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BLOOD ERYTHROCYTIC AND LEUCOCYTIC COMPONENTS OF *Heterobranchus bidorsalis* JUVENILES STOCKED IN WATER POLLUTED WITH CRUDE OIL FRACTIONS

[1]NWAMBA Helen Ogochukwu, [2]UGWU Lawrence Linus Chukwuma, [3]MGBENKA Bernard Obialo and [1]UKOKO Lucky

[1]Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Nigeria
[2]Department of Animal Production and Fisheries Management, Ebonyi State University, PMB 053, Abakaliki, Nigeria
[3]Department of Zoology, Fish Nutrient and Aquaculture Unit, University of Nigeria, Nsukka

Correspondence Author: Nwamba, H. O. Department of Applied Biology, Enugu State University of Science and Technology, Enugu, Nigeria. Email: honwamba@yahoo.com Phone: 2348036731557

ABSTRACT

The blood erythrocytic (RBC) and leucocytic (WBC) components of Heterobranchus bidorsalis juveniles exposed to different concentration of crude oil fractions were studied. Two study periods namely the toxicity and recovery periods were adopted. Lubricating engine oil (LBO), Bonny-light crude oil (BLCO), Kerosene (DPK) and premium motor spirit (PMS) were respectively applied at four concentrations 1.00, 2.00, 4.00 and 8.00 ml L^{-1},. Lower numbers of RBCs were recorded in fish samples exposed to the crude oil fractions than in the control fish. The comparatively high RBCs in fish during the 4 days toxicity period than during 42 days recovery periods is attributed to the greater destruction of RBCs during the toxicity period. The 4.00 ml L^{-1} LBO and BLCO concentration were apparently preferred by the fish to maintain a higher number of RBCs than with 1.00, 2.00, and 8.00 ml L^{-1} concentrations. Similarly, the 8.00 ml L^{-1} concentrations of DPK and PMS gave better values of RBCs than the 1.00, 2.00 and 4.00 ml L^{-1} concentrations. Reduced number WBCs in the fish blood during the toxicity period at concentrations of 1.00, 4.00 and 8.00 ml L^{-1} LBO; was recorded likely the phagocytic action of the WBCs.

Keywords: Heterobranchus bidorsalis, Erythrocyte, Leucocyte, Toxicity, Crude oil fractions

INTRODUCTION

Fish species are widely used to biologically monitor variations in environmental levels of anthropogenic pollutants (White *et al*., Flammarion, *et al*., 2002; Schmitt, 2004). The warm water fish such as the common carp was chosen as a test organism because it occupies a central position in freshwater food chain especially in inland water systems, which are situated near industrial areas and are influences by various xenobiotics (Schmitt, 2004).

Biomarkers are defined as physiological, biological, or histopathological alterations which occur in organisms as a result of exposure to environmental pollutants (Mayer *et al*., 2004). The potential utility of biomarkers for monitoring both environmental quality and health of organisms inhabiting polluted system has received increasing attention during recent years (Lopes *et al*., 2001; Smecka and Kempers, 2003; Gautheir *et al*., 2004). The effects of xenobiotics contamination in an ecosystem can be estimated through analysis of biochemical changes in organisms inhabiting the systems. (Norris *et al*., 2001; Brewer *et al*., 2001). The responses of aquatic organisms to pollution are noticed through expression of several key enzymes, especially those of biotransformation systems (Nwamba *et al*., 2006).

Heterobranchus bidorsalis juveniles are very delicate and sensitive to aquatic pollutants including crude oil and its fractions. An understanding of the effects of crude oil on development, growth, feeding energetic and swimming activity of fish is essential in assessing the impact of oil pollution on fish populations (Anderson *et al*., 1974). Although the uptake of crude oil and compounds from water is very rapid and bioaccumulations do occur, much is not known about what happens to these compounds within the fish (Stageman and Sabo, 1976). 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 *H. bidorsalis.* The high demand of the giant African catfish (*H. bidorsalis),* due to its good flavor, informs the need to investigate aspects of hematological parameters and survival of this choice fish species in polluted water. Owing to incessant oil spills in Nigeria, resulting in environmental degradation, deprivation and spoilage of valuable food fish, the need to study the impact of crude oil fractions on the hematology of *H. bidorsalis* becomes imperative. Therefore, this investigated the effect of the oil fractions on the blood erythrocytic and leucocytic components of *H. bidorsalis* juveniles. The aim was to ascertain the dynamics of these blood components in the face of crude oil pollution and the consequences of this xenobiotic contaminants on fish survival.

MATERIALS AND METHODS

Six hundred (600) juveniles of *Heterobranchus bidorsalis* Geoffrey St. Hilaire, 1809 (means weight, 13.07 + 0.26g) were purchased from Phinomar Fish farm, Emene Enugu, Nigeria and transported in five plastic containers (25l) to the Fisheries Laboratory, Enugu, State University of Science and Technology, Enugu. The fishes were acclimatized for 14 days on a 38% crude protein diet at 5% body weight per day ($bw.d^{-1}$). Subsequently, they were stoc0ked by a completely Randomized Block Design (CRBD) into 60 plastic containers (25l) with 24L of dechlorinated tap water at 10 fish per container.

Crude Oil Fractions: Four samples of crude oil fractions were used for the study namely; Bonny-light crude oil (BLCO), premium motor spirit (PMS), dual purpose kerosene (DPK) and lubricating engine oil (LBO). Aliquots of these four oil samples were introduced in triplicates to 48 plastic containers with fish at the rate of 1.00, 2.00, 4.00 and 8.00 $m1L^{-1}$: twelve (12) plastic containers with fish were not contained with any oil samples (0.00 mlL^{-1}) and were left as the controls.

Experimental Periods: Two experimental periods were adopted for this study namely; the toxicity period which lasted 4 days (96 hours) and the recovery period (42 days). At the end of the toxicity period, the surviving fish were introduced into unpolluted fresh water (clean tap water) to allow for the 42 days recovery period to commence. Blood samples were collected at 4 days (toxicity period) and on fortnightly basis for the 42 days recovery period. The fish were fed 38 % crude protein diet (Table 1) at the rates of 3 % $bw.d^{-1}$ for the toxicity period and 5 % $bw.d^{-1}$ for the recovery period. Records of the mortality/survival rates were taken, while the feeding and swimming activities of the fish were observed. The water temperature (25 ± 1.50° C) and pH (6.65 ± 0.05) were recorded with the aid of a maximum–minimum thermometer and a pH meter (Model ph-J-201-1) respectively.

Blood Samples: Blood samples of fish from each triplicate treatment of the crude oil fractions (BLCO, PMS, DPK and LBO) and the control were collected with the aid of 2.50 ml capacity syringes and hypodermal needles. The collection of blood was via the dorso-anterior musculature, just below the dorsal fin and around the operculum. Anti-coagulant (EDTA) fluid was used to condition the syringes and needles prior to blood collection. Analysis of the blood samples were done within 12 hours at the Bronilla Diagnostic Laboratory, Enugu, Nigeria.

At the laboratory, triplicate samples of blood from fish of each triplicate treatment of the crude oil fractions and control were subjected to analysis of their erythrocytic (red blood corpuscles) (RBC) and leucocytic (white blood corpuscles) (WBC) components with the aid of a high powered microscope (Model LS-3AC). Analysis of the RBC and WBC were done through the estimation of the number of each type of blood cells present in $1mm^3$ concentration of blood samples within 12 hours.

Statistical Analysis: The data obtained was subjected to analysis of variance to establish if there were significant differences among treatment means and partitioned with the Duncan's Multiple Range Test (Steel and Torrie, 1990).

RESULTS

Table 1 shows the gross and proximate composition of the experimental diet. The RBC counts in fish exposed to different concentrations of the crude oil fractions and control indicated that the numbers of RBCs in the control fish blood were higher than in those exposed to the crude oil fractions (Table 2) for both the toxicity and recovery periods of the study. 4.00 mlL^{-1} LBO and BLCO concentrations recorded higher numbers of blood RBCs during the toxicity period at 137 ± 6.00 (for BLCO) than the other oil concentrations (1.00, 2.00 and 8.00 mlL^{-1}) (Table 2).

Table 1: Gross and proximate composition of experimental diet

Feed Ingredient	%
Yellow maize	9.29
Soybean meal	54.84
Fish meal	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 extracts	5.68
Ash	10.48
Dry matter	11.80
Nitrogen free extract	32.84
Crude fibre	1.80

This trend was also exhibited by the fish during the 42 days recovery period; that is at days 14, 28, and 42. Similarly, the numbers of RBCs of fish exposed to 8.00 mlL^{-1} concentrations of DPK (1210 ± 6.00) and PMS (1400 ± 6.00) were higher than those exposed to the other oil concentration (1.00, 2.00 and 4.00 $m1L^{-1}$). This trend was also shown in both the toxicity and recovery periods of the study. Significantly, the exposure of the fish to 4.00 mlL^{-1} concentrations of LBO and PMS resulted in higher numbers of the blood RBC of the oil treated fish relative to the control (Table 2). Generally, the numbers of RBCs in *H. bidorsalis* blood at both the toxicity and recovery periods varied significantly ($p < 0.05$).

The blood WBCs in fish exposed to 2.00 $m1L^{-1}$ LBO concentration (7,900 ± 30.00 WBCs) were higher than the WBC values recorded for 1.00, 4.00 and 8.00 ml L^{-1} LBO concentrations during the 4 days toxicity period (Table 3). This trend was also demonstrated as the fish recovered from day 14 to day 42. The highest numbers of blood WBCs were also recorded in fish exposed to BLCO, DPK and PMS

Table 2: Red blood corpuscles (RBC) count of *H. bidorsalis* juveniles mm^{-3} concentration of blood sample within 12 hours of collection[1]

Period	Duration (days)	Crude oil fraction	Oil concentrations (m1 L^{-1})					Overall mean
			0.0 (Control)	1.00	2.00	4.00	8.00	
Toxicity	4	LBO	1280 ± 6.00^{e}	870 ± 5.00^{a}	780 ± 4.00^{b}	1370 ± 4.00^{c}	790 ± 4.00^{d}	1018±5.00
		BLCO	1280 ± 6.00^{e}	978 ±6.00^{a}	980 ± 5.00^{b}	1180 ± 5.00^{c}	610 ± 3.00^{c}	1005±5.00
		DPK	1280 ± 6.00^{e}	1190 ±7.00^{a}	980 ± 6.00^{b}	970 ± 5.00^{c}	1210 ± 6.00^{d}	1126±6.00
		PMS	1289 ± 7.00^{e}	820 ±4.00^{a}	1200 ± 7.00^{b}	1300 ± 7.00^{b}	1400 ± 6.00^{d}	1200±6.00
	14	LBO	1536 ± 8.00^{e}	1044 ± 6.00^{a}	936 ± 5.00^{b}	1644 ± 8.00^{c}	948 ± 5.00^{d}	1222±6.00
		BLCO	1538 ± 8.00^{d}	1171 ± 7.00^{a}	1176 ± 6.00^{a}	1416 ± 8.00^{b}	732 ± 3.00^{c}	1207±6.00
		DPK	1542 ± 9.00^{e}	1428 ± 8.00^{a}	1176 ± 6.00^{a}	1164 ± 6.00^{c}	1452 ± 7.00^{d}	1352±7.00
		PMS	1540 ± 8.00^{e}	984 ± 5.00^{a}	1440 ± 8.00^{b}	1560 ± 7.00^{c}	1680 ± 7.00^{d}	1441±7.00
Recovery	28	LBO	1690 ± 8.00^{d}	1149 ± 6.00^{a}	1030 ± 5.00^{b}	1808 ± 8.00^{c}	1043 ± 6.00^{a}	1344±7.00
		BLCO	1689 ± 7.00^{e}	1288 ± 7.00^{a}	1294 ± 6.00^{b}	1558 ± 7.00^{c}	805 ± 5.00^{c}	1327±6.00
		DPK	1696 ± 8.00^{d}	1571 ± 8.00^{a}	1294 ± 7.00^{b}	1280 ± 6.00^{b}	1579 ± 7.00^{c}	1488±7.00
		PMS	1694 ± 9.00^{e}	985 ± 6.00^{a}	1320 ± 7.00^{b}	1716 ± 8.00^{c}	1848 ± 8.00^{d}	1513±8.00
	42	LBO	17755 ±10.00^{e}	1207 ± 7.00^{a}	1032 ± 5.00^{b}	1898 ± 9.00^{c}	1095 ± 6.00^{d}	1411±8.00
		BLCO	1774 ± 9.00^{d}	1352 ± 7.00^{a}	1369 ± 6.00^{b}	1636 ± 7.00^{b}	845 ± 4.00^{c}	1393±6.00
		DPK	17815 ± 9.00^{e}	1650 ± 8.00^{a}	1289 ± 7.00^{b}	1344 ± 6.00^{c}	1677 ± 8.00^{d}	1564±8.00
		PMS	1779 ± 10.00^{e}	1034 ± 6.00^{a}	1386 ± 6.00^{b}	1802 ± 9.00^{c}	1940 ± 9.00^{d}	1588±8.00

[1]LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit. Means in the same row with different superscripts differ significantly (P < 0.01).

Table 3: White blood corpuscles (WBC) count of *H. bidorsalis* juveniles mm^{-3} concentration of blood sample within 12 hours of collection[1]

Period	Duration (days)	Crude oil fraction	Oil concentrations (m1 L^{-1})					Overall mean
			0.0 (Control)	1.00	2.00	4.00	8.00	
Toxicity	4	LBO	1,500 ± 8.00^{e}	7.700 ± 30.00^{a}	7,900 ± 35.00^{b}	1,110 ± 7.00^{c}	1,400 ± 8.00^{d}	3,922 ± 15.00
		BLCO	1,500 ± 7.00^{c}	1.300 ± 5.00^{a}	1,500 ± 6.00^{a}	2,000 ± 3.00^{c}	300 ± 2.00^{d}	1,320 ± 4.00
		DPK	1,500 ± 10.00^{c}	1.200 ± 7.00^{a}	6,000 ± 3.00^{b}	800 ± 4.00^{c}	7.00 ± 5.00^{d}	960 ± 5,00
		PMS	1,650 ± 8.00^{e}	1,435 ± 6.00^{a}	1,200 ± 3.00^{b}	1,320 ± 9.00^{b}	1,210 ± 8.00^{d}	1,363 ± 7.00
	14	LBO	1,652 ± 8.00^{e}	8,470 ± 33.00^{a}	8,690 ± 39.00^{b}	1,210 ± 8.00^{c}	1,542 ± 9.00^{d}	4,313 ± 19.00
		BLCO	1,664 ± 7.00^{e}	1,430 ± 6.00^{a}	1,650 ± 7.00^{b}	2,202 ± 4.00^{c}	335 ± 2.00^{d}	1456 ± 5.00
		DPK	1,500 ± 10.00^{e}	1.300 ± 7.00^{a}	600 ± 3.00^{b}	800 ± 4.00^{c}	7.00 ± 5.00^{d}	960d ± 5,00
		PMS	1,650 ± 8.00^{e}	1,435 ± 6.00^{a}	1,200 ± 3.00^{b}	1,320 ± 3.00^{b}	1,210 ± 8.00^{d}	1,363 ± 7.00
Recovery	28	LBO	1735 ± 8.00^{e}	8,694 ± 36.00^{a}	8,892 ± 41.00^{b}	1,271 ± 9.00^{c}	1,619 ± 10.00^{d}	4,442 ± 21.00
		BLCO	1747 ± 6.00^{e}	1,506 ± 7.00^{a}	1,733 ± 8.00^{b}	2,312 ± 4.00^{c}	352 ± 2.00^{d}	1,529 ± 5.00
		DPK	1734 ± 8.00^{e}	1,386 ± 8.00^{a}	693 ± 4.00^{b}	930 ± 6.00^{c}	812 ± 6.00^{d}	1,111 ± 6.00
		PMS	1,733 ± 6.00^{e}	1,507 ± 6.00^{a}	1,260 ± 3.00^{b}	1,380 ± 10.00^{c}	1,271 ± 9.00^{d}	1,431 ± 7.00
	42	LBO	1838 ± 6.99^{e}	9,228 ± 35.00^{a}	9,414 ± 4.00^{b}	1,347 ± 7.00^{c}	1,716 ± 8.00^{d}	4,708 ± 20.00
		BLCO	1838 ± 5.00^{e}	1,698 ± 8.00^{a}	1,738 ± 6.00^{b}	2,450 ± 4.00^{c}	373 ± 4.00^{d}	1,502 ± 7.00
		DPK	1838 ± 7.00^{e}	1,469 ± 6.00^{a}	735 ± 6.00^{b}	986 ± 5.00^{c}	860 ± 5.00^{d}	1,176 ± 6.00
		PMS	1837 ± 8.00^{e}	1,521 ± 6.00^{a}	1,336 ± 4.00^{b}	1,469 ± 9.00^{c}	1,347 ± 6.00^{d}	1,502 ± 7.00

[1]LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit. Means in the same row with different superscripts differ significantly (P < 0.01).

Table 4: Percentage mortality and survival *H. bidorsalis* juveniles exposed to different concentrations of crude oil fractions[1]

Period	Duration (days)	Crude oil fraction	% Mortality Concentration of crude oil and fractions (ml L^{-1})					% Survival Concentration of crude oil and fractions (ml L^{-1})				
			0.0 (Control)	1.00	2.00	4.00	8.00	0.0 (Control)	1.00	2.00	4.00	8.00
Toxicity	4	LBO	0.00	10.00	0.00	40.00	50.00	100.00	90.00	100.00	60.00	50.00
		BLCO	0.00	0.00	0.00	20.00	30.00	100.00	100.00	100.00	80.00	70.00
		DPK	0.00	0.00	0.00	40.00	50.00	100.00	100.00	100.00	60.00	50.00
		PMS	0.00	0.00	10.00	50.00	70.00	100.00	100.00	90.00	50.00	30.00
	14	LBO	0.00	8.00	6.00	32.00	40.00	100.00	92.00	94.00	68.00	60.00
		BLCO	0.00	5.00	4.00	16.00	24.00	100.00	95.00	96.00	84.00	76.00
		DPK	0.00	3.00	2.00	33.00	42.00	100.00	97.00	98.00	67.00	58.00
		PMS	0.00	4.00	3.00	42.00	55.00	100.00	96.00	97.00	58.00	45.00
Recovery	28	LBO	0.00	2.00	1.00	24.00	36.00	100.00	98.00	99.00	76.00	64.00
		BLCO	0.00	2.00	1.00	12.00	18.00	100.00	98.00	99.00	88.00	82.00
		DPK	0.00	1.00	1.00	26.00	34.00	100.00	99.00	99.00	74.00	66.00
		PMS	0.00	1.00	1.00	35.00	42.00	100.00	99.00	100.00	65.00	58.00
	42	LBO	0.00	1.00	0.00	16.00	26.00	100.00	99.00	100.00	84.00	74.00
		BLCO	0.00	0.00	0.00	6.00	14.00	100.00	100.00	100.00	94.00	86.00
		DPK	0.00	0.00	0.00	18.00	24.00	100.00	100.00	100.00	82.00	76.00
		PMS	0.00	0.00	0.00	25.00	36.00	100.00	100.00	100.00	75.00	64.00

[1]*LBO = Lubricating engine oil, BLCO = Bonny-light crude oil, DPK = Kerosene, PMS = Premium motor spirit.*

during the toxicity period at concentrations of 4.00ml L^{-1} (2000 ± 6.00 WBCs), 1.00 ml L^{-1} (1200 ± 7.00 WBCs) and 1.00ml (1,300 ± 6.00 WBCs) respectively (Table 3). Whereas the blood WBC number in *H. bidorsalis* exposed to 2.00ml L^{-1} BLCO (1500 ± 6.00 WBCs) paralleled that in the control fish, the WBCs in fish exposed to 1.00 ml L^{-1} LBO (7,700 3.00 WBCs and 2.00 ml L^{-1} LBO (7,900 ± 35.00 WBCs) were higher than those in the control fish both for the toxicity and recovery periods of the study (Table 3). The blood RBCs and WBCs in the fish blood for both the toxicity and recovery periods varied significantly ($P < 0.05$) as *H. bidorsalis* juveniles were exposed to the different concentrations of LBO, BLCO, DPK and PMS and the control.

The fish exposed to 4.00 and 8.00 mlL^{-1} of the crude oil fractions (LBO, BLCO, DPK and PMS) recorded higher percent mortality and lower survival than those exposed to the other oil concentrations and the control (Table 4). This state of affairs was prevalent both at the toxicity and at the recovery periods of the study. Lower percent mortality of fish was recorded as the fish recovered on a fortnightly basis than for the 4 days toxicity period (Table 4). In the same vein, higher percent survivals were recorded during the recovery period than during the toxicity period (Table 4). From these results it was evident that the 4.00 and 8.00 mlL^{-1} concentrations of LBO, BLCO, PMS and PMS caused the fish to die more and survive less during the toxicity and recovery periods of the study.

DISCUSSION

Stone *et al*. (2002) stated that about 5 million (RBCs) were present in each cubic millimeter (mm^3) of the blood in vertebrates, while the white blood corpuscles (WBCs) are less numerous than the RBCs (that is, 5000 -10,000 m^{-3}). The uptake and translocation of crude in fish may be through the gills or the intestinal wall (Roubal *et al*., 1977). The parent compounds readily solublize in cell membranes and are probably carried via the erythrocytes to the general circulation of blood. The lower numbers of RBCs recorded in fish samples exposed to different concentrations of crude oil fractions than in the control fish (Table 2) might be due to the destruction of RBCs in fish exposed to the oil fractions. This result is consistent with the report of Neff and Anderson (1981) who stated that the exposure of fish to crude oil compounds resulted in the destruction of WBCs important for the immune response of fish.

Incidentally for this study, except for the control fish, the destruction of the RBCs was higher in the fish exposed to oil pollutants during the 4 days toxicity period than in those recuperating from the oil stress during the 42 days recovery period (Table 2). The lower numbers of RBCs in the fish exposed to 2.00 and 8.00 mlL^{-1} LBO and BLCO than in 4.00 ml L^{-1} of the oil pollutants during the toxicity period implies that the later oil concentration (4.00 ml L^{-1}) was preferred by the fish to maintain a comparatively high number of RBCs in its blood relative to the control in terms of physiological adaptation. This trend was extended to the recovery period while the fish recuperated from the stress of oil pollution. This reason could also be attributed to the higher numbers of RBCs in fish exposed to DPK and PMS 8.00 ml L^{-1} than at the other oil concentrations applied. There were increases in the mortality rate and changes in the haemoglobin content of *H. bidorsalis* juveniles exposed to crude oil higher than 2.00 mlL^{-1}.

The reduced numbers of WBCs (Table 3) recorded in the blood of fish samples during the toxicity periods, and when the four oil types were applied at 1.00, 4.00 and 8.00 mlL^{-1} LBO; 1.00, 2.00 and 8.00 ml L^{-1} BLCO, 4.00, 2.00 and 8.00 DPK and 4.00, 2.00 and 8.00 PMS might be due to the phagocytic action of the WBCs (Stone *et al*., 2002) in the face of an infiltration of crude oil compounds into the blood. It could be that the WBCs engulfed the crude oil compounds with such intensity that resulted in their mortality and consequent reduction in number. The results of this study also show that the WBC in the fish blood started to build up with time (Table 3) especially as the fishes recuperated from the oil pollution stress. The effect of the contamination of the fish blood by the crude oil compound was probably reduced by the oxidative enzymes which metabolically detoxified xenobiotics especially the aromatic hydrocarbons (Payne and Penrose, 1975).

The higher percent mortality and the lower percent survival of *H. bidorsalis* juveniles when exposed to 4.00 and 8.00 mlL^{-1} concentrations of LBO, BLCO, DPK and PMS than when exposed to the other oil concentrations (Table 4) imply that the effect of the oil contaminants on the test fish was most pronounced between 4.00 and 8.00 mlL^{-1} oil concentrations. Neff and Anderson (1981) enumerated some ailments and metabolic malfunctions that resulted in fish exposed to crude oil compounds to include: alteration of liver metabolism, adrenal tissue damage, congested lungs, intestinal damage and hemorrhaging. These physiological problems must have been highly expressed when the fishes were exposed to 4.00 and 8.00 mlL^{-1} oil concentrations and consequently resulted in high mortality and low survival.

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