Eco-toxicological assessment of local and industrial refined kerosene on pollution bio-monitor *Pseudomonas* sp. in tri-aquatic ecosystem

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.

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 sensivity 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.

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

21 solvents. Kerosene possesses moderate to high acute toxicity to biota, with product-specific toxicity related
22 to the type and concentration of aromatic compounds [3]. Kerosene serves as spray oil to combat insects
23 on citrus plants. Numerous application of kerosene include: aircraft gas turbine, as jet fuel for commercial
24 airlines and military services [4]. Due to its wide application in several forms of transportation, there is
25 increase in its production demand for transport, stockpiling, and distribution. This has brought with it an
26 ever increasing problem of environmental pollution. Kerosene as well as other hydrocarbon spillage may
27 result in damaging effect on associated microbial community due to its suffocation and toxic properties.
28 Industrial refined kerosene properly refined devoid of impurities could also constitute environmental hazard
29 due to poor handling and leakages from pipelines conveying them. Also kerosene has been refined locally
30 due to the activities of economic saboteurs especially in the Niger Delta states such as Rivers, Bayelsa,
31 and Delta leading to mass environmental pollution in the area. This kind is refined by unskilled personnel
32 with improvised equipments, leading to the production of kerosene full of some impurities, and some other
33 hydrocarbons. The traditional treatment of petroleum polluted water, such as containment and collection
34 using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil
35 thoroughly [5][6]. So far, biodegradation suggests an effective method. During biodegradation, crude oil is
36 used as an organic carbon source by a microbial process, resulting in the breakdown of crude oil
37 components to low molecular weight compounds. However, *Pseudomonas* sp. have been found to
38 degrade kerosene [7]. The degradation of kerosene is a sequential process in which n-alkanes are
39 generally removed first followed by the degradation of iso-alkanes, cycloalkanes, 1-3 ring aromatics [8].
40 The aim of this study is to determine the toxicity of local and industrial kerosene on *Pseudomonas* sp. in
41 tri-aquatic ecosystem.

2. MATERIALS AND METHOD

2.1 Study site and Sample Collection

47 Samples were collected asceptically with sterile four litre (4L) plastic container. Fresh water sample was
48 collected from Asarama Andoni; brackish water was gotten from Eagle Island while marine water was
49 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)

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

2.3 Isolation of Test Organism

58 The test organism *Pseudomonas* sp. was isolated using   *Pseudomonas* agar with composition: Gelatin
59 peptone (16g), Casein hydrolysate (10g), Potassium sulphate anhydrous (K_2SO_4) (10g), Magnesium
60 Chloride anhydrous ($MgCl_2$) (1.4g), Glycerol (10ml), Agar (15g), Reagent grade water
61 (1L), final pH 7.1±0.2
62 The base ingredients or the dehydrated complete base medium were suspended in 1L of water, heated to
63 boiling in order to dissolve completely and sterilized at 121 ± 3° C/15mins. The medium was allowed to cool
64 (45-50^oC); then CN supplement rehydrated in 2ml of sterile reagent grade water was added. It was then
65 pour into plates immediately because the medium cannot be reheated. The plates were stored in the dark,
66 protected from desiccation at 5 ± 3° C and used within one month.
67 ***Pseudomonas* broth**
68 After *Pseudomonas* growth was observed and properly identified, the colony was aseptically transferred
69 into *Pseudomonas* broth using a wire loop and incubated at 30^oC for 24hrs; after which turbidity was
70 observed, dilution that gave 35-50 colonies per aliquot (0.1ml) of inoculums on agar were used.

2.4 Toxicity Test Procedure for the bio-monitor *Pseudomonas* species

74 The acute toxicity bioassays were determined for a duration of 24hours as described in the guidelines
75 APHA, [9]; DPR, [10] (formally NNPC Inspectorate Division). The test was carried out in separate test
76 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 (0.1ml) 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 24hours onto *Pseudomonas* agar and incubated at 28 ± 2^0C for 24 hours. Plates were then counted as colony forming unit per millilitre (CFU/ml).

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

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

2.5 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

3

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:

% log survival = $\dfrac{\text{Log C}}{\text{Log c}}$ x100

2.6 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 concentrations.

2.7 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 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 table 2-4.

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

	FW+Pseu+LRK						FW+Pseu+IRK				
	0h	4h	8h	12h	24h		0h	4h	8h	12h	24h
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				
	0h	4h	8h	12h	24h		0h	4h	8h	12h	24h
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				
	0h	4h	8h	12h	24h		0h	4h	8h	12h	24h
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

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].

Table 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.

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

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

Fig 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.
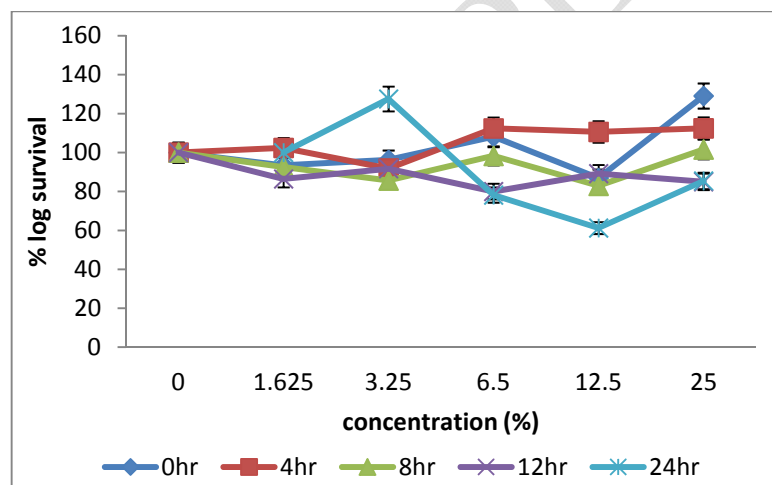

Fig. 1: Lethal toxicity of local refined kerosene on *Pseudomonas* sp. in fresh water.
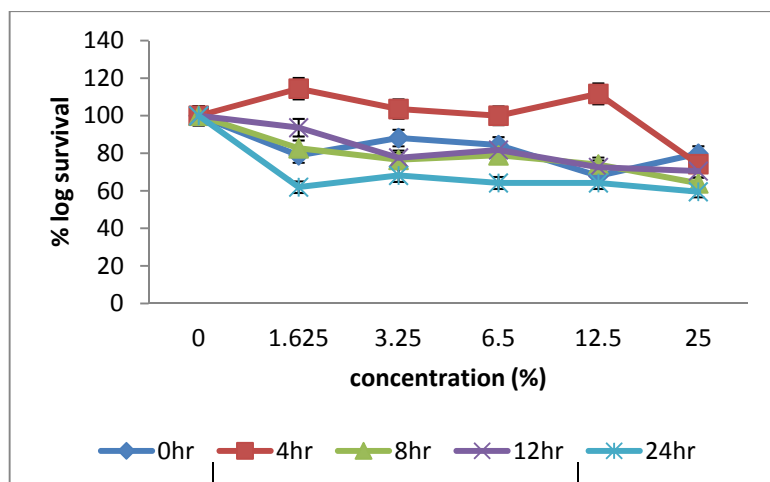
Fig 2: Lethal toxicity of industrial refined kerosene on *Pseudomonas* sp. in fresh water.
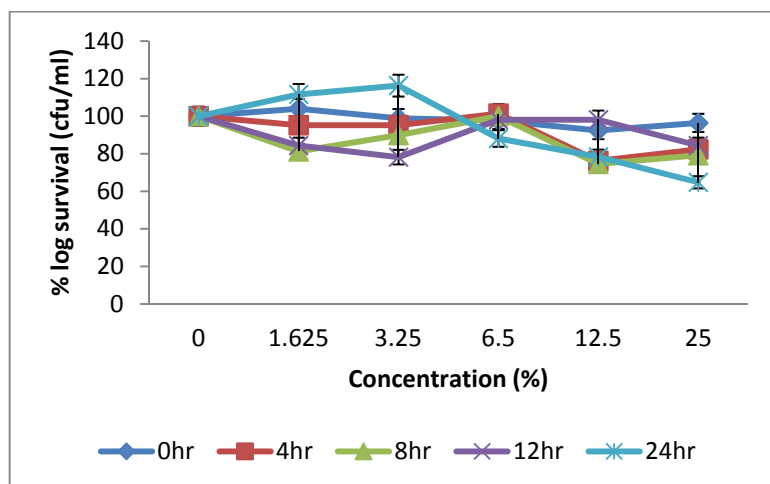

Fig 3: Lethal toxicity of local refined kerosene on *Pseudomonas* sp. in brackish water.
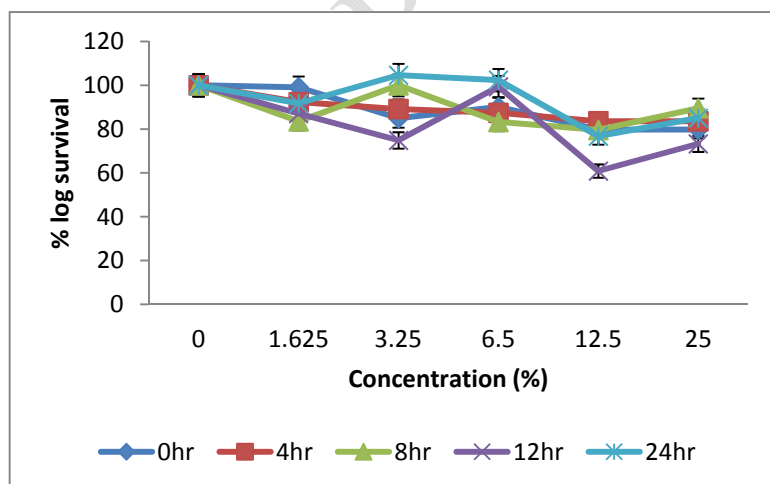

Fig. 4: Lethal toxicity of industrial refined kerosene on *Pseudomonas* sp. in brackish water.
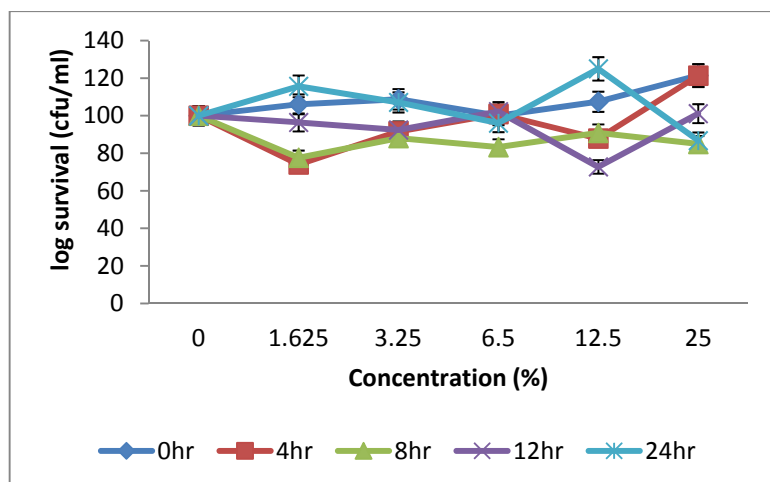
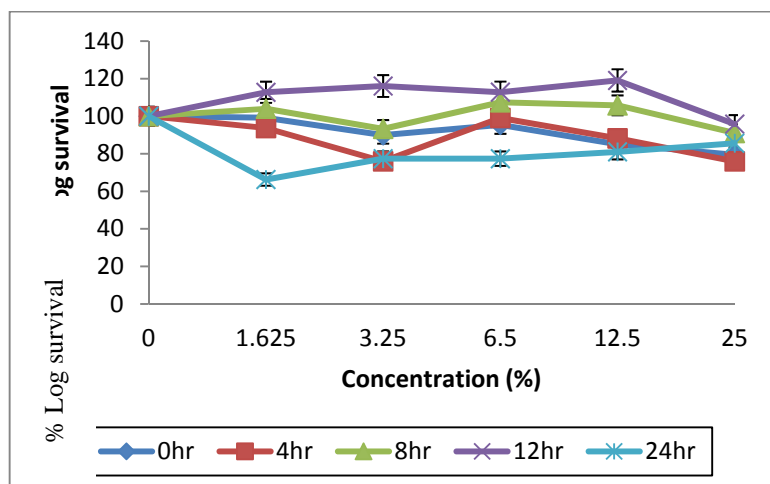Fig 5: Lethal toxicity of local refined kerosene on *Pseudomonas* sp. in marine water

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

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%).
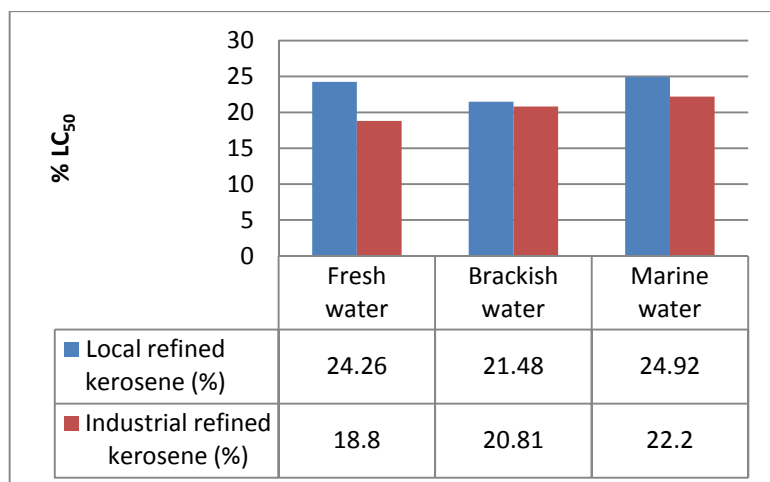
% LC_{50}	Fresh water	Brackish water	Marine water
■ Local refined kerosene (%)	24.26	21.48	24.92
■ Industrial refined kerosene (%)	18.8	20.81	22.2

Fig. 7 Median lethal concentration (LC_{50}) of local and industrial refined kerosene on *Pseudomonas* sp. in fresh, brackish and marine water

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. 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.

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.

221   4. Gouda, M.K., Omar, S.H., Nour-Eldin, H. M. and Chekroud, Z. A. (2008). Sequential hydrocarbon
222   biodegradation in a soil from arid coastal Australia, treated with oil under laboratory controlled
223   conditions. Organic Geochemistry. 2008;39:1336-1346.
224   5. Ollis D. Slick solutions for oil spills. Nature, 1992;358:453–454.
225   6. Nrior RR, Akani NP, Wilcox A. Ecotoxicological Assessment of Nigeria Locally Refined Diesel and
226   Kerosene on Aspergillus niger a Key Fungal Pollution Biomarker Asian Journal of Biology
227   2018;6(4):1-8.
228   7. Chailan F, Fleche AL, Bury E, Phantavong Y, Grimont P, Saliot A, Oudot J. Bioremediation of
229   kerosene II: A case study in contaminated clay (laboratory and field: scale microcosms). World
230   Journal Microbial Biotechnology, 2004;24:1451-1460.
231   8. Greenwood PF, Wibrow S, George SJ, Tibbet M. Identification and biodegradation potential of
232   tropical aerobic hydrocarbon   degrading microorganisms. Research Microbiology, 2008;155:
233   587-595.
234   9. APHA, AWWA and WEF. American Public Health Asssociation, American Water Works
235   Association, and Water Environ- ment Federation), Standard Methods for the Examination of
236   Water and Waste Water. 21st ed., APHA, AWWA, WEF, Washington, DC; 2005.
237   10. Department of Petroleum Resources (DPR). Environmental guidelines and standards for the
238   petroleum industry in Nigeria (EGASPIN) Revised Edition. 2002;277-288.

239   11. Williamson KJ, Johnson OG. A bacterial bioassay for assessment of wastewater toxicity. Water
240   Research, 1981;15: 383 – 390.
241   12. Nrior RR, Obire O. Toxicity of domestic washing bleach (Calcium hypochloride) and detergents on
242   Escherichia coli. Journal of International Society of Comparative Education, Science and
243   Technology (ICEST). 2015;2(1):124-135.
244   13. Reish OL, Oshida OS. Manual of method in aquatic Environment research. Part 10 – short-term
245   static bioassays. FAO fisheries Technical Paper No. 247 Rome. 1987;62.

246   14. Ikpeme EM, Nfongeh JF, Etim L. Comparative remediation enhancement procedures on kerosene
247   polluted utisol from Niger Delta Region, Southern Nigeria. Research Journal of Microbiology,
248   2007;2 (11): 856-860.
249   15. Alexander M. Biodegradation and bioremediation. San Diego, CA: Academic Press, Inc. 1994;302.

250   16. Asitok AD, Antai SP. Petroleum hydrocarbon utilization and biosurfactant production by
251   Pseudomonas and Bacillus species. Nigeria Journal of Microbiology, 2006;20:824-883.
252   17. Alekshun MN, Levy SB. Molecular mechanisms of antimicrobial multidrug resistance. Cell,
253   2007;128(6):1037-1050.

254   18. Anderson DL. The biological cost of mutational antibiotic resistance: any practical conclusion?
255   Current Opinion in Microbiology. 2006;9(3):461-464.
256   19. Levy SB. (1994). Balancing the drug-resistant equation. Trends Microbiology, 1994;10:341-342.
257   20. Prescott LM, Harley JP, Klien DA. (2005). Antimicrobial chemotherapy. Microbiology. 6th ed.
258   McGraw-Hill, New York. 2005;779-796
259   21. Atlas RM, Bartha R. Biodegradation of petroleum in soil environment at low temperatures. Journal
260   of Microbiology, 1992;17: 1652-1857.