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Relative toxicity of spent lubricant oil and detergent against benthic macro-invertebrates of a west african estuarine lagoon

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Abstract: *The relative acute toxicity of spent lubricant oil and detergent was evaluated against hermit crab, Clibanarius africanus (Aurivillus) and periwinkle, Tympanotonus fuscatus (L) from the Lagos lagoon in laboratory bioassays. Based on the derived toxicity indices, the detergent (96 hr LC_{50} = 5.77ml/l) was found to be 1.73 times more toxic than spent engine oil (96 hr LC_{50} = 10.01ml/l) when acting singly against C africanus and 18.73 times (96 hr LC_{50}-48.67 ml/l) more toxic (96 hr LC_{50} = 911.57 ml/l) when acting singly against T. fuscatus. On the basis of the computed susceptibility factors, C.africanus was found to be about eight times and ninety-one times more susceptible to the toxic effect of detergent and spent lubricant oil respectively. The randomized analysis of variance (ANOVA) showed that there was significant difference (F_{cal} 58.83 F_{tab} 3.87; DF 13; p>0.05) between all treatments of spent lubricant oil and detergent during the 96 hr exposure period of test animals. At 5% level of significance the Student Neuman-Keuls (SNK) test further revealed significant differences in the mean mortality response of test animals exposed to toxicants at all concentrations and untreated control. The results obtained in this study suggest that the estuarine benthic macroinvertebrates, which play key roles in the environment, may serve as useful in-situ sentinels for biomonitoring studies of petroleum pollutants in fragile aquatic ecosystems such as the Lagos lagoon.*

Key words: *Relative toxicity, Spent oil, Detergent, Macroinvertebrate.*

Introduction

The advancement of technology in the world has brought about rapid increase in demand for utilization of petroleum and petroleum products. This has resulted in a corresponding and steady increase of petroleum contamination of the marine, estuarine, fresh water and terrestrial ecosystems. Pollution from spent engine oil is one of the important environmental problems of urban centres and coastal regions especially estuaries, lagoons and mangrove swamps in Nigeria. This is as a result of continual rise in human population density, increase in number of motor vehicles that drive the highways and roads daily and prevailing poor disposal of such oils used. In Nigeria, for instance, spent engine oil is improperly discarded by mechanics along highways, street corners or into drainage water in open gutters and streams where the oil floats into bigger rivers, lagoons, estuaries and even the seas. The spent engine oil is again released into the aquatic environment directly through ship harbours or boat jetties, which are located on or in close proximity to the water bodies. The largest amounts of oil from land reach the water bodies along with domestic or industrial effluents, which run off into the rivers or seas.

When spillage of refined petroleum products including spent engine oil occurs in an aquatic ecosystem, steps are usually taken to clean up. Several methods may be adopted in such clean up exercises including mechanical containment and recovery, use of sinkers bioremediation and burning or chemical dispersants.(Westermeyer, 1991) The use of chemical dispersant quickly solves the aesthetic problems of oil pollution. 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. 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).

Most assays on the toxicity of oil and oil dispersants (e.g. detergents) all over the world involve the use of aquatic animals (Swedmark *et al*., 1973; Akintonwa and Ebere, 1990). These tests are characterized by highly variable results that are dependent on the type of test organism and life stage as well as the origin and solubility of the oil and dispersant being assayed. In carrying out acute toxicity tests, it is particularly important to consider benthic organisms which are particularly vulnerable to oil spills (Sprague *et al*., 1981) and which forage the bottom sediment into most pollutants (degradable and non degradable).

The Lagos lagoon forms a natural habitat for a number of benthic organisms like periwinkle (*Tympanotonus fuscatus),* hermit crab (*Clibanarius africanus), Pachymelania aurita*, *Thais haemastoma, Ostrea coculata, Dorsinia isocardia* (Ogunwenmo and Kusemiju, 2004). Hermit crabs are conspicuous members of the benthic community in Nigerian coastal waters as they are found aggregating in groups of four or five or as many as fifty on stones, tree trunks and roots. They are important deposit feeders in the aquatic environment (Greenwood, 1972). *Tympanotonus fuscatus* commonly called periwinkles is restricted to the mangrove swamps and mudflats in low salinity areas of the central part of Lagos lagoon system and Niger delta. (Oyenekan, 1987; Egonmwan, 1986). *Tympanotonus fuscatus* is a dominant benthic specie in West African coastal lagoon 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 objective of this paper, therefore, is to investigate the relative acute toxic effects of spent lubricant oil and a Nigerian brand of detergent, against benthic macroinvertebrates *Clibanarius africanus* and *Tympanotonus fuscatus,* (L), which oftentimes, are discharged either accidentally or deliberately into the estuarine lagoon ecosystems.

Materials and Methods

Test animals: The test animals used for this assay were adult stages of *Clibanarius africanus* (Aurivillus) (hermit crab) (Arthropoda; Crustacea, Decapoda, Paguridae) and *Tympanotonus fuscatus (*L*)* (periwinkle). (Mollusca; Gastropoda, Mesogastropoda, Potamididae) They were handpicked into a plastic bucket from the shores of the Lagos lagoon at low tide. These animals were collected from the same site to reduce the variability in biotype.

Acclimatization of animals: The test animals were transported to the laboratory and kept in holding glass tanks (113 cm x 54 cm x 80 cm) half filled with lagoon water. Sediment from the site of collection was placed at the bottom of the holding tanks serving as their substrate. Water in these holding tanks were aerated with a 220 V air pump and then changed every 48 hr to prevent accumulation of toxic waste metabolites. The animals were left to acclimatize to laboratory conditions (28^{0}C + 2^{0}C; 72.2% R.H) for 7 days before using them in laboratory bioassays in accordance with guidelines for bioassay technique (APHA, 1985). Hermit Crabs *(Clibanarius africanus*) and periwinkle (*Tympanotonus fuscatus*) of approximately the same size range (length of shell 30± 0.5 mm) were selected for all experiments.

Test compounds:

Spent engine oil: Spent engine oil was collected in gallons from different mechanic workshops around the Lagos metropolis. The mixture of the different used engine oil served as the test compound. The mixing of the oil was to simulate the natural situation, since the spent engine oil that impacts aquatic organisms comes from different sources such as mechanic workshops and boat jetties

Detergent: The detergent used in this study was a brand known as Omo. It is a blue powdered detergent manufactured by Unilever Nig Plc. The composition as stated by the manufacturers includes active detergent, cationic, enzyme, antiredepository agent, perfume, optical brightners, colourant.

General bioassay procedures:

Bioassay containers: The bioassays were carried out in glass tanks (22 cm x 15 cm x 18 cm). These glass tanks were preferred to plastic containers as they minimize absorption of toxicants and prevents risk of corrosion and chemical reactions. Some plastics are known to react with some crude oil components (Don – Pedro, 1989).

Preparation of substrate: The use of sediments as substrate in toxicity testing has been observed to increase the sensitivity of benthic organisms including hermit crabs and periwinkles to toxicant in laboratory bioassays (Otitoloju and Don-Pedro, 2002). The substrate used, however was collected from the site of collection of test animals and was subjected to its standardization procedure after Otitiloju (2002). A weighed mass of sieved soil (100 g) was used as substrate in each bioassay container.

Application of toxicants to test media: Lagoon water was used as the medium for all the bioassay tests conducted. Pre-determined volumes of prepared spent engine oil mixtures were measured using a measuring cylinder and introduced into the soil substrate and the volume made up to 100ml/l by adding appropriate volumes of lagoon water. There was control in which test medium substrate was similar but no detergent or spent engine oil was introduced.

Again a pre-determined volume of prepared detergent solution was measured. About 100 g of detergent (Omo) 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 lagoon water. A glass rod was used to stir the mixture.

Assessment of quantal response:

T. fuscatus: This animal 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 15 min in an untreated dilution water (salinity 16$^0/_{00}$) in a Petri dish; or if the foot was retracted at the start of observation and the animal failed to respond by withdrawing the foot into the shell on prodding with a glass rod (Otitoloju, 2002).

***C. africanus*.:** This animal was taken to be dead if it failed to retract its protruded body into its shell upon prodding with a glass rod or failed to emerge or protrude its legs/body during an observation period of 3-4 min in untreated brackish water at salinity of 16$^0/_{00}$ placed in an observatory petri dish. Mortality assessments were carried out at defined time intervals of 24, 48, 72 and 96 hr.

Bioassays:

Relative acute toxicity of spent engine oil and detergent against test animals: Test animals of similar sizes were placed randomly in treated and untreated test media in bioassay tanks already holding soil substrate. In all cases a total of 45 test animals of C.*africanus* and T. *fuscatus* were exposed per

Table – 1: Relative acute toxicity of spent engine oil and detergent against *Clibanarius africanus.*

Test compounds	LC_{50}(95%CL)	Lc_{95}(95%CL)	LC_{5}(95%CL)	Slope ± SD	D F	Probit equation	TF_1	TF_2
Spent engine oil								
24	21.196 (44.38-10.17)	54.01 (561.73-5.24)	8.32 (21.134-3.27)	4.06±.58	1	Y = 0.387+4.062X	1	
48	14.85 (17.05-12.94)	27.72 (45.94-16.71)	7.96 (11.27-5.63)	6.09±2.05	2	Y = -2.13+6.09X	1.4	
72	12.28 (13.53-11.15)	22.33 (28.71-17.34)	6.75 (8.36-5.47)	6.35±1.15	4	Y = -1.92+6.35X	1.7	
96	10.01 (11.11-9.02)	19.65 (24.89-15.49)	5.10 (6.67-3.91)	5.63±0.98	4	Y =-0.64+5.633	2	1.73
Detergent								
24	31.310 (58.51-17.03)	152.78 (874.45-27.81)	6.42 (12.45-3.28)	2.397±0.882	3	Y =1.415+2.397X	1.00	
48	19.46 (29.96-12.86)	172.79 (767.11-42.02)	2.19 (5.51-0.84)	1.740±0.4604	4	Y = 2.757+1.740X	1.61	
72	11.02 (15.39-7.90)	108.80 (356.89-35.65)	1.12 (3.25-0.36)	1.659±0.3924	4	Y=3.271+1.659X	2.84	
96	5.77 (8.02-4.09)	38.36 (73.12-20.82)	0.87 (2.15-0.33)	2.005±0.386	4	Y = 3.474+2.005X	5.43	1

TF_1 = Toxicity factor = $\frac{LC_{50} \text{ of test compound at 24 hr}}{LC_{50} \text{ of test compound at other hr (48, 72,96).}}$

TF_2 = Toxicity factor = $\frac{96 \text{ hr } LC_{50} \text{ of detergent}}{96 \text{ hr } LC_{50} \text{ of spent engine oil}}$

SD = Standard deviation
DF = Degree of freedom
CL = Confidence limit
LC = Lethal concentration

treatment including untreated control in three replicates (15 animals per replicate) per treatment. The test animals were exposed to various concentrations of test compound a follows:

- Spent engine oil against *Clibanarius africanus* 6, 8, 10, 12, 14, 16 ml/l and untreated control.
- Spent engine oil against *Tympanotonus fuscatus*, 820, 860, 900, 940, 980 1000 ml/l and untreated control.
- Detergent against *Clibanarius africanus,* 2, 6, 10, 14, 18, 22, ml/l and untreated control.
- Detergent against *Tympanotonus fuscatus* 30, 40, 50, 60, 70, 80 ml/l and untreated control.

Statistical analysis:

Dose – response data analysis: Toxicological dose-response data involving quantal response (mortality) were analyzed by probit analysis (Finney, 1991) based on a computer program written by Ge Le Pattourel, imperial college, London as adopted by Don- Pedro (1989). The indices of toxicity measurement derived from this analysis were:

LC_{50} = Median lethal concentration that causes 50% response (mortality) of exposed organism.

LC_{95} = Lethal concentration that causes 95% response (mortality) of exposed organisms.

LC_{5} = Sub lethal concentration that causes 5% response (mortality) of exposed organisms and their 95% confidence limits (CL).

T F = Toxicity factor of relative potency measurements e.g. 96hr LC_{50} of a compound / 96hr LC_{50} of another compound tested against same species. One way analysis of variance (ANOVA) and comparison of means by Student Neuman – Keuls (SNK) test were used to test for statistical differences in the results of 96 hr toxicity tests.

Results and Discussion

Relative acute toxicity of spent engine oil and detergent against test animals:

C. africanus: On the basis of 96 hr LC_{50} values, the detergent (96hr LC_{50} = 5.77 ml/l) was found to be more toxic than the spent lubricant oil (96 hr LC_{50} = 10.01 ml/l) when tested against *C. africanus.* (Table 1). The detergent was significantly (no overlap in 95% C.L. of 96 hr LC_{50} values) more toxic to spent lubricant oil against *C. africanus.* Computed toxicity factor (96 hr LC_{50}) showed that the detergent was about 1.73 times more toxic than the spent lubricant oil when tested against *C. africanus,* (Table 1).

The randomized analysis of variance (ANOVA) showed that there was significant difference (F_{cal} 43.58, F_{tab} 3.87; DF 7; $p<0.05$) between all the treatments (concentrations) at 24,48,72 and 96 hr of exposure of *C. africanus* to toxicants. Using Student Neumans-Keuls (SNK) test at 5% level of significance, the mean mortality response of *C. africanus* at 96hr of exposure to all concentrations of test media were significantly different from the control.

T. fuscatus: On the basis of 96 hr LC_{50} values, detergent with a toxicity value of 48.67 ml/l was more toxic than the spent lubricant oil when tested against *T. fuscatus* (Table 2). Computed toxicity factor (96 hr LC_{50} ratio) showed that the

Table – 2: Relative toxicity of spent engine oil and detergent against *Tympanotonus fuscatus*.

Test compounds (hr)	LC_{50}(95%CL)	LC_{95} (95%CL)	LC_5 (95%CL)	Slope±SD00	DF	Probit equation	TF_1	TF_2
Spent engine oil								
24	1176.99 (1683.86-822.73)	1632.63 (4088.77-650.66)	845.51 (8061.97-679.29)	11.610±10.25	2	Y = 30.651+ 11.610x	1.00	
48	1020.66 (1084.43-959.52)	1279.99 (1531.79-1068.14)	812.92 (885.59-747.24)	16.737±4.79	4	Y = 45.354 +16737x	1.15	
72	977.90 (1031.12-927.42)	1295.59 (1584.50-1052.65)	739.83 (849.35-645-52)	13.618±4.14	4	Y = 35.721+13.618x	120	
96	911.57 (945.64-874.71)	1182.54 (1361.96-1025.36)	702.68 (813.57-607.55)	14.597±3.99	4	Y = 38.204+14.597x	1.29	18.7
Detergent								
24	96.29 (156.00-63.28)	247.18 (963.89-53.32)	39.89 (68.90-23.18)	4.165±2.18	2	Y = 13.318+4.165x	1.00	
48	74.97 (89.97-62.50)	173.25 (324.87-92.13)	32.45 (48.24-21.90)	4.536±1.37	3	Y = 3.504+4.536x	1.32	
72	62.21 (69.73-55.51)	126.67 (175.16-91.31)	30.56 (39.71-23.59)	5.343±1.04	4	Y = 4.585+5.343x	160	
96	48.67 (53.93-43.91)	93.33 (116.07-74.80)	25.38 (32.92-19.62)	5.835±0.99	4	Y = 4.845+5.835x	2.04	1

TF_1 = Toxicity factor = $\frac{LC_{50} \text{ of test compound at 24 hr}}{LC_{50} \text{ of test compound at other hs (48,72, 96)}}$

TF_2 = Toxicity factor = $\frac{96h\ LC_{50} \text{ of detergent}}{96h\ LC_{50} \text{ of spent engine oil}}$

SD = Standard deviation
DF = Degree of freedom
CL = Confidence limit
LC = Lethal concentration

Table – 3: Relative susceptibility of test benthic macroinvertebrates against spent lubricant oil and detergent based on the 96 hr mortality data.

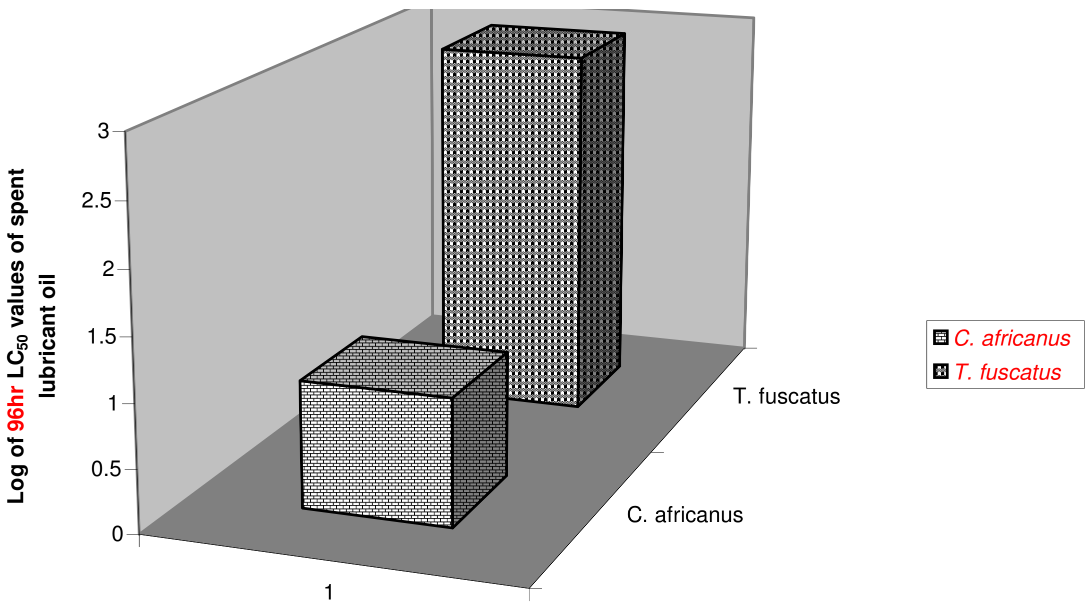
Animals	LC_{50} (95%CL) ml/l	Slope ± SD	DF	Probit line equation	SF
Spent lubricant oil					
C. africanus	10.01(11.11 – 9.02)	5.63±0.98	4	Y=0.64+5.633X	1
T. fuscatus	911.57(945.64 – 874.71)	14.597±3.99	4	Y=38.204+14.597X	91.06
Detergent					
C. africanus	5.77 (8.02 – 4.09)	2.005±0.386	4	Y=3.474+2.005X	1
T. fuscatus	48.67 (53.93 – 43.91)	5.835±0.99	4	Y=4.845+5.835X	8.46

S.F = Susceptibility/sensitivity factor = $\frac{96hr\ LC_{50} \text{ of other test animals}}{96hr\ LC_{50} \text{ of the most sensitive test animals}}$

detergent was about 18.73 times more toxic than spent lubricant oil against *T. fuscatus* (Table 2).

The analysis of variance (ANOVA) showed that there was significant difference (F_{cal} 36.73, F_{tab} 3.87; DF 7; $p<0.05$) between all concentrations at 24, 48, 72 and 96 hr of exposure of *T. fuscatus* to test media. Using Student Neuman – Keuls (SNK) test at p = 0.05, the mean mortality response of *T. fuscatus* exposure to toxicants was significantly different from that of the control. The median lethal concentration of the toxicants decreased as the duration of exposure increased denoting an increase in toxicity as the period of exposure increases. Analyses of variance showed a significant difference ($p< 0.05$) between all treatment mean mortality response of test animals at 24, 48, 72 and 96 hr of exposure to test media. Chukwu (2001), Nwaokoro (2001) Chukwu and Ugbeva (2003) have also demonstrated that the LC_{50} values of toxicants in exposed animals such as *Macrobrachium vollenhoevenii, Tilapia guineensis* and *Clibanarius africanus* deceased with time of exposure and test medium concentration.

The relative acute toxicity of spent lubricant oil and detergent against estuarine benthic macroinvertebrates, *C. africanus* (Aurivillus) and *T. fuscatus* revealed that the detergent was more toxic to the test organisms. The toxicity of dispersant against *C. africanus* 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 (1990) and Westermeyer (1991) could be attributed to the differences in the physical



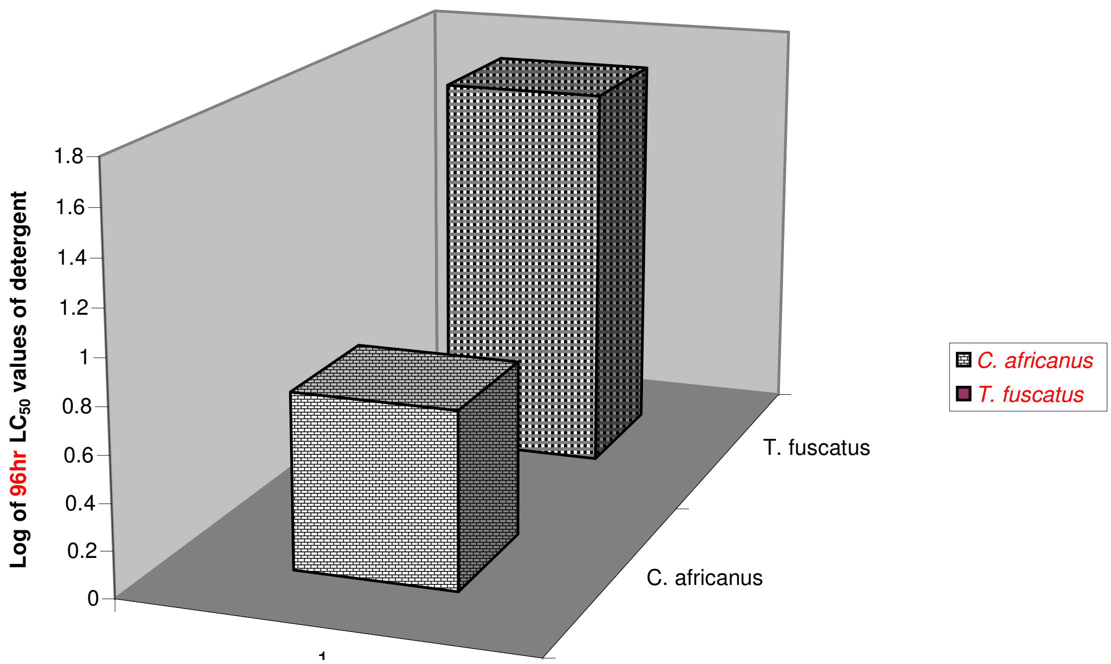


Fig.1: Relative toxicity of spent lubricant oil and detergent against test animal species based on 96 hr LC_{50} (sensitivity scale).

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 in flux of toxicants (Beynon and Cowell, 1974).

Relative sensitivity of test animal species to spent lubricant oil and detergent:

Detergent: *C. africanus* was found to be the more sensitive test species to the spent lubricant oil with a 96hr LC_{50} value of 5.77 ml/l followed by *T.fuscatus*, (Table 3; Fig. 1). On the basis of the computed sensitivity factors, *C. africanus* was found to be about 8.46 times more susceptible to the toxic effect of the detergent than *T. fuscatus* (Table 3).

Spent lubricant oil: *C. africanus* was also found to be the most sensitive test animal to the spent lubricant oil with a 96 hr LC_{50} value of 10.01 ml/l followed by *T. fuscatus* (Table 3; Fig. 1). On the basis of computed sensitivity factors, *C. fuscatus* was found to be about 91.06 times more susceptible to the toxic effect of spent lubricant oil than *T. fuscatus.*(Table 3).

The toxicity sensitivity ranking order for the test animals revealed that *C.africanus* was more susceptible to the toxicant than *T. fuscatus.* This result is consistent with the findings of other workers on the susceptibility patterns of benthic organisms of Lagos lagoon to toxicants (Otitoloju and Don-Pedro, 2002; Oyewo and Don-Pedro, 2003). It is suggested that the relatively lower tolerance or higher sensitivity of *C. africanus* to the toxic effect of petroleum toxicants may probably be due to the fact that the hermit crab tends to withdraw out of their acquired shells when stressed, thus making them more vulnerable to the toxicant (Chukwu, 2003).

The results obtained in this study suggest that the estuarine benthic macroinvertebrates, which play key roles in the environment, may serve as useful *in-situ* sentinels for biomonitoring studies of petroleum pollutants in fragile aquatic ecosystems such as the Lagos lagoon.

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