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Original Research Article

QUANTAL RESPONSE OF PERIWINKLE (*TYMPANOTONUS FUSCATUS*) AFTER EXPOSURE TO KEROSENE

ABSTRACT

Periwinkles (*Tympanotonus fuscatus*) handpicked from the New Calabar River were acclimated to laboratory conditions in the research laboratory of the Chemistry Department of the Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt. They were subjected to concentrations (60, 90, 120, 150 and 200 ml/L) of a petroleum product, kerosene and a control to examine the effect of acute exposure on mortality of the periwinkles. The mean mortality of the periwinkles increased with the concentration of the kerosene and the exposure time. The mean lethal concentration (96 hr LC_{50}) of the kerosene was 111.14ml/L, while the 96 hr LC_{99} was 433.94 ml/L and the probit equation at that hour, Y= -0.80 + 0.007X was significant. The mean lethal time (MLT_{50}) at 60, 90, 120, 150 and 200 with the associated confidence limits were 90.13 (52.94 – 126.45), 84.06 (61.40 – 110.50), 79.02 (42.00 – 105.06), 73.27 (40.74 – 96.30) and 70.17 (39.84 – 94.20 mg/L) respectively. The data obtained from the laboratory is an indication that kerosene is toxic to periwinkles which con be extrapolated to field conditions in the event of spill.

Keywords: Kerosene, periwinkle, environment, quantal response, toxicity.

INTRODUCTION

Due to human influence and the need for technological advancement, there is an increase in petroleum and petroleum products related pollution in the coastal areas the world over. However, information regarding the effects of these pollutants on aquatic organisms are of critical interest due to there increased occurrence. The control of such pollution problems in the aquatic environment is almost impossible (difficult) due to the large number of input sources (Howard *et al*, 2009). Studies on the accidental and intentional release of petroleum and petroleum based products and their effects on aquatic environment reliably shows that aquatic organisms bioaccumulate these substances (Benson *et al*., 2008) and are found to be toxic to aquatic species (Moles and Norcoss, 1998).

Nigeria with a wide range of pipeline network and depots for distributing refined products have its coastal waters at risk of contamination (Renner et al., 2008). Most of these pipelines are old and poorly maintained, thereby resulting in corrosion and leakages which culminates in oil spills (Brume, 2004). Recently, indiscriminate vandalisation of these pipelines for siphoning the products for illegal sales have resulted in oil/ petroleum products spills along the coastal waters (Dwire and Kaufman, 2003). Death of aquatic/ terrestrial fauna and flora from oil spills is very common in the Niger Delta region of Nigeria where there is extensive oil exploration, exploitation and refining of the crude oil (Nwankwo and Irrechukwu, 1981). Apart from death of aquatic organisms and other effects on aquatic life, oil contamination of coastal amenities has adverse effect on tourism, recreation and aesthetics of the affected area. This affects substantially on a community whose economy depends on tourism (Howard *et al*., 2009).

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Tympanotonus fuscatus is a prosobranch gastropod common in many brackishwater, creeks, estuaries and mangrove swamps within the Niger Delta. *Tympanotonus fuscatus* is known locally as periwinkle. It is a relatively cheap source of animal protein and its shell can be used as source of calcium in animal feeds. It is a delicacy especially in the Niger Delta area of Nigeria where the collection and marketing of periwinkles form an important industry (Egonmwan, 1983; Doerffer, 1992).

This study was carried out to examine the toxicity of kerosene to this prosobranch, periwinkle (*Tympanotonus fuscatus*) after acute exposure.

MATERIALS AND METHODS

Periwinkles (*Tympanotonus fuscatus*) of size between 4.5 - 5.5cm were handpicked at the Eagle Cement area of the New Calabar River near the Ignatius Ajuru University of Education Rumuolumeni, Port Harcourt. They were transported in plastic buckets to the Chemistry Department Laboratory of the University. Two hundred apparently healthy periwinkles were acclimated to laboratory conditions in plastic tanks of six litre capacity. The tanks were half filled with brackish water and sediments collected from same source. The acclimation was done for seven days. The substrate was prepared by air drying the sediment and then macerated in a mortar and sieved in 2mm mesh.

About250g of finely prepared sediment were put into each of the plastic tanks to serve as the substrate base. Completely randomized design (CRD) was used for the experiment. The experiment was divided into five treatment levels with three replicates. The test media were prepared in the following concentrations: 60.00ml/L, 90.00ml/L, 120.00ml/L, 150.00ml/L and 200.00ml/L of kerosene and a control (0.00ml/L). Ten of the test animals were introduced into each of the toxicant media. Dead periwinkles were ascertained if the animal has completely retracted into the shell or if it fails to respond to prodding of a glass rod for a period of 15minutes. Mortality assessment was carried out at defined intervals of 24, 48, 72 and 96 hours.

The data obtained were subjected to analysis of variance (ANOVA) to determine if significant differences existed between the means in the mortality at different levels of contamination. Where differences existed, Duncan's multiple range test (DMRT) was used to compare the means (Zar, 1984). Toxicological response data involving quantal response (mortality) was analysed using probit analysis (Finney, 1991) to determine the lethal concentrations (LCs) and median lethal times (MLTs).

RESULTS

The total mortality of the periwinkles (*Tympanotonus fuscatus*) in the various concentrations of kerosene at different time intervals showed that the death rate is time and concentration dependent. However, between 60-120ml/L there seem to be some irregularities in the mortality pattern. The data showed that the mortality of *Tympanotonus fuscatus* in a particular concentration was time dependent (Table 1). The mean mortality of the

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periwinkles (*Tympanotonus fuscatus*) were significant at various time intervals and were concentrations dependent (Table 2).

The lethal effects of the kerosene on the periwinkles were expressed as LC_{50}, LC_{90}, LC_{95} and LC_{99} respectively for 48, 72 and 96 hrs with the associated 95% confidence limit. The result showed that there was great variation between the 48th and the 96th hour. The values of the associated lethality decreased progressively as the exposure duration increased. The 96hr LC_{50} was 111.14ml/L as against the 96hr LC_{99} of 433.94ml/L, while the 48hr LC_{50} was 306.16ml/L and the 48th LC_{99} was 1079.11ml/L. The probit equation were Y= -0.92+0.00X, -0.83+0.04X and -0.080+0.007X for 48, 72 and 96 hrs respectively (Table 3).

The MLT_{50}, $_{90}$, $_{95}$ and $_{99}$ in the exposure concentrations decreased with time and varied appreciably. The probit equation for the variation were however significant. The MLT_{50} data obtained with the lower and upper limits were 90.13 (52.94-126.45), 84.06 (61.40-102.58), 79.02 (42.00-105.0), 73.27 (40.74-96.30) and 70.17 (38.84-94.20) hours for 60, 90, 120, 150 and 200ml/L respectively. The MLT_{99} varied from 208.21 (127.56-409.69) to 155.71 (12870-234.72) hrs between 60.00ml/L to 200.00ml/L of the kerosene concentrations (Table 4).

Table 1: Total mortality of *Tympanotonus fuscatus* in different concentrations of kerosene After acute exposure.

Time Duration (Minutes)	Concentration of kerosene in mg/L 60	90	120	150	200
24	4^{c}	5^{b}	6^{b}	7^{c}	9^{cd}
48	10^{b}	11^{ab}	15^{a}	12^{b}	14^{c}
72	14^{ab}	12^{ab}	16^{a}	16^{ab}	23^{b}
96	19^{a}	16^{a}	17^{a}	22^{a}	30^{a}

Means with the same alphabet in the same column are not significantly different (P>0.05)

Table 2: Mean mortality of *Tympanotonus fuscatus* in different concentrations of kerosene After acute exposure.

Time Duration (Minutes)	Concentration of kerosene in mg/L 60	90	120	150	200
24	1.33 ± 0.11^{c}	1.67 ± 0.36	2.00 ± 0.00^{b}	2.33 ± 1.01^{c}	3.00 ± 0.00^{cd}
48	3.33 ± 1.23^{b}	3.67 ± 1.11^{ab}	5.00 ± 1.05^{a}	$4.00 \pm 0{,}00^{b}$	4.67 ± 1.36^{c}
72	4.67 ± 1.56^{ab}	4.00 ± 0.45^{ab}	5.33 ± 1.23^{a}	5.33 ± 1.88^{ab}	7.67 ± 2.43^{b}
96	6.33 ± 1.67^{a}	5.33 ± 1.34^{a}	5.67 ± 1.10^{a}	7.33 ± 2.31^{a}	10.00 ± 0.00^{a}

Means with the same alphabet in the same column are not significantly different (P>0.05)

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Table 3. Mean lethal concentration of kerosene with associated 95% confidence interval to *Tympanotonus fuscatus* exposed 96 hours

Exposure Duration (hrs)	Lethal concentrations (ml/L) with associated 95% confidence interval LC_{50}	LC_{90}	LC_{95}	LC_{99}	Probit equation	Test sig.
48	306.16	731.97	852.68	1079.11	Y= - 0.92+0.003X	** (ns)
72	191.02	486.26	569.96	726.96	Y= - 0.83+0.04X	**
96	111.14	289.00	339.38	433.94	Y= - 0.80+0.007X	***

ns = non significant, ** or *** = significant

Table 4. Median lethal time of kerosene with associated 95% confidence interval to *Tympanotonus fuscatus* after exposure to various concentrations of kerosene

Concentration of kerosene (ml/L)	Median lethal time (hrs) and associated 95% confidence interval MLT_{50}	MLT_{90}	MLT_{95}	MLT_{99}	Probit equation	Test sig.
60.00	90.13 (52.94-126.45)	155.18 (120.89-272.16)	173.62 (139.16-319.46)	208.21 (140.56-409.67)	Y= - 1.70+0.23X	***
90.00	84.06 (61.40-110.58)	139.84 (-)	159.59 (-)	196.64 (-)	Y= - 1.78+0.20X	***
120.00	79.02 (42.00-105.06)	137.99 (101.68-214.05)	154.70 (123.42-251.68)	186.06 (145.54-324.04)	Y= - 1.27+0.018X	***
150.00	73.27 (40.74-96.30)	128.43 (104.14.138.80)	144.06 (116.63-214.09)	173.39 (138.28-272.70)	Y= - 1.72+0.22X	***
200.00	70.17 (39.84-94.20)	123.53 (39.84-94.20)	134.72 (104.58-169.26)	155.71 (128.70-234.72)	Y= - 2.73+0.032X	***

*** = significant

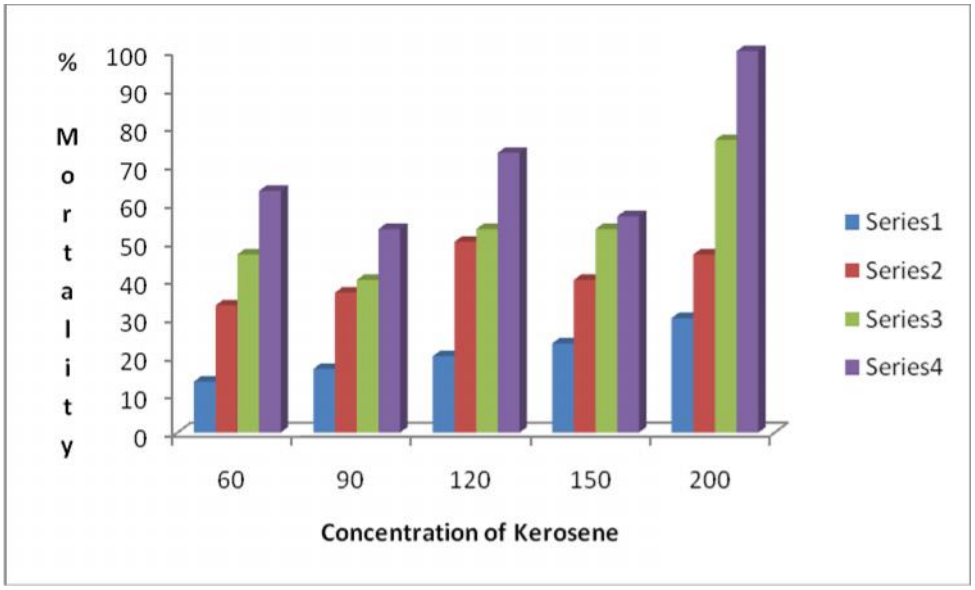

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Figure 1: Percentage mortality of *Tympanotonus Fuscatus* in different concentrations of kerosene for 96 hours. Series 1 =24 hours, series 2 =48 hours, series 3 = 72 hours, series 4 = 96 hours.

DISCUSSION

According to Sprague *et al*., (1981) benthic organisms are particularly vulnerable to oil spills and forage the bottom sediments into most pollutants. The mortality of periwinkles may have resulted from the action of kerosene on the organism. Generally, it is known that petroleum products exhibits the mechanism of limiting gaseous exchange between organisms and the aquatic environment by coating the surfaces and thereby suffocating the organism to death (Chukwu and Odunzeh, 2006). The limiting of oxygen supply through this process leads to asphyxiation in the organism which finally culminates in death (Osuji and Mbata, 2004). One method by which crude oil and its products cause damage to aquatic organisms is because oxygen is not soluble in them and therefore limits the amount of oxygen made available to the water body from the atmosphere (Nwamba *et al*., 2006). Petroleum products penetrates into the metabolic pathways of aquatic animals and thereby alters the metabolic action of the affector sites and hence exerts their action on the exposed organism (Chukwu and Odunzeh, 2006).

As deposit feeders, periwinkles may have incorporated part of the dissolved components of the kerosene into its tissues (Ewa-Oboho and Otogo, 2009) which eventually may have altered the normal body physiology and biochemistry of the periwinkle, thereby leading to death. The mortality pattern in this study is in accordance with the findings of Ewa-Oboho and Otogo, (2009), Renner *et al*., (2008) and Chukwu and Odunzeh, (2006) who observed that mortality of periwinkle from petroleum products depends on the concentration of the products and time duration of exposure. The kerosene prevents gaseous exchange between the respiratory organs and the affector sites thereby hindering the oxygen changing capacity of the periwinkle which lead to death (Fafioye *et al*., 2001). The kerosene also may have excited the medium which controls choline and catecholamine and the system which controls fluid distribution (Smith, 1984; Gabriel and Edori, 2010).

The 96hr LC_{50} (111.14ml/L) observed in this study was lower than the 48hr LC_{50} which implies that high concentration of kerosene in the environment resulting from spill and accidental discharge can easily sweep off this specie from marine ecosystem. The observed 96hr LC_{50} (111.14ml/L) on the periwinkle was lower than that reported for spent engine oil on periwinkle, which was 911.57mg/L (Chukwu and Odunzeh, 2006). However, this value was higher than the LC_{50} of detergent on periwinkle which was 48.67mg/L (Chukwu and Odunzeh, 2006) and petrol on periwinkle which was 104.68mg/L (Renner *et al*., 2008), showing that kerosene is more toxic to periwinkles than spent lubricant but less toxic than detergent and petrol. The decrease in LC_{50} values of kerosene in this study with time is in consonance with the findings of other authors (Nwaokoro,2001; Gabriel *et al*., 2010; Chukwu and Ugbeva, 2003). The 96hr MLT_{50} observed, showed that higher concentrations took lesser time to effect mortal damage in the organism (Gabriel and Edori, 2010).

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Therefore, effort should be taken to prevent kerosene spill in our environment. In situations where spill has occurred, immediate and adequate measure of clean-up should be taken to preserve the environment from decay and devastation.

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