods.

Blood samples of fish from each triplicate treatment of BLCO and the control were collected with hypodermic syringes from the dorso-anterior musculature below the dorsal fin. Blood sampling was done at day 4 (for the chronic toxicity period) and at days 14, 28 and 42 (for the recovery period). Anti-coagulant (EDTA) fluid was used to condition the syringes, needles and microfuge tubes before blood collection. Analysis of the acid phosphate concentration in each sample was carried out within 12 h at the Bronilla Diagnostic Laboratory, Abakpa-Nike, Enugu, Nigeria. Here, the blood was centrifuged at 1000 rpm for 15 min to obtain the serum.

Laboratory assay for the Acid Phosphate Concentration (APC) of the blood serum was carried out with the following chemical: 10 N tetraoxosulphate (IV) acid (H_2SO_4), ammonium molybdate solution, metol (P-dimethylamino phenol sulphate) and a standard phosphate (KH_2PO_4) solution. The protein content of the blood serum of each triplicate treatment of BLCO was removed by adding 9.00 mL of 10% trichloroacetic acid to 1.00 mL serum contained in a microfuge tube and both were mixed thoroughly by inversion. The mixture was subsequently centrifuged at 1000 rpm for 15 min.

Table 1: Gross composition and proximate composition of experimental diet fed to adult *Heterobranchus bidorsalis*

Ingredients	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
Nutrient	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Moisture	11.30
Nitrogen free extract	35.46
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose (mg kg^{-1} of diet): Thiamin, 10; riboflavin, 20; pyridoxine, 10; folacin, 5; Pantothenic acid, 40; choline chloride, 3000; niacin, 150; vitamin B_{12}, 0.06; menadione-N-bisulphate, 80; inositol, 400; biotin, 2; vitamin C, 200; alpha tocopherol, 50; cholecalcipherol (1000,000 g^{-1}) and retinyl acetate (500,000 iu g^{-1}), 6 [2]Contained as g kg^{-1} of premix: $FeSO_4 \cdot 7H_2O$, 5; $MgSO_4 \cdot 7H_2O$, 132; K_2SO_4, 329.90; KI, 0.15; $MnSO_4 \cdot H_2O$, 0.7; and cellulose, 380.97

The supernatant of each triplicate treatment was distributed into 3 test tubes (3.00 mL each) for the acid phosphate assay. The principle underlying this assay was that the supernatant obtained from removing the protein present in the blood serum (by means of trichloroacetic acid) was treated with an acid molybdate reagent. This reacted with inorganic phosphate to form phosphomolybdic acid. This hexavalent molybdenum of the phosphomolybdic acid was reduced by a metal to give a blue compound estimated colorimetrically (Windham, 1996). Three replicate test tubes each were used to carry out the actual test, the standard test and the blank test as follows:

For the actual test: Five militer supernatant fluid, 1.00 mL ammonium molybdate and 1.00 mL metal were placed in three replicate test tubes. For the standard test, 5.00 mL standard phosphate, 1.00 mL ammonium molybdate and 1.00 mL metol were used, while 5.00 mL trichloroacetic acid, 1.00 mL ammonium molybdate and 1.00 mL metol were used for the blank test. All the mixtures for the actual, standard and blank test were thorough and were allowed to stand for 30 min. Subsequently, the mixtures were read in colorimeter at 680 nanometers (nm).

Calculations of the acid phosphate concentrations in the blood serum were carried out as follows:

$$\text{mg } 100\text{ mL}^{-1}\text{ APC} = \frac{\text{Total value}}{\text{Standard value}} \times 0.025 \times \frac{100}{0.50}$$

$$= \frac{\text{Total value}}{\text{Standard value}} \times \frac{5}{1}$$

Where, the total value was the colorimeter reading when the supernatant, ammonium molybdate and metol were used for the assay and the standard value was the colorimeter reading when the standard phosphate, ammonium molybdate and metol were used for the assay.

The Analysis of Variance (ANOVA) was used to determine statistical differences between treatment means ($p<0.05$) (Steel and Torrie, 1980). Simple percentages were also used to explain the analyzed data were appropriate.

RESULTS

Table 1 shows the gross and proximate compositions of the experimental diet fed to *H. bidorsalis* adults during the study periods. Table 2 shows the APC (g 100 mL^{-1}) of the blood of the fish injected with 10.00-50.00 µL g^{-1} BLCO. The water temperature was 26±0.3°C and the pH was 6.82±0.02. Table 3 shows the percent mortality and percent survival of the fish. The control fish recorded significantly ($p<0.01$) higher values of APC in the blood than those injected with the different concentrations of BLCO (Table 2). This situation was prevalent even during the 42 days recovery period of the study (Table 2). Nevertheless, the recovery period of the fish (14-42 days) provided comparatively higher values of APC in the fish blood than what was obtained during the chronic toxicity period (Table 2). Generally, increases in the APC values of the fish blood during the recovery period were recorded at 5% (day 14), 12% (day 28) and 19% (day 42). Nonetheless, the highest APC value (2.46±0.04 g 100 mL^{-1}) (Table 2) recorded at day 42 with the fish recovering form 10.00 µL g^{-1} BLCO injection was lower than the corresponding APC values of the control fish (4.93±0.04 g 100 mL^{-1}).

This study also recorded significant decreases ($p<0.01$) in the APC values of the fish blood during the 4 days chronic toxicity period as the injected BLCO concentrations increased from 10.00 to 50.00 µL g^{-1} (Table 2). As was recorded during the recovery period, the APC value of the control fish (3.52±0.04 g 100 mL^{-1}) was higher than those of the contaminated fish. Hence, the control fish showed highest survival (100.00%) and nil mortality (0.00%) during the study (Table 3). Among the injected fish, however, those treated with 40.00 and 50.00 µL g^{-1} BLCO died more and survived less than those treated with between 10.00 and 30.00 µL g^{-1} BLCO (Table 3).

DISCUSSION

Increases in the values of Acid Phosphate Content (APC) of the blood in adults *H. bidorsalis* during the recovery period (Table 2) could be linked with the reduction in the toxic effect of BLCO with time. The declining toxic effect of BLCO between days 14 and 42 reduced the biochemical interactions between the globular blood proteins and the acid phosphate in the blood serum. The circumstance must have resulted in the availability of higher acid phosphate concentration in the blood of fishes during the recovery period than during the chronic toxicity period. This result is consistent with the report that pollutants generally predispose fish to stress and diseases and usually elicit quick responses in the physiology of the fish especially the haematological parameters (Oluah, 2001).

The damage done to the physiological processes in fish by crude oil compounds is therefore likely to commence in the blood since Roubal *et al.* (1977) stated that the translocation of crude oil compounds goes via the erythrocytes in the general circulation of the blood. Since the globular proteins, especially the albumins, are water-soluble and heat-coagulable

Table 2: Acid phosphate concentration g 100 mL^{-1}) of blood of *H. bidorsalis* adults injected with different concentrations of bonny-light crude oil

		BLCO[1] concentration (µL g^{-1})						
Study period	Duration (Days)	10.00	20.00	30.00	40.00	50.00	Control 0.00	Overall Mean ±
Toxicity period	4	1.76±0.03^{a}	0.88±0.02^{b}	0.57±0.01^{c}	0.37±0.01^{d}	0.24±0.01^{e}	3.52±0.04^{f}	1.22±0.02
Recovery period	14	1.85±0.04^{a}	0.92±0.02^{b}	0.60±0.02^{c}	0.39±0.01^{d}	0.25±0.01^{e}	3.70±0.03^{f}	1.29±0.03
	28	2.07±0.04^{a}	1.03±0.03^{b}	0.67±0.03^{c}	0.44±0.02^{d}	0.28±0.02^{e}	4.14±0.05^{f}	1.44±0.03
	42	2.46±0.04^{a}	1.23±0.03^{b}	0.79±0.02^{c}	0.52±0.01^{d}	0.33±0.01^{e}	4.93±0.04^{f}	1.71±0.03

[1]Bonny-Light crude oil, Numbers in the same row with different superscripts differ significantly ($p<0.01$)

Table 3: Percent mortality and percent survival of *H. bidorsalis* adults injected with different concentrations of bonny-light crude oil within 4 days (Toxicity) and 42 days (Recovery) periods

		Mortality (%)						Survival (%)					
		BLCO[1] Concentration (µL g^{-1})						BLCO Concentration (µL g^{-1})					
Study period	Duration (days)	10.00	20.00	30.00	40.00	50.00	Control 0.00	10.00	20.00	30.00	40.00	50.00	Control 0.00
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	1.00	26.00	26.00	0.00	100.00	99.00	99.00	84.00	74.00	100.00

[1]Bonny-Light crude oil

(McDonald *et al*., 1995) it was possible that their biochemical interactions with the acid phosphate in the blood were jeopardized by the presence of potentially harmful Aromatic Compounds (ACs) in the crude oil (NRC, 1985). Additionally, the decreasing values of APC in the fish blood as the BLCO concentrations increased (Table 2) are consistent with the report that fish exposures to even sublethal concentrations of pollutants induce stress and perturbations in the functional integrity o the physiological processes in fishes. Sotherton (1991) posited that the exposure of life forms (including fish) to hazardous chemicals caused cell injury and death to even non-target organisms. This situation was exemplified in this study by the percent mortality recorded with virtually all the BLCO concentrations applied except the control (Table 3). The comparatively lower APC values recorded in fish injected with 40.00-50.00 μL g^{-1} (Table 2) than in those injected with 10.00-30.00 μL g^{-1} could be corroborated with the higher percent mortality recorded with fish injected with 40.00-50.00 μL g^{-1} than with 10.00-30.00 μL g^{-1} (Table 3).

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