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Eco-Toxicological Assessment of Local and Industrial Refined Kerosene on Pollution Bio-Monitor *Pseudomonas sp.* in Tri-Aquatic Ecosystem

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Authors' contributions

This work was carried out in collaboration between all authors. Author RRN designed the study, authors GEL and RRN performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors RRN, CGDO and GEL managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate eco-toxicity of local and industrial refined kerosene on pollution bio-monitor *Pseudomonas sp.* in tri-aquatic ecosystem (Marine, brackish and freshwater).
Study Design: The study employs experimental examination and statistical analysis of the data and interpretation. It was designed to evaluate the different kerosene concentration and the duration of exposure that could cause potential toxicological effect on *Pseudomonas sp.* in tri-aquatic ecosystem.
Place of Study: Fresh water, brackish water, and marine water samples were collected in four litre (4L) sterile containers. Fresh water sample was collected from Asarama Andoni; brackish water from Eagle Island while marine water was collected from Bonny River in Bonny L.G.A., all in Rivers state, Southern, Nigeria. The locally refined kerosene was gotten from Okrika mainland, while the industrially refined kerosene was obtained from Chinda filling station, UST roundabout, Mile 3 Port Harcourt. The study lasted for three months.

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Methodology: Standard microbiological techniques were used; toxicity procedure were applied using local and industrial refined kerosene; prepared at concentrations of 1.625%, 3.25%, 6.5%, 12.5% and 25% in fresh, brackish and marine water. These were tested with *Pseudomonas sp.* for 0, 4, 8, 12, and 24h separately for each toxicant. The cultures were incubated at 35°C for 24 hours. The median lethal concentration (LC_{50}) was employed to compute the toxicities of the different toxicants on the test organism.
Results: The results specify that percentage (%) logarithm of mortality of *Pseudomonas sp.* increases with increased toxicants concentration and exposure time. The pollution bio-monitor *Pseudomonas sp.* demonstrated sensitivity to the toxicity of local and industrially refined kerosene. The sensitivity showed variations, toxic level decreased in the following order (noting that the lower the LC_{50}, the more toxic the toxicants): Industrial refined kerosene in fresh water (18.80%) > Industrial refined kerosene in brackish water (20.81%) > Local refined kerosene in brackish water (21.48%) > Industrial refined kerosene in marine water (22.20%) > Local refined kerosene (24.26) > Local refined kerosene in marine water (24.92%). Industrial refined kerosene was seen to be more toxic in fresh water and local refined kerosene was found to be least toxic in marine water.
Conclusion: The study showed that industrial refined kerosene in fresh water (LC_{50} = 18.8%) has the highest toxicity strength while local refined kerosene in marine water (LC_{50} = 24.92%) has the least toxicity strength on *Pseudomonas sp.* in the tri-aquatic ecosystem. These results show that local and industrial refined kerosene can inhibit the growth of *Pseudomonas sp.* in an aquatic ecosystem; noting that *Pseudomonas sp.* is one of the most effective biodegrading bacteria in ecological biogeochemical cycles, pollutant removal/remediation and a key pollution bio-monitor.

Keywords: Local and industrial refined kerosene; eco-toxicity; median lethal concentration (LC_{50}), Pseudomonas sp.; fresh water; brackish water; marine water; tri-aquatic ecosystem.

1. INTRODUCTION

Petroleum-based products are the major source of energy for industry and daily life. Petroleum products consist of extremely complex mixture of aliphatic and aromatic hydrocarbons. The kerosene fractions, have been described as one of the greatest pollution problems in the environment [1,2]. Kerosene is a colourless, flammable hydrocarbon liquid derived from fractional distillation of petroleum at 150-275˚C. It consist of a characteristic odour and taste. Kerosene is insoluble in water, but miscible with most organic solvents. Kerosene possesses moderate to high acute toxicity to biota, with product-specific toxicity related to the type and concentration of aromatic compounds [3]. Kerosene serves as spray oil to combat insects on citrus plants. Numerous application of kerosene include: Aircraft gas turbine, as jet fuel for commercial airlines and military services [4]. Due to its wide application in several forms of transportation, there is increase in its production demand for transport, stockpiling, and distribution. This has brought with it an ever increasing problem of environmental pollution. Kerosene as well as other hydrocarbon spillage may result in damaging effect on associated microbial community due to its suffocation and toxic properties. Industrial refined kerosene properly refined devoid of impurities could also constitute environmental hazard due to poor handling and leakages from pipelines conveying them. Also kerosene has been refined locally due to the activities of economic saboteurs especially in the Niger Delta states such as Rivers, Bayelsa, and Delta leading to mass environmental pollution in the area. This kind is refined by unskilled personnel with improvised equipments, leading to the production of kerosene full of some impurities, and some other hydrocarbons. The traditional treatment of petroleum polluted water, such as containment and collection using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly [5,6]. So far, biodegradation suggests an effective method. During biodegradation, crude oil is used as an organic carbon source by a microbial process, resulting in the breakdown of crude oil components to low molecular weight compounds. However, *Pseudomonas sp.* have been found to degrade kerosene [7]. The degradation of kerosene is a sequential process in which n-alkanes are generally removed first followed by the degradation of iso-alkanes, cycloalkanes, 1-3 ring aromatics [8].

The aim of this study is to determine the toxicity of local and industrial kerosene on *Pseudomonas sp.* in tri-aquatic ecosystem.

2. MATERIALS AND METHODS

2.1 Study Site and Sample Collection

Samples were collected asceptically with sterile four litre (4L) plastic container. Fresh water sample was collected from Asarama Andoni; brackish water was gotten from Eagle Island while marine water was collected from Bonny River in Bonny L.G.A., all in Rivers state, South South, Nigeria.

2.2 Source of Toxicant (Local and Industrial Refined Kerosene)

The locally refined kerosene was gotten from Okrika mainland, while the industrially refined kerosene was obtained from Chinda filling station, UST roundabout, Mile 3 Port Harcourt

2.3 Isolation of Test Organism

The test organism *Pseudomonas sp.* was isolated using Pseudomonas agar with composition: Gelatin peptone (16 g), Casein hydrolysate (10 g), Potassium sulphate anhydrous (K2SO4) (10 g), Magnesium Chloride anhydrous (MgCl2) (1.4 g), Glycerol (10 ml), Agar (15 g), Reagent grade water (1 L), final pH 7.1±0.2

The base ingredients or the dehydrated complete base medium were suspended in 1L of water, heated to boiling in order to dissolve completely and sterilized at 121±3˚C/15 mins. The medium was allowed to cool (45-50˚C); then CN supplement rehydrated in 2 ml of sterile reagent grade water was added. It was then pour into plates immediately because the medium cannot be reheated. The plates were stored in the dark, protected from desiccation at 5±3˚C and used within one month.

2.4 Pseudomonas Broth

After Pseudomonas growth was observed and properly identified, the colony was aseptically transferred into Pseudomonas broth using a wire loop and incubated at 30˚C for 24 hrs; after which turbidity was observed, dilution that gave 35-50 colonies per aliquot (0.1 ml) of inoculums on agar were used.

2.5 Toxicity Test Procedure for the Bio-Monitor Pseudomonas Species

The acute toxicity bioassays were determined for a duration of 24 hours as described in the guidelines APHA, [9]; DPR, [10] (formally NNPC Inspectorate Division). The test was carried out in separate test tubes containing appropriate volume of filtered waters; FW, BW and MW from the organism's habitat. For each of the experimental set up, a toxicant in percentage (%) concentrations of 0, 1.625, 3.25, 6.5, 12.5 and 25 were added into tubes later inoculated with 1ml of test organism and loosely plugged with cotton wool and repeated for the other toxicant (as illustrated in Table 1). Aliquot (0.1 ml) of each concentration of the effluent was plated out immediately after inoculated onto Pseudomonas agar, this is known as zero hour count plating, then each was plated out after 4, 8, 12 and 24 hours onto Pseudomonas agar and incubated at 28± 2˚C for 24 hours. Plates were then counted as colony forming unit per millilitre (CFU/ml).

2.6 The Percentage Log Survival of Nitrobacter in Kerosene

The percentage log survival of the bacterial isolates in the kerosene effluent used in the study was calculated using the formula adopted by Williamson and Johnson [11]; Nrior and Obire, [12]. The percentage log survival of the bacterial isolates in the effluent was calculated by obtaining the log of the count in each toxicant concentrations (Log C), divided by the log of the count in the zero toxicant concentration (Log c) and multiplying by 100. Thus:

$$\% \text{ log survival} = (\text{Log C} / \text{Log c}) \times 100$$

2.7 The Percentage Log Mortality of Nitrobacter in Kerosene

The formula for the calculation of percentage (%) mortality was adopted from APHA, [9]. The percentage (%) log mortality was done by using the percentage (%) log survival in zero toxicant concentration to subtract the percentage (%) log survival. Thus: percentage (%) log mortality = % log survival in zero toxicant concentration (100) - percentage (%) log survival in test concentra-tions.

2.8 Statistical Analysis

The results from toxicity screening were subjected to statistical analysis using Analysis of Variance (ANOVA) and student t-test at 0.05 confidence limit (Reish and Oshida, [13]) to determine the significant difference between

Table 1. Toxicity test set-up using industrial and local refined kerosene on *Pseudomonas sp.* in Fresh Water (FW), Brackish Water (BW) and Marine Water (MW)

	Industrial refined kerosene (IRK)					Local refined kerosene (LRK)			
1	**Control (0%)**	**0.0 ml IRK**	**10 ml FW**	**1 ml**	**19**	**Control (0%)**	**0.0 ml LRK**	**10 ml FW**	**1 ml**
2	1.625%	0.16 ml IRK	9.84 ml FW	1 ml		1.625%	0.16 ml LRK	9.84 ml FW	1 ml
3	3.25%	0.33 ml IRK	9.67 ml FW	1 ml	**20**	3.25%	0.33 ml LRK	9.67 ml FW	1 ml
4	6.5%	0.65 ml IRK	9.35 ml FW	1 ml	**21**	6.5%	0.65 ml LRK	9.35 ml FW	1 ml
5	12.5%	1.25 ml IRK	8.75 ml FW	1 ml	**22**	12.5%	1.25 ml LRK	8.75 ml FW	1 ml
6	25%	2.5 ml IRK	7.5 ml FW	1 ml	**23**	25%	2.5 ml LRK	7.5 ml FW	1 ml
7	**Control (0%)**	**0.0 ml IRK**	**10 ml BW**	**1 ml**	**25**	**Control (0%)**	**0.0 ml LRK**	**10 ml BW**	**1 ml**
8	1.625%	0.16 ml IRK	9.84 ml BW	1 ml	**26**	1.625%	0.16 ml LRK	9.84 ml BW	1 ml
9	3.25%	0.33 ml IRK	9.67 ml BW	1 ml	**27**	3.25%	0.33 ml LRK	9.67 ml BW	1 ml
10	6.5%	0.65 ml IRK	9.35 ml BW	1 ml	**28**	6.5%	0.65 ml LRK	9.35 ml BW	1 ml
11	12.5%	1.25 ml IRK	8.75 ml BW	1 ml	**29**	12.5%	1.25 ml LRK	8.75 ml BW	1 ml
12	25%	2.5 ml IRK	7.5 ml BW	1 ml	**30**	25%	2.5 ml LRK	7.5 ml BW	1 ml
13	**Control (0%)**	**0.0 ml IRK**	**10ml MW**	**1ml**	**31**	**Control (0%)**	**0.0 ml LRK**	**10 ml MW**	**1 ml**
14	1.625%	0.16 ml IRK	9.84 ml MW	1 ml	**32**	1.625%	0.16 ml LRK	9.84 ml MW	1 ml
15	3.25%	0.33 ml IRK	9.67 ml MW	1 ml	**33**	3.25%	0.33 ml LRK	9.67 ml MW	1 ml
16	6.5%	0.65 ml IRK	9.35 ml MW	1 ml	**34**	6.5%	0.65 ml LRK	9.35 ml MW	1 ml
17	12.5%	1.25 ml IRK	8.75 ml MW	1 ml	**35**	12.5%	1.25ml LRK	8.75 ml MW	1 ml
18	25%	2.5 ml IRK	7.5 ml MW	1 ml	**36**	25%	2.5ml LRK	7.5 ml MW	1 ml

mortality of the test bacterium and toxicants, kerosene. The median lethal concentrations of toxicants with respect to bacterium with respect were calculated using regression analysis.

3. RESULTS AND DISCUSSION

Log survival count of *Pseudomonas sp.* at different concentrations of local and industrial refined kerosene.

The log survival count of *Pseudomonas sp.* at different concentrations (1.625, 3.25, 6.5, 12.5 and 25%) of petroleum products (local and industrial refined kerosene) at 0, 4, 8, 12, and 24h exposure in fresh, brackish and marine water as shown in Tables 2-4.

The effects of the release of kerosene into aquatic ecosystem were investigated. Rapidity, simplicity, low cost, small space and short generation time are among the many advantages in the use of bacteria as bioassay organism. It was observed that the microbial composition of chronic kerosene contaminated water samples include *Pseudomonas sp.* [14].

Tables 5-7 represent lethal toxicity as calculated from the log survival count of *Pseudomonas sp.* in tri-aquatic ecosystem using local and industrial refined kerosene.

The results of the log survival count show that *Pseudomonas sp.* has a very high kerosene tolerant capability using kerosene as its carbon

Table 2. Log survival count of *Pseudomonas sp.* in freshwater with local and industrial refined kerosene

	FW+Pseu+LRK						FW+Pseu+IRK				
	0 h	**4 h**	**8 h**	**12 h**	**24 h**		**0 h**	**4 h**	**8 h**	**12 h**	**24 h**
Ctrl 0%	1.415	1.176	1.301	1.505	1.380	**Ctrl 0%**	2.107	1.623	2.033	1.903	1.681
1.625%	1.322	1.204	1.204	1.301	1.380	**1.625%**	1.591	1.857	1.681	1.839	1.041
3.25%	1.362	1.080	1.114	1.380	1.176	**3.25%**	1.857	1.681	1.556	1.477	1.146
6.5%	1.531	1.322	1.279	1.204	1.079	**6.5%**	1.778	1.623	1.602	1.556	1.079
12.5%	1.230	1.301	1.079	1.342	0.845	**12.5%**	1.431	1.820	1.505	1.380	1.079
25%	1.478	1.322	1.322	1.279	1.176	**25%**	1.681	1.204	1.301	1.342	1

Key: FW= Freshwater, Pseu= Pseudomonas sp., LRK= Local Refined Kerosene, IRK= Industrial Refined Kerosene

Table 3. Log survival count of *Pseudomonas sp.* in brackish water with local and industrial refined kerosene

	BW+Pseu+LRK						BW+Pseu+IRK				
	0 h	4 h	8 h	12 h	24 h		0 h	4 h	8 h	12 h	24 h
Ctrl 0%	1.431	1.580	1.447	1.279	1.079	Ctrl 0%	1.886	1.672	1.477	1.643	1.176
1.625%	1.491	1.505	1.176	1.079	1.204	1.625%	1.869	1.544	1.398	1.431	1.079
3.25%	1.415	1.505	1.301	1	1.255	3.25%	1.602	1.491	1.477	1.230	1.230
6.5%	1.396	1.602	1.477	1.255	0.954	6.5%	1.699	1.462	1.230	1.623	1.204
12.5%	1.322	1.204	1.079	1.255	0.845	12.5%	1.505	1.398	1.176	1	0.903
25%	1.380	1.301	1.146	1.079	0.699	25%	1.505	1.398	1.322	1.204	1

Key: BW= Brackish water, Pseu= Pseudomonas sp., LRK= Local Refined Kerosene, IRK= Industrial Refined Kerosene

Table 4. Log survival count of *Pseudomonas sp.* in marine water with local and industrial refined kerosene

	MW+Pseu+LRK						MW+Pseu+IRK				
	0 h	4 h	8 h	12 h	24 h		0 h	4 h	8 h	12 h	24 h
Ctrl 0%	1.301	1.505	1.477	1.431	1.041	Ctrl 0%	2.017	1.982	1.681	1.380	1.681
1.625%	1.380	1.114	1.146	1.380	1.204	1.625%	2	1.857	1.748	1.556	1.114
3.25%	1.415	1.380	1.301	1.322	1.114	3.25%	1.813	1.505	1.568	1.602	1.301
6.5%	1.301	1.519	1.230	1.462	1	6.5%	1.924	1.964	1.806	1.681	1.301
12.5%	1.398	1.322	1.342	1.401	1.301	12.5%	1.716	1.748	1.778	1.643	1.362
25%	1.580	1.826	1.255	1.447	0.903	25%	1.602	1.505	1.531	1.322	1

Key: MW= Marine water, Pseu= Pseudomonas sp., LRK= Local Refined Kerosene, IRK= Industrial Refined Kerosene

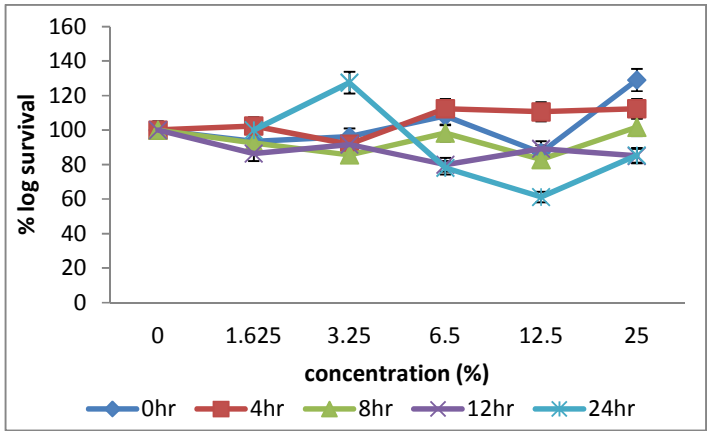

Fig. 1. Lethal toxicity of local refined kerosene on *Pseudomonas sp.* in fresh water

source. This result confirms the report of Alexander [15] and Nrior et al. [2] that certain bacteria do utilize petroleum hydrocarbons. Fig. 4 shows that the test organism showed reasonable growth even at 6.5% and 12.5% concentration of toxicant at 12h, Asikot and Antai, [16] reported a high optical density of 1.976 for *Pseudomonas sp.* hence their survivability in kerosene concentration at a high level of concentration. The ability of this hydrocarbon degrader to survive kerosene toxicity may be due to a number of resistance mechanisms such as efflux pump, enzyme-linked mediated resistance, genetic adaptation, level of metabolic activity within the biofilm, outer membrane structure [17,18,19,20].

Figs. 1-6 shows the percentage log survival of *Pseudomonas sp.* with different concentration of the toxicant (local and industrial refined kerosene) in fresh, brackish and marine water.

Table 5. Lethal toxicity of local and industrial refined kerosene on *Pseudomonas sp.* in fresh water

FW+Pseu+LRK						**FW+Pseu+IRK**				
Concentration	1.625%	3.25%	6.5%	12.5%	25%	1.625%	3.25%	6.5%	12.5%	25%
Control (%)	100	100	100	100	100	100	100	100	100	100
0 h										
% log survival	93.43	96.25	108.20	86.93	129.08	78.88	88.13	84.39	67.92	79.78
% log mortality	6.57	3.75	-8	13.07	-29.08	21.12	11.87	15.61	32.08	20.22
4 h										
% log survival	102.38	91.84	112.42	110.63	112.42	114.42	103.57	100.00	111.65	74.18
% log mortality	-2.38	8.16	-12.42	-10.63	-12.42	-14.42	-3.57	0	-11.65	25.82
8 h										
% log survival	92.54	85.63	98.31	82.94	101.61	82.69	76.54	78.92	74.03	63.99
% log mortality	7.46	14.37	1.69	17.06	-1.61	17.31	23.46	21.08	25.97	36.01
12 h										
% log survival	86.45	91.69	80.00	89.17	84.98	93.64	77.61	81.77	72.52	70.52
% log mortality	13.55	8.31	20	10.9	15.02	6.36	22.39	18.23	27.48	29.48
24 h										
% log survival	100.00	127.54	78.19	61.23	85.43	61.94	68.17	64.19	64.19	59.49
% log mortality	0	-27.54	21.81	38.77	14.57	38.06	31.83	35.81	35.81	40.51

Table 6. Lethal toxicity of local and industrial refined kerosene on *Pseudomonas sp.* in brackish water

FW+Pseu+LRK						**FW+Pseu+IRK**				
Concentration	1.625%	3.25%	6.5%	12.5%	25%	1.625%	3.25%	6.5%	12.5%	25%
Control (%)	100	100	100	100	100	100	100	100	100	100
0 h										
% log survival	104.19	98.88	97.69	92.38	96.44	99.10	84.94	90.08	79.80	79.80
% log mortality	-4.19	1.12	2.31	7.68	3.56	0.9	15.06	9.92	20.2	20.2
4 h										
% log survival	95.25	95.25	101.39	76.20	82.34	92.34	89.17	87.44	83.61	83.61
% log mortality	4.75	4.75	-1.39	23.8	17.66	7.66	10.83	12.56	16.39	16.39
8 h										
% log survival	81.27	89.91	100	74.57	79.20	83.61	100	83.28	79.62	89.51
% log mortality	18.73	10.09	0	25.43	20.8	16.39	0	16.27	20.38	10.49
12 h										
% log survival	84.36	78.19	98.12	98.12	84.36	87.10	74.86	99.33	60.86	73.28
% log mortality	15.64	20.81	1.88	1.88	15.64	12.9	25.14	0.67	39.14	26.72
24 h										
% log survival	111.58	116.31	88.12	78.13	64.78	91.75	104.59	102.38	76.79	85.03
% log mortality	-11.58	-16.31	11.88	21.69	35.22	8.25	-4.59	-2.38	23.21	14.97

Table 7. Lethal toxicity of local and industrial refined kerosene on *Pseudomonas sp.* in marine water

FW+Pseu+LRK						**FW+Pseu+IRK**				
Concentration	1.625%	3.25%	6.5%	12.5%	25%	1.625%	3.25%	6.5%	12.5%	25%
Control (%)	100	100	100	100	100	100	100	100	100	100
0 h										
% log survival	106.07	108.76	100	107.46	121.45	99.16	89.89	95.39	85.08	79.42
% log mortality	-6.07	-8.76	0	-7.46	-21.33	0.84	10.11	4.61	14.94	20.58
4 h										
% log survival	74.02	91.64	100.93	87.84	121.33	93.69	75.93	99.09	88.19	75.93
% log mortality	25.98	8.36	-0.93	12.16	-21.33	6.31	24.07	0.91	11.81	24.07
8 h										
% log survival	77.59	88.08	83.28	90.86	84.97	103.99	93.28	107.44	105.77	91.08
% log mortality	22.41	11.92	16.72	9.14	15.03	-3.99	6.72	-7.44	-5.77	8.92
12 h										
% log survival	96.44	92.38	102.17	72.75	101.12	112.75	116.06	112.81	119.06	95.80
% log mortality	3.56	7.62	-2.17	27.25	-1.12	-12.75	-16.09	-12.81	-19.06	4.2
24 h										
% log survival	115.66	107.01	96.06	124.98	86.74	66.27	77.39	77.39	81.02	85.61
% log mortality	-15.66	-7.01	3.94	-24.98	13.26	33.73	22.61	22.61	18.98	14.39

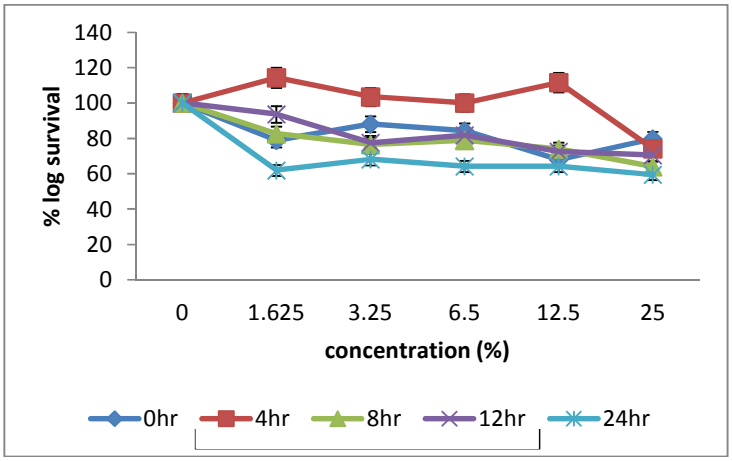

Fig. 2. Lethal toxicity of industrial refined kerosene on *Pseudomonas sp.* in fresh water

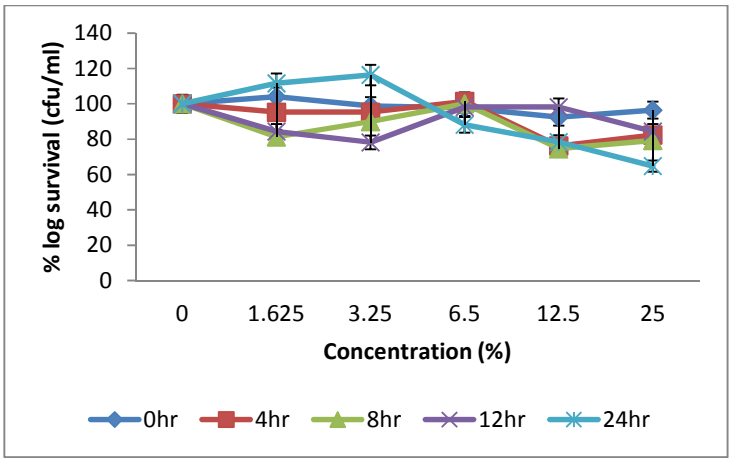

Fig. 3. Lethal toxicity of local refined kerosene on *Pseudomonas sp.* in brackish water

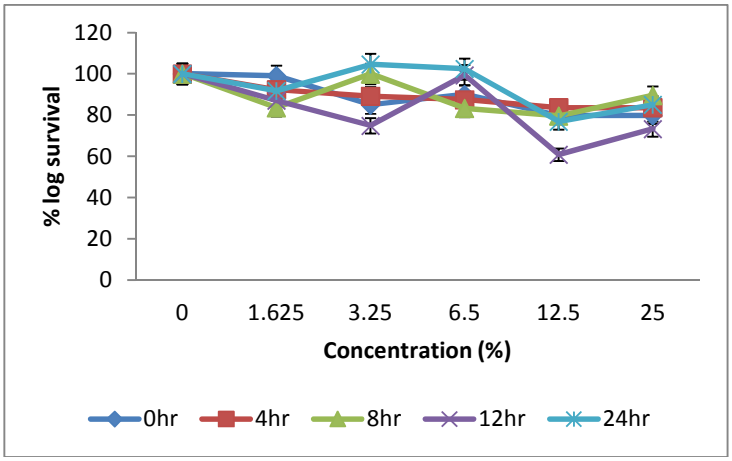

Fig. 4. Lethal toxicity of industrial refined kerosene on *Pseudomonas sp.* in brackish water

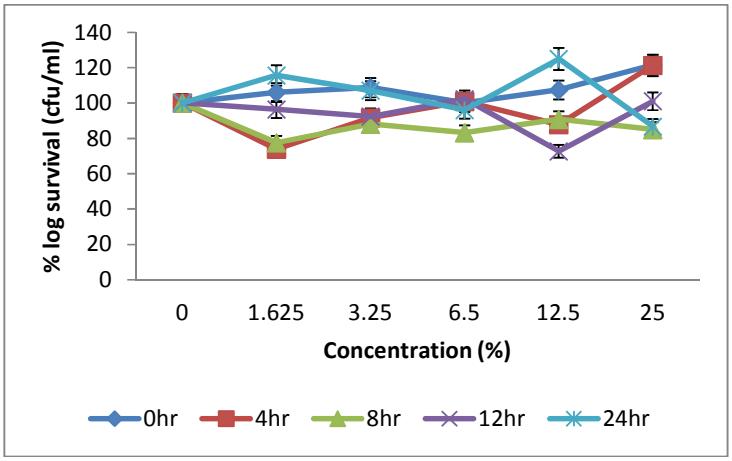

Fig. 5. Lethal toxicity of local refined kerosene on *Pseudomonas sp.* in marine water

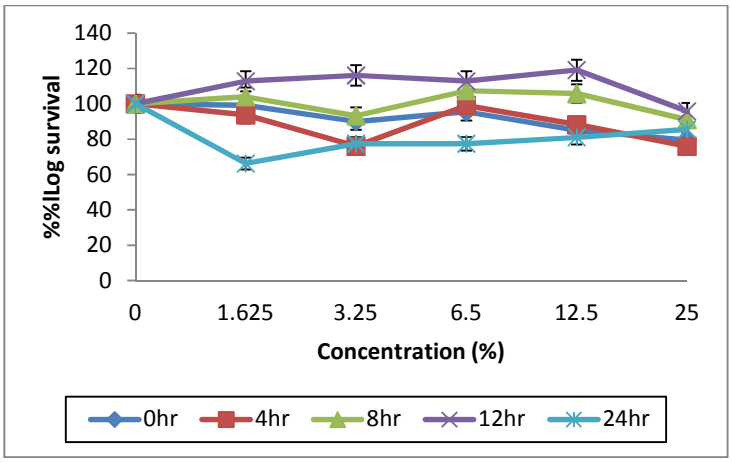

Fig. 6. Lethal toxicity of industrial refined kerosene on *Pseudomonas sp.* in marine water

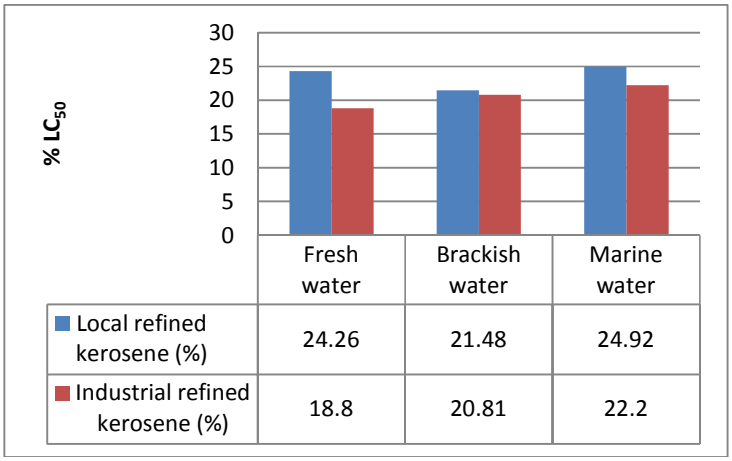

Fig. 7. Median lethal concentration (LC50) of local and industrial refined kerosene on *Pseudomonas sp.* in fresh, brackish and marine water

The sensitivity showed variations, toxic level decreased in the following order (noting that the lower the LC50, the more toxic the toxicants): Industrial refined kerosene in fresh water (18.80%) > Industrial refined kerosene in brackish water (20.81%) > Local refined kerosene in brackish water (21.48%) > Industrial refined kerosene in marine water (22.20%) > Local refined kerosene > (24.26) > Local refined kerosene in marine water (24.92%).

Industrial refined kerosene was seen to be more toxic in fresh water and local refined kerosene was found to be least toxic in marine water as shown in Fig. 7. The degree of degradation of kerosene and hence survivability of the microorganisms is largely dependent on the concentration of the kerosene contaminant in the medium with respect to the duration of exposure. Thus, it is expected that an increase in the concentration of the contaminant would result in further decrease in percentage log-survival of these bacterial. Hence, a decrease in microbial counts is indicative of susceptibility to kerosene toxicity.

4. CONCLUSION

The investigation revealed that due to the presence of more hydrocarbons in the local refined kerosene, than the industrial refined kerosene there was more colony count of *Pseudomonas sp.* in local refined kerosene contaminated water (marine, brackish and freshwater) than that of industrial refined kerosene. This investigation provides information that would lead to selection of bacterial species/strains that could be employed for bioremediation in environments polluted with petroleum and petroleum products, hydrocarbon utilizing microorganisms are important in combating the problem of oil pollution [21]. However, further studies need to be carried out to develop strains that would be more efficient in the utilization of the different fractions of petroleum hydrocarbons.

It is therefore recommended that: Routine monitoring of both physicochemical and microbial parameters of the aquatic ecosystem should be carried out so that any alteration of the parameters from the standard acceptable limit will be discovered and rectified immediately; avoidance of indiscriminate discharge of kerosene into the aquatic ecosystem, Government and oil companies should engage the services of qualified microbiologist to periodically evaluate the state of the aquatic ecosystem with respect to kerosene discharge and also discourage the activities of illegal bunkers so as to limit the incessant discharge of kerosene into the water ways.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salona-Serena F, Marchal R, Lebeault MJ, Vandeecasteele JP. Selection of microbial populations degrading recalcitrant hydrocarbons of gasoline by culture head space monitoring. Letters in Applied Microbiology. 2000;30:19-22.
2. Nrior RR, Ngerebara NN, Baraol RT, Amadi LO. Ecotoxicity of local and industrial refined kerosene on key environmental pollution monitor, *Nitrobacter* sp. in tri-aquatic systems in Nigeria. International Research Journal of Public and Environmental Health. 2017; 4(9):199-204.
3. Agarry SE, Owabor CN, Yusuf RO. Enhanced bioremediation of soil artificially contaminated with kerosene: Optimization of biostimulation agents through statistical experimental design. Journal of Petroleum Environmental Biotechnology. 2012;3:120.
4. Gouda MK, Omar SH, Nour-Eldin HM, Chekroud ZA. Sequential hydrocarbon biodegradation in a soil from arid coastal Australia, treated with oil under laboratory controlled conditions. Organic Geochemistry. 2008;39:1336-1346.
5. Ollis D. Slick solutions for oil spills. Nature. 1992;358:453–454.
6. Nrior RR, Akani NP, Wilcox A. Ecotoxicological assessment of Nigeria locally refined diesel and kerosene on *Aspergillus niger* a key fungal pollution biomarker. Asian Journal of Biology. 2018;6(4):1-8.
7. Chailan F, Fleche AL, Bury E, Phantavong Y, Grimont P, Saliot A, Oudot J. Bioremediation of kerosene II: A case study in contaminated clay (laboratory and field: Scale microcosms). World Journal Microbial Biotechnology. 2004;24:1451-1460.
8. Greenwood PF, Wibrow S, George SJ, Tibbet M. Identification and biodegradetion potential of tropical aerobic hydrocarbon degrading microorganisms.

Research Microbiology. 2008;155:587-595.
9. APHA, AWWA, WEF. American public health asssociation, american water works association, and water environment federation), standard methods for the examination of water and waste water. 21st ed., APHA, AWWA, WEF, Washington, DC; 2005.
10. Department of Petroleum Resources (DPR). Environmental guidelines and standards for the petroleum industry in Nigeria (EGASPIN) Revised Edition. 2002;277-288.
11. Williamson KJ, Johnson OG. A bacterial bioassay for assessment of wastewater toxicity. Water Research. 1981;15:383–390.
12. Nrior RR, Obire O. Toxicity of domestic washing bleach (*Calcium hypochloride*) and detergents on *Escherichia coli*. Journal of International Society of Comparative Education, Science and Technology (ICEST). 2015;2(1):124-135.
13. Reish OL, Oshida OS. Manual of method in aquatic Environment research. Part 10 – short-term static bioassays. FAO fisheries Technical Paper No. 247 Rome. 1987;62.
14. Ikpeme EM, Nfongeh JF, Etim L. Comparative remediation enhancement procedures on kerosene polluted utisol from Niger Delta Region, Southern Nigeria. Research Journal of Microbiology. 2007; 2(11):856-860.
15. Alexander M. Biodegradation and biore-mediation. San Diego, CA: Academic Press, Inc. 1994;302.
16. Asitok AD, Antai SP. Petroleum hydrocarbon utilization and biosurfactant production by *Pseudomonas* and *Bacillus* species. Nigeria Journal of Microbiology. 2006;20:824-883.
17. Alekshun MN, Levy SB. Molecular mechanisms of antimicrobial multidrug resistance. Cell. 2007;128(6):1037-1050.
18. Anderson DL. The biological cost of mutational antibiotic resistance: Any practical conclusion? Current Opinion in Microbiology. 2006;9(3):461-464.
19. Levy SB. Balancing the drug-resistant equation. Trends Microbiology. 1994;10: 341-342.
20. Prescott LM, Harley JP, Klien DA. Antimicrobial chemotherapy. Microbiology. 6th ed. McGraw-Hill, New York. 2005;779-796.
21. Atlas RM, Bartha R. Biodegradation of petroleum in soil environment at low temperatures. Journal of Microbiology. 1992;17:1652-1857.

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