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Acute Toxicity Tests of Different Concentrations of Diesel Fuel on the Mudskipper, *Periophthalmus koelreuteri* (Pallas 1770): (Gobiidae)

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ABSTRACT Acute toxicity test of water soluble fraction (WSF) of diesel fuel was carried out to determine its effect on the mudskipper, *Periophthalmus koelreuteri* (Pallas). Each of the six experimental tanks containing 20 mudskippers were subjected to graded levels of water soluble fractions (WSF) of diesel fuel with the following concentraction: oppm; 5.20ppm; 8.33ppm, 12.50ppm; 16.66ppm and 29.16 ppm respectively, oppm serves as the control, without any toxicant. The results indicate that despite its amphibious nature, the mudskipper is highly vulnerable to even low concentrations of water-soluble fractions of diesel fuel. The LD_{50} was determined at 4.58ppm. It was suggested that effort should be made to check pollution of the brackish water ecosystem by petroleum hydrocarbons in order to maintain the biodiversity of *P. koelreuteri* in the Niger Delta.

INTRODUCTION

The mudskippers, (Periophthalmus) are of different species found on all tropical seacoasts of the world and one species *Periophthalmus koelreuteri* (Pallas) lives in the West African coast. This fish is important and is a delicacy of the riverine communities of the Niger Delta. In recent times, this fish has become very scarce and expensive and is no more within the reach of the ordinary citizens. These fishes are differentiated by arm-like elongated pectoral fins with which they move along a dry surface. In some species the pelvic fins have developed into two independent active arms (Harris 1960; Swanson and Gibb 2004). The mudskippers inhabit the Niger Delta mangrove swamps which are subject to the influence of tidal fluctuations, and when in danger, they jump into the open sea or move onto land. The mudskippers can move rapidly on the mud which is rarely accessible to man (Ross 2007). They may lie there or roll about in the right sunlight, interrupting their resting periods by hunting for insects and crustaceans, with playful movements.

The fish has ability to resist oil pollution in the open rivers and creeks because of their ability to move out of water for some time, but mudskippers cannot resist oil pollution in a restricted environment. The Niger Delta is characterized by exploration and exploitation and exportation of crude oil and it related activities.

These activities have impacted negatively on the aquatic fauna in the Niger Delta environment and constitute a threat to the biodiversity of the mudskippers. Some works have been done on the toxic effect of petroleum hydrocarbons on aquatic organisms (Oladimeji and Onwumere 1988; Wang 2002; Paris-Palacios et al. 2004; Kadar et al. 2005). There is dearth of information on the toxicity of petroleum hydrocarbon on the mudskipper *Periophthalmus koelreuteri*.

Objectives

The main focus of this paper is to assess the effect of graded levels of diesel fuel on the brackish water mudskipper *Periophthalmus koelreuteri* (Pallas), with a view to simulating the likely effect of petroleum hydrocarbons in its natural environment.

This will enable us assess its biodiversity status and thus take necessary precausion towards its being endangered or driven into extinction.

MATERIALS AND METHODS

The experimental fish were collected from the Rumuolumeni creek besides the University of Education Main Campus, during low tide when the intertidal mud flats were exposed. Cane traps were used to catch the fish. A total of one hundred and twenty (120) mudskippers were caught.

The refined petroleum product (diesel) was obtained from a nearby filling station. The fish had a mean weight of 17.80g.

Five concrete tanks in the Department of Biology, Rivers State University of Education Port Harcourt were used for the experiment; some mud was collected from the mudflat in addition to some mangrove stems with leaves as well as brackish water to simulate the natural environment in the concrete tanks. The fish were left to acclimatize for 5 days before the commencement of the test. During this period, the fish were fed with their natural diet of mashed mangrove crab. Five fish died during acclimatization.

Preparation of Water Soluble Fraction (WSF) of Diesel Fuel

One litre (1 part) of the fresh diesel fuel obtained from a filling station was diluted with four litres (4-parts) of the brackish water from where the fish were obtained, which was also used as the culture medium, in a 6 litre flask according to Baden (1982). By means of a Gallenkamp magnetic stirrer the diesel-water mixture was slowly stirred for 24 hours. This was aimed at enhancing the dissolution in the water, of the water soluble fraction of the diesel fuel. To obtain a clear oil-water interphase, the mixture was left to stand for 3 hours before it was poured into separating funnels, and allowed to stand overnight. The lower layer of water containing the WSF of the diesel was decanted several times into containers until sufficient quantity was obtained for the experiment (Afolabi et al. 1985; Dede and Kaglo 2001).

Exposure of Test Organisms

Each of the experimental tanks contained 20 mudskippers. The tanks were numbered X_1, X_2, X_3, X_4, X_5 and X_6; X_2 to X_6 contained graded levels of the WSF of diesel fuel 5.20 ppm 8.33 ppm, 12.50 ppm, 16.66ppm and 29.16ppm respectively, while X_1 was the control, with no toxicant.

The mudskippers were observed for 96 hours. Fish were confirmed dead when they no longer responded to prodding (floating in water). Dead fish was removed from each of the tanks, immediately it was noticed.

LD_{50} Determination

By means of a table, the numbers of dead fishes in each group were recorded against the time of their death according to Sprague (1972). The median lethal dose (LD_{50}) was calculated using this data of the water soluble fraction (WSF) on *P. koelreuteri* with the Arithmetic method of (Dede and Kaglo 2001).

RESULTS

Toxicity Testing of Water Soluble Fraction of Diesel Fuel on Periophthalmus koelreuteri Within 24 Hours Within 24 hours dead fish was observed in 5.20ppm (1 death); 8.33ppm (2 deaths); 12.50ppm (5 deaths); 16.66ppm (7 deaths); 29.16ppm (8 deaths) (Table 1).

Toxicity Testing of the Water Soluble Fraction of Diesel Fuel on Periopthalmus Koelreuteri Within 48 Hours. Within 48 hours the following deaths were observed 5.20ppm (4 deaths); 8.33 ppm (7 deaths); 12.50 ppm (8 death); 16.66ppm (11 deaths); 29.16 ppm (13 deaths) (Table 2).

Toxicity Testing of the Water Soluble Fraction of Diesel Fuel on Periophthalmus Koelreuteri, Within 72 Hours. Within 72 hours the following deaths were observed. 5.20ppm (7 deaths); 8.33 ppm (10 deaths); 12.50 ppm (11 deaths); 16.66 ppm (14 deaths); 29.16ppm (16 deaths) (Table 3).

Toxicity Testing of Water Soluble Fraction of Diesel Fuel on Periophthalmus Koelreuteri Within 96 Hours. Within 96 hours the following deaths were recorded; 5.20ppm (12 deaths); 8.33 ppm (15 deaths); 12.50 ppm (19 deaths); 16.66 ppm (20 deaths, all died); 29.16 ppm (20 deaths all died) (Table 4).

No deaths were observed in the control tank.

Table 5 was generated from the number of dead fish in each group against the time of death and dose difference. This was used for the LD_{50} calculation.

Table 1: Toxicity testing at 24 hours

Conc. (ppm)	*No. surviving*	*% alive*	*% dead*
0	20	100	0
5.20	19	95	5
8.33	18	90	10
12.50	15	75	25
16.66	13	65	35
29.16	12	60	40

Table 2: Toxicity testing at 48 hours

Conc. (ppm)	*No. surviving*	*% alive*	*% dead*
0	20	100	0
5.20	16	80	20
8.33	13	65	35
12.50	12	60	40
16.66	9	45	55
29.16	7	35	60

Table 3: Toxicity testing at 72 hours

Conc. (ppm)	*No. surviving*	*% alive*	*% dead*
0	20	100	0
5.20	13	65	35
8.33	10	50	50
12.50	9	45	55
16.66	6	30	60
29.16	4	20	80

Table 4: Toxicity testing at 96 hours

Conc. (ppm)	*No. surviving*	*% alive*	*% dead*
0	20	100	0
5.20	8	40	60
8.33	5	25	75
12.50	1	5	95
16.66	0	0	100
29.16	0	0	100

Table 5: 96 hour LD50 determination based on the method of Dede and Kaglo (2001)

Conc. (ppm)	*Conc. difference*	*No. alive*	*No. dead*	*Mean death*	*Mean death X dose difference*
0(Control)	-	20	0	-	0
5.20	5.20	8	12	11	57.20
8.33	3.13	5	15	13.5	42.26
12.50	4.17	1	19	18	75.06
16.66	4.16	0	20	18.5	76.96
29.16	13.0	0	20	18.5	240.05
					491.53

$$LD_{50} = LD_{100} - \Sigma \frac{\text{Mean death X Conc. Diff}}{\text{No of organisms per group}}$$

$$= 29.16 - \frac{491.53}{20}$$

$$= 29.16 - 24.58$$

$$LD_{50} = 4.58 \text{ ppm}$$

DISCUSSION

Within the first 24 hours and within the concentration range of 5.20 ppm and 29.16 ppm the mortality ranged between 5 – 40%. In 48 hours within the same concentration range, a mortality range of between 20-60% was observed. In 72 hours, 35 – 80% mortality was noticed within the same toxicant concentration range. In 96 hours, the specified range of concentration of the toxicants gave a mortality range of between 60 to 100%.

Although mudskippers are known to be amphibious and have the ability to breathe through their skin and lining of their mouth (the mucosa) and throat (the pharynx), the high mortality rate is an indication of the clogging of these respiratory structures by the water soluble fraction (WSF) of the diesel fuel. It is possible that the fish had suffered from oxygen stress induced by the organic compounds in the WSF of fuel. Similar oxygen stress imparted by water soluble fractions of petroleum hydrocarbon on aquatic life has been reported earlier (Baden 1982; Igloh et al. 2001; Dede and Kaglo 2001).

Also the trend in the mortality rate indicated by this study agrees with the earlier work of Horsefall and Spiff (1998) that the extent of depletion of oxygen in the water is often a function of the concentration of the organic pollutant in it.

The result indicates that the death rate increased with increase in the concentration of the toxicant. The lowest concentration (5.20ppm) recorded 1, 4, 7 and 12 deaths respectively within the 96 hours while the highest concentration (29.16ppm) recorded 8, 13, 16 and 20 deaths respectively within 96hours.

From the international classification of toxicity of substances based on their medium lethal dose (LD_{50}) the water soluble fraction (WSF) of diesel fuel is slightly toxic to *P. Koelreuteri*. The exposure of *P. koelreuteri* to water soluble fraction of diesel fuel showed mortality even at low concentrations. This agrees with earlier reports on the effect of water-soluble components of hydrocarbon on aquatic life (Oladimeji and Onwumere 1988; Dede and Kaglo 2001; Fafioye 2006).

It could be deduced from the findings in this work that under confinement, even low concentrations of water soluble fractions of petroleum hydrocarbon could be lethal to *P. koelreuteri*. This is capable of affecting the biodiversity of the species. Attempts should therefore be made by the oil companies operating in the Niger Delta region of Nigeria to minimize pollution of the aquatic environment to ensure the biodiversity of this fish and prevent them from being endangered and subsequent extinction.

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