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Comparative Toxicity of Three Petroleum Products against Benthic Macroinvertebrate *Clibanarius africanus* of Lagos Lagoon

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Comparative toxicity testing of three petroleum products (Kerosene, Petrol and Diesel) were evaluated against hermit crab, *Clibanarius africanus* (Aurivillus) from University of Lagos Lagoon front within the Lagos Lagoon was carried out in the ecotoxicological laboratory using semi-static toxicity testing technique. The resulting lethal concentration (LC_{50}) for petrol was 22.08 ml/L; which was relatively more toxic compared to kerosene and diesel (33.50 ml/L and 36.08 ml/L) respectively. ANOVA analysis indicates that ($P<0.05$) was significant difference between the three petroleum products used throughout the acute toxicity test against *C. africanus*. Duncan test further divulged significant difference ($p<0.05$) in the mean mortality of the test organisms treated with the three petroleum products at all concentration including the control. The toxicity of the test chemicals increased with the period of exposure. Hence, deliberate or accidental discharges of petroleum products can cause serious hazard to the aquatic biota.

Keywords: Comparative acute toxicity; petroleum products; Clibanarius africanus; Lagos Lagoon; macro benthic invertebrate.

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1. INTRODUCTION

The current technological advancement in Nigeria has resulted in increased usage of petroleum products. Hence, high demand for petroleum products has subsequent effect on the aquatic and terrestrial environment. The constituent of Petroleum oil composed of various range of hydrocarbons compounds [1]. Nigeria is one of the major oil producing nations with large number of on/off shore oil installations [2]. The majority of bioassay on the toxicity of petroleum products required the use of aquatic animals [3]. These toxicity tests are illustrate by extremely variable outcome that are contingent with the nature of test organism and lifespan including the type and solubility of the petroleum products being assayed. Hermit crabs are decapods crustaceans; is one of the most common benthic organisms in Lagos Lagoon [4]. *Clibanarius africanus* is the most prominent species in Lagos Lagoon among the members of the benthic community in Nigerian coastal waters. Usually found aggregating in cluster of four or five on wood, stones and roots of plants. Hermit crabs are majorly deposit feeders and their abundance dependent on the food availability in the aquatic ecosystem [5]. Due to the potentially poisonous nature of refined petroleum products, it is imperative to ascertain the lethal concentration when sensitive species are exposed to different concentrations in order to infer the dose response relationship. Consequently, the derived lethal concentration based on the resulting toxicity indices are used as a tool in petroleum products contamination classification in order to obtained environmental safe limits as well as established toxicity scales of the pollutants [6]. These toxicity scales as the potential to provide significant possible environmental parameter of lowest risk to aquatic ecosystem. The objective of this study therefore; was to evaluate and compare acute toxic effects of three selected petroleum products against benthic macro invertebrate *Clibanarius africanus.*

2. MATERIALS AND METHODS

2.1 Study Area

Lagos lagoon is relatively among the largest Lagoon in the Gulf of guinea. Yewa, Ogun, Oshun and Ona rivers empty into the lagoon. Lagos Lagoon is surrounded by the Lagos Island and it's located between longitude 30 10' and 30 4' SE and latitude 60 5' and 60 36' N (see Fig. 1).

2.1.1 Test animals

C. africanus juvenile was used in these bioassay tests. They are specially selected during low tide at the shore of the University of Lagos Lagoon Front within the Lagos Lagoon into a plastic buckets with the sediment of the Lagoon acting as a substrate.

2.1.2 Test animals acclimatization

C. africanus collected were transported into the ecotoxicological laboratory and held in glass tanks of 113 cm x 80 cm half filled with Lagoon water. The experimental set-up in the glass tanks was aerated with a 220 V air pump and then Lagoon water was changed every 48 hr to avoid toxic waste metabolites accumulation. The test animals were left to adapt to laboratory conditions (28ºC±1ºC; 72.2% R.H) for 7 days before definitive test was commenced on hermit crabs in the bioassays laboratory in agreement with guidelines for bioassay technique [7]. The same range sizes of *Clibanarius africanus* with length of shell approximately 28±0.1 mm were selected for this bioassay.

2.1.3 Test chemical

Three petroleum products (kerosene, petrol and diesel) were purchased in gallon from nearby fuel station around the Lagos metropolis.

2.1.4 Bioassay containers

The experimental set-up of the assays was performed in glass tanks (22 cm x 18 cm). Tanks made with glass were chosen in order to reduce toxicants absorption and avoid risk of corrosion associated with plastic tanks.

2.1.5 Substrate preparation

Lagoon sediments are collected from the test organism sampling station and they were used as substrate in order to increase the sensitivity of the test organism to the toxicant in bioassay laboratory toxicity testing [8]. The collected sediment were sieved and weighed, 100 g of the sediment was used as substrate in each glass tanks according to procedural guideline [9].

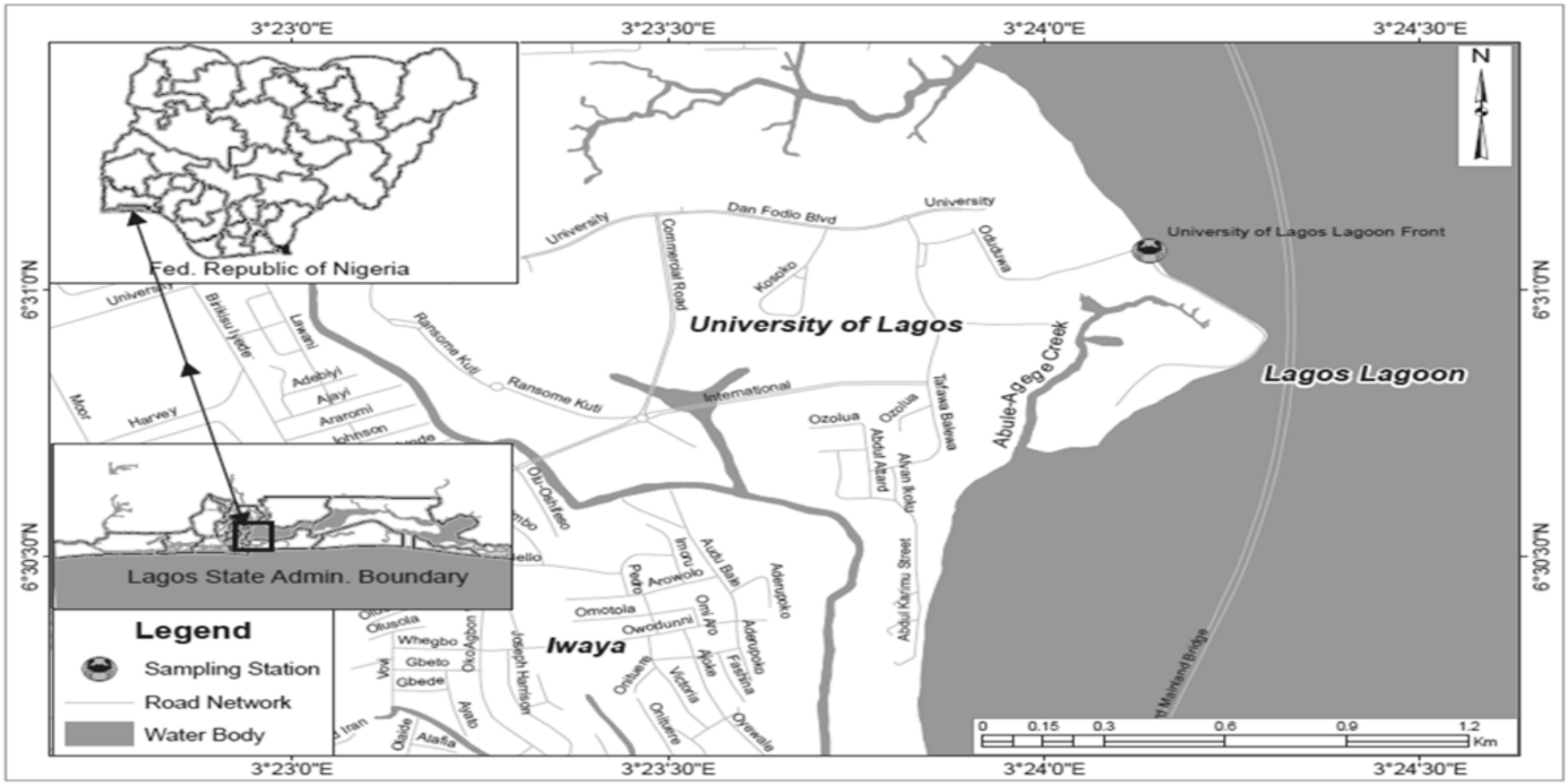

Fig. 1. Depicting the study area

2.1.6 Toxicants application to test media

Water from the sampling station was used as the medium for all the toxicity tests performed. Volumes of kerosene, petrol and diesel predetermined according to the range finding tests were measured using a measuring cylinder and introduced into the sediment substrate and approximately 1liter of the lagoon water were adding to each of the bioassay containers including the control tank. Assessment of quantal response: The test animals were placed in an observatory Petri dish and assumed to be dead if it failed to withdraw its legs into its shell upon poking with a glass rod during an observation period of 3-4 min. Assessment of mortality were carried out at defined time intervals of 24, 48, 72 and 96 hr.

2.1.7 Bioassays

C. africanus were treated with kerosene, petrol and diesel in a comparative acute toxicity. Hermit crabs of related sizes were placed randomly in the assays tanks. A total of 45 test organisms were exposed each of the test media including the control in replicate. Various concentrations were used against the test organisms. Each petroleum products used are as follows:

Kerosene: 20, 40, 60, 80, 100 ml/l and untreated control.
Diesel : 20, 40, 60, 80, 100 ml/l and untreated control.
Petrol : 20, 40, 60, 80, 100 ml/l and untreated control.

Analysis of toxicological dose response: Dose response relationships were analyzed by Probit equation [10]. Toxicity measurement indices derived from these analyses include:

Lethal concentration (LC_5) = concentration that cause 5% mortality of the exposed test animals to the test media.

(LC_{50}) = concentration that cause 50% mortality of the exposed test animals to the test media.

(LC_{95}) = concentration that cause 95% mortality of the exposed test animals to the test media.

Toxicity factor (T.F): comparative potency of an index chemical on the same test organisms.

2.2 Statistical Data Analysis

One way analysis of variance (ANOVA) using post hoc comparison of Duncan to test statistical difference in the derived toxicity results obtained during the 96hr exposure period.

3. RESULTS

Based on the derived toxicity results obtained during the period of exposure the lethal concentration (LC_{50}) values obtained for petrol (22.08ml/l;) was more toxic compared to kerosene (33.50ml/l) and diesel (36.08ml/l) when tested against C*. africanus* (Table 1). Figs. 2, 3 and 4 shows the progression of the lethal concentration for 96hr from period of exposure at 24hr interval in petrol, kerosene and diesel respectively. Fig. 5, depicts relative acute toxicity for *C. africanus treated with* three petroleum products. The statistical test on analysis of variance (ANOVA) indicate that there was significant difference ($p<0.05$) between all concentrations in each of the petroleum products used against *C. africanus* at 24, 48, 72 and 96hr of exposure. ANOVA post hoc comparison of Duncan test was significantly different ($P < 0.05$) from that of the control at mean quantal response of *C. africanus* exposure to the test media. The derived toxicity factor computed (96 hr LC_{50}) signified that the petrol was about 1.52 times more toxic than kerosene and 1.63 times more toxic than the diesel when tested against *C. africanus*, (Table 1). The mortality rate of hermit crab at different concentrations and time are shown on the table. No mortality was observed in the control samples during the experiment.

4. DISCUSSION

The relative acute toxicity of three petroleum products against estuarine benthic macro invertebrate, *C. africanus* indicated that the toxicity of petrol was higher on the hermit crabs compared to kerosene and diesel respectively. This agreed with the results obtained by other researcher such as Adeoye [11], and Ihedike [12]. The diversity of toxicity obtained in this study can be associated with the fact that petroleum products have various physical and chemical characteristics which influence the mode of action on the test organisms and the ability of the *C. africanus* to excrete and metabolized them especially the effect observed on petrol. It has been ascertain that petroleum products exert its toxicity effect on exposed organisms by coating the respiratory surface of the exposed animals thus restricting gaseous exchange [13]. This may possibly be attributed to the delayed quantal response of *C. africanus* to diesel and kerosene oil observed in this study.

Table 1. Relative toxicity of petroleum products against *C. africanus*

Test chemical	LC_5 (95%CL)	LC_{50} (95%CL)	LC_{95} (95%CL)	Slope±S.E	Probit equation	DF	TF
Diesel							
24hr	44.48 (0.39-6.35)	215.35 (19.59-139.07)	1042.46 (282.69-564.76)	0.761±0.11	Y=2.501*X+-5.802	3	1.82
48hr	20.81 (1.97-34.65)	134.04 (89.31-136.87)	8893.39 (289.93-218.65)	0.981±0.70	Y=2.1429*X+-4.535	3	1.79
72hr	9.99 (0.74-19.96)	74.09 (54.06-68.03)	549.32 (225.96-1659.75)	0.989±0.50	Y=2.1429*X+-3.857	3	1.92
96hr	8.15 (2.16-14.95)	36.08 (24.73-45.87)	152.98 (102.28-890.22)	0.896±0.51	Y=4.2851*X+-6.5714	3	1.63
Kerosene							
24hr	33.32 (4.24-50.92	118.24 (82.06-97.84)	419.61 (174.93-720.52)	0.999±0.30	Y=3.021*X+-6.201	3	1
48hr	19.86 (1.84-33.47)	129.27 (75.06-95.27)	841.52 (286.86-1318.91)	0.998±0.60	Y=2.143*X+-4.536	3	1.73
72hr	18.12 (4.96-28.49)	80.29 (62.65-125.61)	355.78 (189.03-2333.83)	0.908±0.45	Y=3.5714*X+-6.643	3	2.08
96hr	6.32 (0.89-12.77)	33.50 (20.23-44.14)	177.56 (109.86-637.13)	0.950±0.50	Y=2.8571*X+-4.214	3	1.52
Petrol							
24hr	18.64 (3.87-26.81)	167.43 (73.91-532.01)	1504.33 (87.35-123.61)	0.972±0.03	Y=2.012*X+-4.014	3	1.42
48hr	8.18 (0.07-18.24)	74.88 (49.41-9953.23)	685.93 (166.34-1648.10)	0.952±0.80	Y=2.453*X+-3.801	3	1
72hr	4.66 (0.10-13.29)	38.67 (22.75-75.25)	320.53 (117.06-269.56)	0.951±0.70	Y=3.12*X+-3.206	3	1
96hr	6.35 (0.78-11.81)	22.08 (11.93-27.98)	76.78 (54.84-260.62)	0.914±0.30	Y=4.273*X+-5.2574	3	1

$$TF = Toxicity\ factor = \frac{LC50\ of\ the\ test\ chemical\ \ less\ toxic\ at\ 24/48/72/96\ hr}{LC50\ of\ the\ test\ chemical\ most\ toxic\ at\ 24/48/72/\ 96hr}$$

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

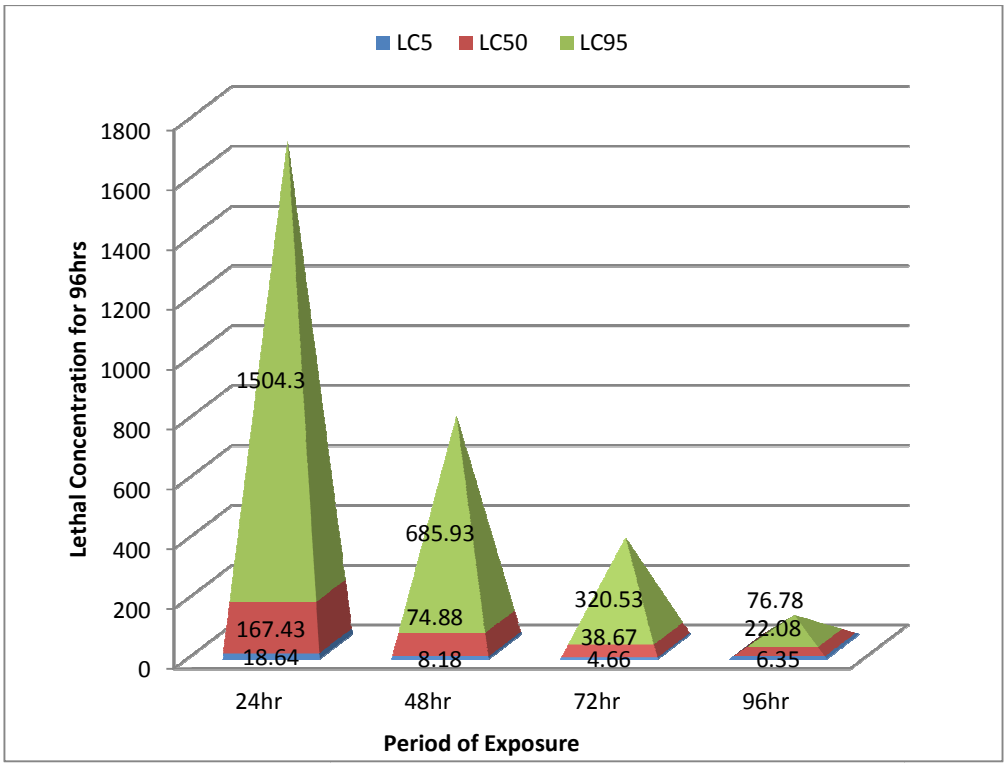

Fig. 2. Acute toxicity for *C. africanus* treated with petrol for 96 hr

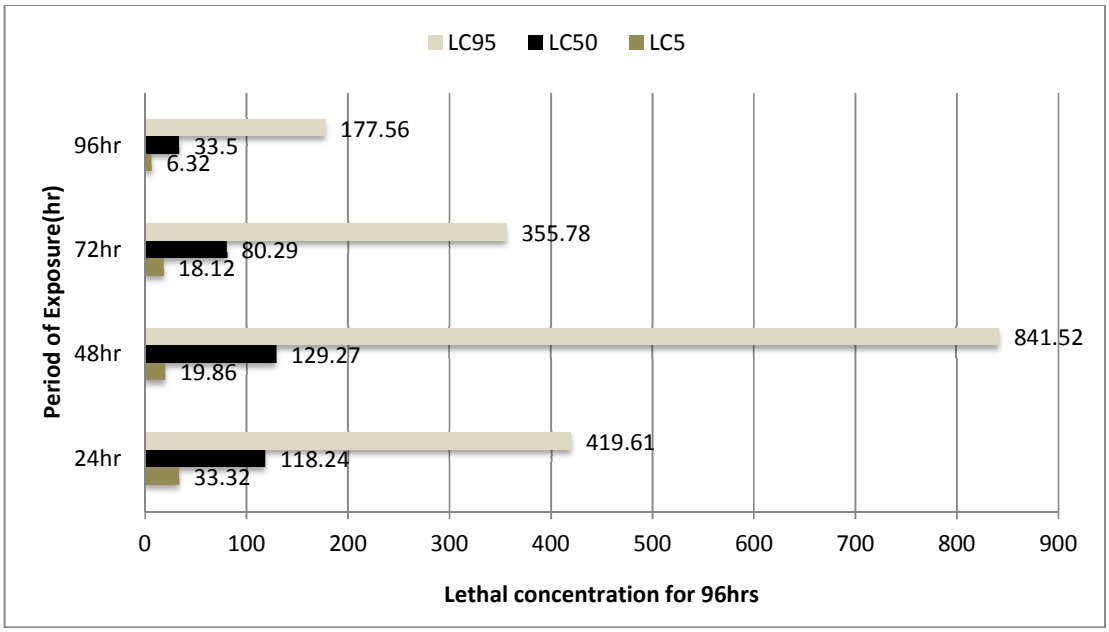

Fig. 3. Acute toxicity for *C. africanus* treated with Kerosene for 96 hr

Other factors that may account for the variation among toxicity of *C. africanus* against the three petroleum products used in this assays and its toxicity against other petroleum products include chemical composition, evaporization and weathering for instance Chukwu and Odunzeh

[13], these author obtained LC_{50} of 10.01 ml/l for *C. africanus treated with* spent lubricant oil in 96 hr exposure while Oribhabor et al. [14] derived LC_{50} of 45.2 ml/l for

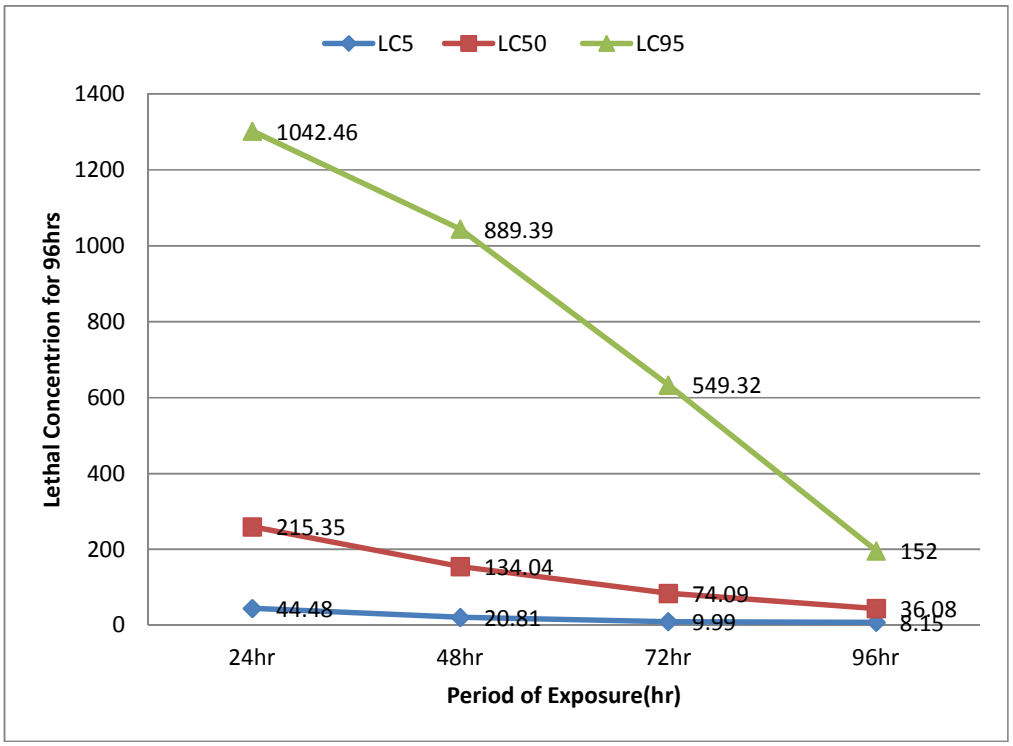

Fig. 4. Acute toxicity for *C. africanus* treated with diesel for 96 hr

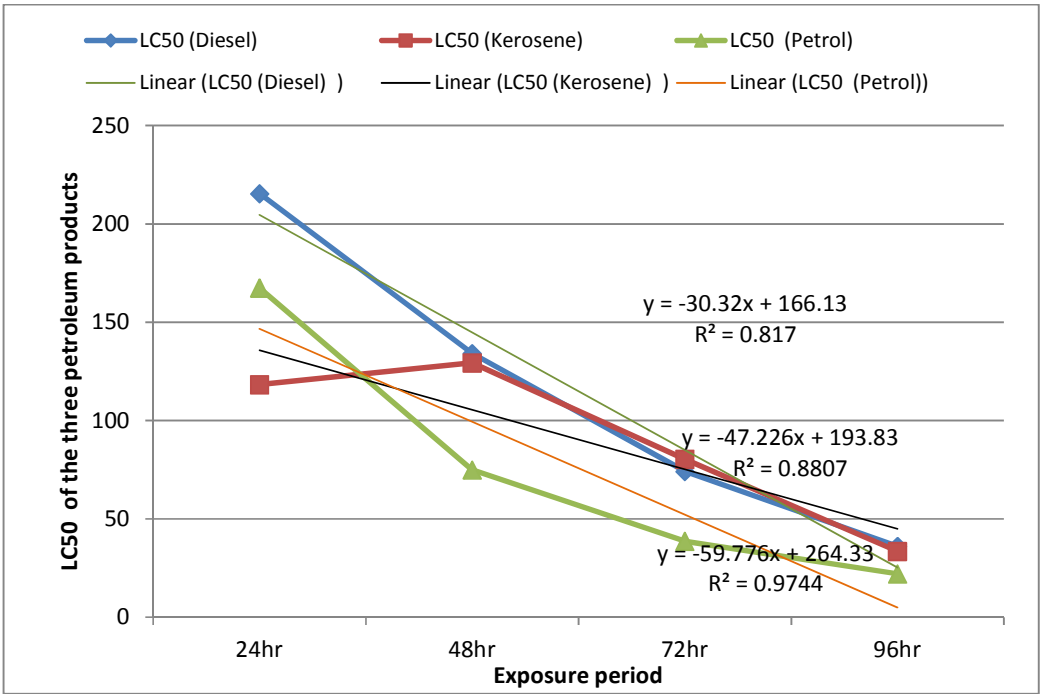

Fig. 5. Relative acute toxicity for *C. africanus treated with* three petroleum products for 96 hr

C. africanus (*hermit crabs*) against Bonny light crude oil for 96hr exposure period. These lethal concentrations obtained also differ compared to LC_{50} value results obtained for other crustacean in preceding studies. Ndimele et al. [15], the author reported 126 mg/l for shrimp *Desmocaris trispinosa* treated with Bonny light crude oil for 96 hr. Comparism of toxicological effects of petroleum products are difficult, because petroleum products have different hydrocarbon concentrations present in them and they varying according to their origin [16]. These properly result in difference in the LC_{50} results obtained for *C. africanus* toxicity using diverse petroleum products. The basis derived toxicity result obtained revealed that lethal concentration of each of the petroleum products used reduced as the period of exposure increased indicating an increase in toxicity potency of the test media.

5. CONCLUSION

The derived toxicity observed in these assays indicates that introduction of these refined petroleum products into the natural water bodies poses a serious threat to the biodiversity and general ecology of the aquatic ecosystem. Hence, stringent laws regarding the indiscriminate disposal and discharge of petroleum products either deliberately or accidentally into the aquatic biota should be enforced in order to achieve effective aquatic environmental protection.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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