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Laboratory assessment of the effects of Nigerian crude oil and industrial detergent on *Tympanotonus fuscatus* (Gastropoda: Potamididae) in the Cross River Estuary, South-South Nigeria

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Periwinkle, *Tympanotonus fuscatus*, was collected from the Cross River Estuary, allowed to acclimate to laboratory conditions for 7 days and exposed to Nigerian crude oil and detergent (Klin) at different concentration intervals of 50, 100, 150, 200, 250 and 20, 40, 60, 80, 100 ml/L of water, respectively for a period of 96 h. A total of 176 mortalities were observed for detergent out of 250 individuals during the entire experiment and 161 for crude oil with higher concentrations recording higher mortalities. Based on the derived toxicity indices, the detergent [96 h lethal concentration (LC_{50}) = 12.42 ml/L] was found to be 7.1 times more toxic than Nigerian crude oil (96 h LC_{50} = 10.01 ml/L) and 5.8 times (96 h LC_{95} = 68.83 ml/L) more toxic (96 h LC_{95} = 396.7 ml/L). The two-way without replication analysis of variance (ANOVA) showed that there was significant difference (F_{crit} = 3.5, P > 0.05) between all treatment of crude oil and detergent. One-sample t-test showed significant difference within 24 to 96 h (P < 0.05) mortality response of test animals exposed to toxicants at all concentrations. It is concluded that the detergent used as first generation oil dispersant is more toxic to shellfish than Nigerian light crude oil even at low concentration 20 ml/L of the estuarine water. Also, periwinkle may serve as useful *in-situ* sentinels for health assessment studies of organic pollutants in aquatic ecosystems.

Key words: Ecosystems, periwinkle, petroleum, crude oil.

INTRODUCTION

The crude oil comprises different kinds of organic compounds, many of which are highly toxic. Oil is acutely lethal to marine organisms. Petroleum hydrocarbons in the estuary arise from different sources including releases of crude oil from drilling rigs, railcars, pipelines, tankers, offshore platforms and wells. Enin (1997) reported that offshore petroleum production facilities located adjacent to the mouth of the Cross River Estuary introduce small-scale oil spills and leakages into the estuary by wave and tides. Bunkering activities along Calabar and Great Kwa Rivers which empties into the

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Atlantic Ocean through Cross River Estuary contributed immensely to oil pollution of this estuary.

Oil spills on sea surface undergoes a number of major physical and chemical processes such as drifting, evaporation, dissolution, dispersion, emulsification, sedimentation, biodegradation and photo-oxidation; these processes, whether physical or chemical, help in the distribution of the spills and partitioning of the oil component into the water, air, ambient atmosphere, organisms and sediments as the case may be (Clark, 1992). In Nigerian waters, cases of oil spillage have been recorded between 1958 to date releasing about 2.4 million barrels of crude oil into coastal aquatic environment. Of importance are the Exxon Mobil spills, Idoho disaster, 1998, Ogoni oil spills disasters, 1958 to 2005 (Udo, 2007). This poses a great risk to aquatic organisms like periwinkle, which is a source of protein for the coastal dwellers in Nigeria. Doerffer (1992) reported that oil spill causes substantial mortality among fish and invertebrates. Other effects include changes in species composition, low abundance, loss of species and tainting (Widdows et al., 1982).

Oil pollution and its clean up processes involving chemicals has revealed that both dispersant alone and dispersant - oil mixtures may be more injurious to aquatic organisms than the oil alone (Chukwu and Odunzeh, 2006). Products specifically formulated to be used as oil spill dispersants did not exist during the 1960s. Industrial detergents (First generation “dispersants”) primarily developed for other uses such as cleaning oily machinery or washing vehicles and consisting of a variety of different surfactants dissolved in solvents, were occasionally pressed into service to be used to clean up spilled oil, on a small-scale, whenever the need arose. The Torrey Canyon oil spill in 1967 was the first - and last - occasion that industrial detergents were used in oil spill response on a massive scale. About 10,000 tons of detergents were used to try and clean the estimated 14,000 tons of spilled oil off of the beaches in Cornwall in the UK. After the use of dispersants to clean up the oil spilled from the Torrey Canyon tanker incidence in 1967, it was found that some dispersants increased the biological activity of crude oil (Nielson - Smith, 1968). Several other reports have confirmed this observation and so increased the controversy of the use of dispersants in cleaning up of crude oil and its refined petroleum products in the environment (Beynon and Cowell, 1974; Oyewo, 1986).

The Cross River Estuary lies between latitudes 4°30’ N and 5°15’ N and longitudes 8°00’ E and 8°40’ of the equator (Figure 1). The mineral-rich catchments area in combination with the dense vegetation and frontal rainfall pattern characteristic of this area plays a tremendous role in the biological-chemical regulation of organic and inorganic nutrients in the estuary (Akpan and Ofem, 1993; Asuquo et al., 1998). These nutrients encourage high primary productivity, species abundance and diversity in the estuary. The estuary is also prone to allochthonously imposed negative changes in the environment, principally due to oil pollution activities adjacent to the mouth of the estuary (Enin, 1997). The Cross River Estuary, the largest in Nigeria with tidal amplitude of 3 m (Asuquo et al., 1998) is delineated into three aquatic ecological habitats.

Tympanotonus fuscatus commonly called periwinkle is found in the mangrove swamps and mudflats in low salinity areas of the Cross River Estuary. *T. fuscatus* is a dominant benthic specie in West African coastline and is also a high source of animal protein, which is considered a delicacy especially in the Niger Delta areas where its collection and marketing form an important local fishery industry. The present study is designed to investigate the relative acute toxic effects of Nigerian crude oil and a Nigerian brand of detergent (Klin), against *T. fuscatus* in Cross River Estuary.

MATERIALS AND METHODS

The test organisms, mudflat periwinkles *(T. fuscatus)* were collected from the Cross River Estuary and were transported to the laboratory and kept in glass aquaria half filled with the estuary water. Sediment from the site of collection was placed at the bottom serving as substrate. The samples shell sizes ranged from 14.8 to 24.5 mm. The organisms were left to acclimatize to laboratory conditions for 7 days before using them in laboratory bioassays in accordance with guidelines for bioassay technique (APHA, 1985).

For each experiment, 50 specimens were placed inside all the aquaria (32 × 21 × 19.5 cm) including the control. Some quantity of mud (100 g) was introduced into the aquaria to simulate the natural condition of the shellfish. Before stocking, the aquaria were thoroughly washed and rinsed. Both the control and experimental media were monitored every 24 h for 4 days. Mortality was investigated by careful observations.

Predetermined volumes of Nigerian crude oil were measured using a measuring cylinder and introduced into the sediment substrate and the appropriate volumes of estuary water. The volumes of Nigerian crude oil used were 50, 100, 150, 200 and 250 ml/L of estuary water. Also, a pre-determined volume of prepared detergent solution was measured. About 100 g of detergent (Klin) was measured and dissolved in 100 ml of water and then mixed vigorously. This now served as the second toxicant for the experiment. The volumes of detergent were measured using measuring cylinder and introduced into the substrate and the volume made up to 1000 ml by adding appropriate volumes of estuary water. A glass rod was used to stir the mixture. The volumes of detergent used were, 20, 40, 60, 80 and 100 ml/L. There was control in which test medium substrate was similar but no detergent or crude oil was introduced.

The organisms was taken to be dead when it was observed to be totally retracted into its shell and failed to emerge or protrude its muscular foot during an observation period of 30 min in an untreated dilution water in a Petri dish; or if the foot was retracted at the start of observation and the organisms failed to respond by withdrawing the foot into the shell on prodding with a glass rod (Otitoloju, 2002; Chukwu and Odunzeh, 2006).

The mortality data of periwinkle, *T. fuscatus* exposed to different concentrations of Nigerian crude oil and Nigeria brand detergent (Klin) is presented in Table 1. Toxicological dose-response data involving quantal response (mortality) were analyzed by Probit analysis (Finney, 1971) using SPSS 13.0 computer program. The

Figure 1. Map of the study area.

indices of toxicity measurement derived from this analysis were median lethal concentration that causes 50% response (mortality) of exposed organisms (LC_{50}), lethal concentration that causes 95% response (mortality) of exposed organisms (LC_{95}). Sub lethal concentration that causes 5% response (mortality) of exposed organisms (LC_5) and their 95% confidence limits (CL). Toxicity factor of relative potency measurements (TF) for example, 96 h LC_{50} of a compound/96 h LC_{50} of another compound tested against same species (Table 2). Microsoft excel 2010 was used for analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Relative acute toxicity of Nigerian crude oil against periwinkle

Mortality was absent within the first 24 h in 50, 100, 150 and 200 ml concentrations. The result is similar to that observed by Ewa-Oboho and Otogo (2009). The delay in mortality in the concentrations of 50, 100, 150 and 200 ml

Table 1. The mortality data of *Tympanotonus fuscatus* in Nigeria crude oil and detergent.

Conc. / hours	Crude oil (ml)					Detergent (ml)				
	50	100	150	200	250	20	40	60	80	100
24	0	0	0	0	2	5	6	10	9	11
48	3	2	9	11	14	6	10	10	12	14
72	5	12	14	15	14	11	12	15	12	9
96	8	10	11	15	16	14	12	12	15	16
Total	16	24	34	41	46	36	40	47	48	50

Table 2. Relative toxicity of Nigerian oil and detergent against *Tympanotonus fuscatus*.

Test compound	LC_{50} (95% CL)	LC_{95} (95% CL)	LC_5 (95% CL)	SD	DF	Chi square	TF_1	TF_2
Nigerian crude oil								
24	1805.2 (0)	8644.21 (0)	376.98 (0)	0.89	3	2.010	1.00	
48	503.82 (324.5 - 1767.5)	3992.5 (1317.7 - 117291.8)	63.58 (23.15 - 91.94)	5.17	3	3.416	3.58	
72	192.7 (154.7 - 272.6)	1713.6 (812.12 - 9728.9)	21.67 (5.88 - 38.3)	4.06	3	0.557	9.37	
96	88.14 (70.9 - 103.7)	396.7 (295.1 - 655.8)	19.6 (9.33 - 29.94)	3.39	3	3.21	20.48	7.1
Detergent								
24	1105.3 (0)	172393.7 (0)	7.08 (0)	2.59	3	0.550	1.00	
48	109.02 (73.3 - 414.96)	3914.34 (726.7 - 6925942.1)	3.04 (0.02 - 9.63)	2.97	3	0.178	10.14	
72	26.35 (5.6 - 40.13)	1443.23 (316.1 - 862252.3)	0.48 (0.0003 - 3.25)	2.17	3	1.292	41.94	
96	12.42 (5.42 - 18.18)	68.83 (52.84 - 113.05)	2.24 (0.31 - 5.57)	1.79	3	3.74	88.97	

$$TF_1 = \frac{LC_{50} \text{ of test compound at 24 h}}{LC_{50} \text{ of test compound other hours (48, 72, 96)}}$$

$$TF_2 = \frac{\text{96 h } LC_{50} \text{ of crude oil}}{\text{96 h } LC_{50} \text{ of detergent}}$$

DF, Degree of freedom; **LC,** lethal concentration; **CL,** confidence limit; **SD,** standard deviation; **TF,** toxicity factor.

during the first 24 h could be attributed to the time taken for crude oil to dissolve and residue to emulsify and that the waxy excess oil on the surface could hinder evaporation during the first few hours till the oil

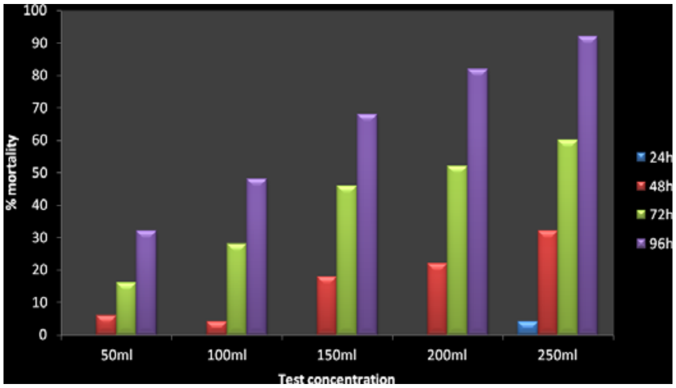

Figure 2. Percentage mortality of periwinkle against crude oil.

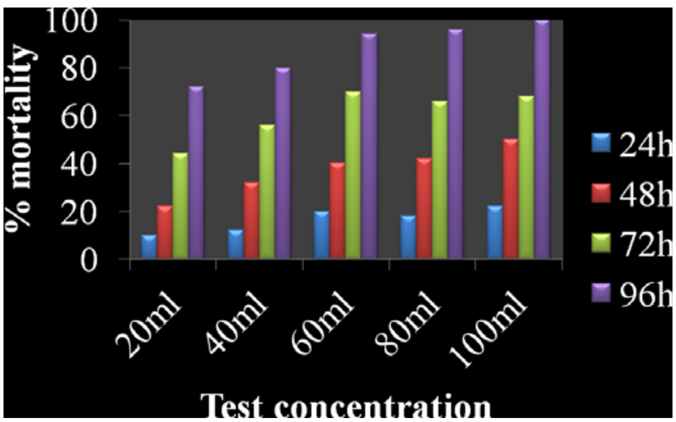

Figure 3. Percentage mortality of detergent against periwinkle.

coagulates into chocolate mouse. Coagulation takes place between 10 to 70 h after oil is spilled (Clark, 1992; Ewa-Oboho and Otogo, 2009). The percentage mortality of Nigerian crude oil against periwinkle is shown in Figure 2. Twenty-four (24) and 48 h LC_{50} and LC_{95} were outside the range of concentrations utilized for the experiments but 48 h LC_5 was within the range of 63.6 ml/L. The 72 and 96 h LC_{50} were 192.7 and 88.14 ml/L, respectively showing high toxicity of Nigerian crude oil against tested organisms. There was no significant difference using homogeneity test (Chi-square, Table 2). T-test (one-sample test) showed significant difference within 24 to 96 h ($P < 0.05$), similar to report by Ewa-Oboho and Otogo (2009). The two-way without replication ANOVA showed that there was significant difference ($F_{crit} = 3.5$, $P > 0.05$) between all treatment of crude oil.

Apart from mortality caused by the toxic effects of soluble oil fraction, a layer of oil in the water column would result in a drop in respiration rate of text organisms leading to death. The rate of mortality of *T. fuscatus* was attributed to the concentration of the oil, the period of exposure, ability of the shellfish to bio-accumulate and the nature of the oil (Clark, 1992; Ewa-oboho and Otogo, 2009; Daka and Ekweozor, 2003).

Relative acute toxicity of detergent (Klin) against periwinkle

Mortality was observed even at low concentration of 20 ml/L of detergent unlike the crude oil indicating toxicity of water soluble sulphonate group attach the alkyl benzene hydrocarbon of the detergent. The percentage mortality of detergent in Figure 3 showed 100% mortality of periwinkle at concentration of 100 ml/L at 96 h suggesting high toxicity level compare to the crude oil. 96 h LC_{50} was found to be 12.42 ml/L in the present study confirming the acute toxicity of detergent at a low concentration (72 h LC_5 = 0.48 ml/L). There was no significant difference using homogeneity test (Chi-square, Table 2). One-sample t-test showed significance difference within 24 to 96 h ($P < 0.05$). Ewa-Oboho and Otogo (2009) also reported similar observation. The two-way without replication ANOVA showed that there was significant difference ($F_{crit} = 3.49$, $P > 0.05$) between all treatment.

From the result, it is clear that the acute toxicity of detergent used as dispersant is more toxic to shellfish than the Nigerian crude oil. Chukwu and Odunzeh (2006) reported that the relative acute toxicity of spent lubricant oil and detergent (Omo) against estuarine benthic macro-invertebrates, *Cucumis*. *Africanus* (Aurivillus) and T. *fuscatus* was more toxic to the test organisms. The toxicity of dispersant against periwinkle in the present study at relatively low concentration is in agreement with the findings of Oyewo (1986) who reported that some dispersants used in Nigeria were relatively highly toxic to some brackish water species. Similarly, Akintonwa and Ebere (1990) reported that the dispersants Teepol and Conco-k were more toxic to Barbus sp fingerlings than Asabo crude oil. The differential toxicity observed between petroleum products and dispersants according to Nelson-Smith (1971) and Westermeyer (1991) could be attributed to the differences in the physical characteristics and chemical composition of test compounds. These chemical and physical characteristics of the chemicals dictate the penetrability of the compounds into living organisms, site of action of metabolism and hence the toxic actions they exert on the exposed organisms. Generally, it has been well established that one of the mechanisms of action of petroleum products against exposed animals is that it limits gaseous exchange by coating the respiratory surfaces such as spiracles, skin, and gills of exposed organisms, while most dispersants act on the surface membrane of exposed organisms thereby disrupting the membrane barrier and thus causing easier influx of toxicants (Beynon and Cowell, 1974).

Conclusion

Nigerian crude oil and industrial detergent used as dispersant were found to be very toxic to *T. fascatus* of the Cross River Estuary even at low concentration. Detergent was more toxic unlike crude oil due to its ability to completely dissolve in polar solvent such as estuary water. This benthic shellfish serves as a major source of protein to most coastal homes in the Niger Delta. The results obtained in this study also confirmed that the estuarine periwinkle serve as useful *in-situ* sentinels for health assessment studies of organic pollutants in aquatic ecosystems.

Conflict of interests

The authors have not declared any conflict of interests.

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