Ecotoxicological Evaluatation Of Artisanal Effluents On *Bacillus, Enterobacter, Amorphotheca, Cladosporium* and *Penicillium* Species In Brackish Water

ABSTRACT

Aim: To assess the effect of the hydrocarbon discharges from the artisanal refineries on the community structure of microbial mats in an aquatic environment

Study design: The study employs experimental design, statistical analysis of the data and interpretation.

Place and Duration of Study: The microbial mats, surface water and sediments samples were collected from four hydrocarbon polluted stations (A, B, C and D) and a control sampling station in Yellow island (Iyalla kiri) in Degema Local Government Area, in Rivers state Nigeria. The samples were immediately transported with ice packs to the Microbiology Laboratory of Rivers State University, Port Harcourt. The study lasted from March 2020 to February 2021, covering both wet and dry seasons.

Methodology: Different concentrations of fresh effluent (0, 1.625, 3.25, 6.5, 12.5, 25, 50 and 75%) were prepared in test tubes to final volume of ~~10ml~~10 mL. Each of the test tubes was inoculated with one milliliter (~~1ml~~1 mL) of the test organism. Five sets of concentrations were prepared for the five test organisms (*Bacillus subtillis_MW808817, Enterobacter ludwigii_MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides_MW793722* and *Penicillium chrysogenum_MN184857*). The organisms were exposed to the pollutant for duration of 0, 4, 8, 12 and 24 h~~ours~~ and plated out using spread plate technique. The cultures were incubated for 24 h~~ours~~ for bacteria and five days for fungi. Median lethal concentration (LC_{50}) was determined using SPSS version 20.

Results: The results showed that the percentage logarithm survival of the test organisms decreased with increase in exposure time and concentration. The LC_{50} of *Bacillus subtillis_MW808817* was 30.93%, *Enterobacter ludwigii_MW767009 was 29.74%, Amorphotheca resinae_EU040230 was 19.65%, Cladosporium cladosporioides_MW793722* was 20.08% and *Penicillium chrysogenum_MN184857* was 17.77%, (noting; the lower the LC_{50} the more toxic the pollutant).

Conclusion: The effluent discharge was more toxic on *Penicillium chrysogenum_MN184857* than the other test organisms. Also, the ecotoxicological evaluation of the effluents on the test organisms isolated from the study area showed that LC_{50} of the effluent was slightly toxic on the microbial population when the results obtained were compared to GESAMP Standard for Toxicity Ranking of Chemicals/Effluents in Marine Environment.

Key words: ~~ecotoxicological~~ Ecotoxicological evaluation, ~~effluents~~Effluents, ~~percentage~~ Percentage mortality, ~~median~~ Median lethal concentration.

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INTRODUCTION

In the past years and even presently, environmental pollution has been acknowledged as one of the world's major concerns. Toxic compounds from industrial, agricultural and other anthropological activities, are released into the environment continuously. These harmful chemical discharges induce strong acute toxic effects and, in some cases, delayed biological responses from the exposed organisms in the environment. However, the consequences are more often delayed due to the

effects of bioaccumulation and biomagnification. Early detection of toxic chemical compounds in the environment particularly in water, and their biological effects on organisms has therefore, become increasingly important. It is interesting to know that chemical analysis of the discharges or pollutants only give an insight into the concentration(s) of the contaminants (whether or not they are above the baseline concentration and by what margin) but do not reveal the toxic effect(s) of the pollutants on the biota (1).

Harmful effects of contaminants on the ecosystem and humans cannot be assessed by standard chemical analyses of environmental samples, therefore, toxicity tests using live organisms or cells represent a vital part of environmental monitoring (1). This therefore, makes toxicological analysis an imperative. Many different biological methods based on the use of indigenous or genetically modified

microorganisms as test-species, have already successfully been applied to environmental toxicity/genotoxicity assessment. An important reason is the modern 3R concept (reduction, replacement, refinement) in toxicology and ecotoxicology, which encourages the use of microorganisms in biotests due to simple cultivation in axenic cultures and due to the lack of ethical problems (1). Initially, toxicity tests for environmental monitoring were based on multi-cellular eukaryotic organisms, particularly fish and mammals. This method was considered to be relatively expensive, time-consuming, difficult to standardize and ethically questionable. The need for alternative biological methods for environmental monitoring based on the 3R strategy soon became evident. The development and standardization of toxicity tests based on prokaryotic (bacteria) or eukaryotic (protozoa, unicellular algae, yeasts) microorganisms instead of higher organisms has enabled fast and inexpensive screening of environmental samples for toxicological analysis (1).

The traditional method of environmental pollution assessment based on chemical analysis of the environmental samples only give an insight on the concentrations of known chemicals in the samples without an adequate interpretation of its toxicity to the organisms in the context of bioavailability. This implies that, it only provides information about the potential, not actual toxicity of the pollutants. Moreover, chemicals that can cause toxic effect below the detection limit of chemical analytical methods or new compounds that are not yet deposited in the databases cannot be detected this way. Another disadvantage of chemical methods of environmental quality monitoring is the lack or scanty information available about the combined toxicity of different pollutants such as additive, synergistic or antagonistic effects (1). This study is therefore, intended to be a reference point in the use of prokaryotes for environmental monitoring protocols and also exposing the actual, not potential, effect of the petroleum releases on the indigenous microbial population in the study area.

MATERIALS AND METHODS

2.1 Study Area and Samples Collection

Petroleum hydrocarbon contaminated microbial mats, surface water and sediments from four sampling stations and control in the study area were examined. The study samples were collected in Yellow island or Iyalla kiri along the New Calabar River in Rivers State, Nigeria. Composite sampling method was adopted. Microbial mats and sediment samples were collected into sterile bottles with the aid of grab sampler. The surface water was also collected in sterile bottles. The samples were immediately conveyed to the laboratory for analysis in a cool box containing ice packs. The sites co-ordinates determined with GPS for sampling stations A, B, C and D were Station A 4.759588E and 6.982448W, Station B 4.754444E and 6.975733W, Station C 4.748003E

2.2 Determination of microbial loads of samples

Serial dilutions of the samples (microbial mats, sediments and surface water) from each of the sampling points including the control samples were done in sterile test tubes, using normal saline as the diluent. Aliquot (0.~~1ml~~1 mL) from $\times 10^5$ test tube was plated out on triplicate nutrient agar plates for total heterotrophic bacteria (THB) and $\times 10^3$ dilution for total fungi, hydrocarbon utilizing fungi (HUF) and hydrocarbon utilizing bacteria (HUB) after an initial range finding test was conducted.

Mineral salts agar (MSA) comprising of ~~Nacl~~NaCl, 10 g ; $MgSO_4.7H_2O$, 0.42 g ; KCl, 0.29 g ; KH_2PO_4, 0.53 g ; K_2HPO_4, ; NH_4NO_3, 0.42 g and 15 g of agar in ~~1litre~~ 1 L of distilled water was used for the cultivation of hydrocarbon utilizers, using vapour transfer technique (2). The enumeration of total heterotrophic bacteria (THB), hydrocarbon utilizing bacteria (HUB) and total heterotrophic fungi were done on the nutrient agar (Oxoid), mineral salts agar and sabouraud dextrose agar (Oxoid), respectively. Sterile filter papers were saturated with the pollutant hydrocarbon and placed inside the cover of the mineral salt agar plates.

The sabouraud dextrose agar plates and the mineral salts agar plates were incubated at inverted positions at room temperature for 2-7 days. The nutrient agar plates were incubated at 35^oC for 24hrs. The mean values of the colony counts from the triplicate plates were recorded.

2.3 Characterization and identification of isolates

The isolates were identified by conventional microbiological methods. They were characterized by cellular morphology, Gram stain reaction, motility and biochemical reactions. The organisms were identified

based on the standard key of Bergey`s Manual of Determinative Bacteriology (3), and molecular identification methods. The molecular identification method included: DNA extraction, 16S rRNA and internal transcribed spacer (ITS) amplifications, sequencing and phylogenetic analysis. The extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit. Pure cultures of the isolates were suspended in isotonic buffer in ZR Bashing Bead Lysis tubes and lysed by centrifuging at 10,000×g for 1 min~~ute~~. DNA elution buffer was added to the column matrix and centrifuged at 10,000x*g* for 30 seconds to elute the DNA. The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F and 1492R primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 20 ~~m~~µL~~icrolitres~~ for 35 cycles.

2.4 Experimental Design for toxicity evaluation

The experimental set up was modified from Obire and Nrior (4). Different concentrations of fresh effluent (0, 1.625, 3.25, 6.5, 12.5, 25, 50 and 75%) were prepared in test tubes to final volume of ~~10ml~~ 10 mL as shown in table 1. Into the test tubes with different effluent concentrations were added 1ml of microbial broth. Aliquots (0.~~1ml~~1 mL) of various concentrations in the different test tubes were immediately inoculated onto triplicate plates of nutrient agar and sabouraud dextrose agar at 0, 4, 8, 12 and 24 h. Colonies were counted in the triplicate plates and mean recorded.

Table 1: Experimental design for toxicity test, Obire and Nrior (9) modified

SN	Concentration (%)	Vol. of Diluent (~~ml~~mL)	Vol. of Effluent (~~ml~~mL)	Final Vol. (~~ml~~mL)	Microbial Broth (~~ml~~mL)
1	0 (control)	10.0	0	10.0	1.0
2	1.625	9.8375	0.1625	10.0	1.0
3	3.25	9.675	0.325	10.0	1.0
4	6.5	9.35	0.65	10.0	1.0
5	12.5	8.75	1.25	10.0	1.0
6	25	7.5	2.5	10.0	1.0
7	50	5.0	5.0	10.0	1.0
8	75	2.5	7.5	10.0	1.0

The percentage (%) log survival and percentage (%) log mortality were calculated according to Nrior *et al* (5). The median lethal concentration, LC_{50} was calculated using the formular:

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\% \text{ Control}}$$

2.4.1 Percentage (%) log survival

The percentage (%) log survival was calculated from the data obtained from the toxicity evaluation using the following formular used by Nrior *et al* (5).

$$\text{Percentage (\%) Log Survival} = \frac{\text{Log } \mathbf{C}}{\text{Log c}} \text{ x } 100$$

Where:
Log C is the log of count in each toxicant concentration
Log c is log of count in zero toxicant concentration (control), Nrior *et al* (5).

2.4.2 Percentage log mortality

The percentage log mortality was calculated using the following formular:
% Log mortality = % log survival in zero toxicant conc - % log survival in each toxicant conc
3
= 100 - % log survival in each toxicant concentration, Nrior *et al* (5)

3.0 RESULTS AND DISCUSSION

The percentage (%) log survival and percentage (%) log mortality of *Bacillus subtilis MW802817, Enterococcus ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides*

MW793722 and *Penicillium chrysogenum MN184857* at different concentrations of hydrocarbon effluents at 0, 4, 8, 12, 24 h exposure time in brackish water are shown in Tables 2-6.

Table 2: Lethal Toxicity Results of Hydrocarbon Effluent on *Bacillus subtilis MW802817* in *brackish water*.

Conc (%)	1.625	3.25	6.5	12.5	25	50	75
Control	100	100	100	100	100	100	100
Start (0 h)							
% Log Survival	83.77	81.14	78.07	74.12	68.85	65.35	59.64
% Log Mortality	16.23	18.86	21.93	25.88	31.15	34.65	40.36
4 h							
% Log Survival	79.74	77.58	73.27	69.82	65.94	59.48	49.13
% Log Mortality	20.26	22.42	26.73	30.18	34.06	40.52	50.87
8h							
% Log Survival	71.00	61.76	54.62	49.15	43.69	32.35	25.21
% Log Mortality	29.00	38.24	45.38	50.85	56.31	67.65	74.79
12 h							
% Log Survival	56.19	51.65	45.86	41.32	34.71	24.79	19.42
% Log Mortality	43.81	48.35	54.14	58.68	65.29	75.21	80.58
24 h							
% Log Survival	48.97	45.83	31.42	28.16	19.18	12.24	12.24
% Log Mortality	51.03	54.17	68.58	71.84	80.82	87.76	87.76

Table 3: Lethal toxicity of effluent on *Enterobacter ludwigii MW767009* in brackish water

Conc (%)	**1.625**	**3.25**	**6.5**	**12.5**	**25**	**50**	**75**
Control	100	100	100	100	100	100	100
Start (0h)							
% Log Survival	88.63	86.81	83.63	80.00	72.72	69.54	65.45
% Log Mortality	11.37	13.19	16.37	20.00	27.28	30.46	34.55
4h							
% Log Survival	75.30	69.95	67.07	63.37	58.02	46.91	41.15
% Log Mortality	24.70	30.05	32.93	36.63	41.98	53.09	58.85
8h							
% Log Survival	68.57	60.00	53.06	47.75	38.77	34.28	28.16
% Log Mortality	31.43	40.00	46.94	52.25	61.23	65.72	71.84
12h							
% Log Survival	48.78	40.65	36.58	31.30	28.04	19.10	19.10
% Log Mortality	51.22	59.35	63.42	68.70	71.96	80.90	80.90
24h							
% Log Survival	38.61	38.61	34.14	28.04	24.39	12.19	12.19
% Log Mortality	61.39	61.39	65.86	71.96	75.61	87.81	87.81

Table 4: Lethal toxicity of effluent on *Amorphotheca resinae EU040230* in brackish water

Conc (%)	1.625	3.25	6.5	12.5	25	50	75
Control	100	100	100	100	100	100	100
Start (0 h)							
% Log Survival	90.00	90.00	77.00	69.00	60.00	47.00	47.00
% Log Mortality	10.00	10.00	23.00	31.00	40.00	53.00	53.00
4 h							
% Log Survival	78.50	71.96	56.07	56.07	43.92	43.92	28.03
% Log Mortality	21.50	28.04	43.93	43.93	56.08	56.08	71.97
8 h							
% Log Survival	62.16	62.16	54.05	54.05	27.02	27.02	0.00
% Log Mortality	37.84	37.84	45.95	45.95	72.98	72.98	100.0
12 h							
% Log Survival	58.97	40.17	40.14	25.64	0.00	0.00	0.00

% Log Mortality	41.03	59.83	59.86	74.36	100.0	100.0	100.0
24 h							
% Log Survival	50.00	39.16	25.00	25.00	0.00	0.00	0.00
% Log Mortality	50.00	60.84	75.00	75.00	100.0	100.0	100.0

Table 5: Lethal toxicity of effluent on *Cladosporium cladosporioides MW793722* in brackish water

Conc (%)	1.625	3.25	6.5	12.5	25	50	75
Control	100	100	100	100	100	100	100
Start (0 h)							
% Log Survival	94.87	85.47	76.92	71.79	58.97	51.28	51.28
% Log Mortality	5.13	14.53	23.08	28.21	41.03	48.72	48.72
4 h							
% Log Survival	83.20	76.00	67.20	55.20	37.60	37.60	24.00
% Log Mortality	16.80	24.00	32.80	44.80	62.40	62.40	76.00
8 h							
% Log Survival	67.16	62.68	51.49	44.77	35.07	22.38	22.38
% Log Mortality	32.84	37.32	48.51	55.23	64.93	77.62	77.62
12 h							
% Log Survival	55.39	49.64	33.81	21.58	0.00	0.00	0.00
% Log Mortality	44.61	50.36	66.19	78.42	100.0	100.0	100.0
24 h							
% Log Survival	47.26	41.09	20.54	20.54	0.00	0.00	0.00
% Log Mortality	52.74	58.91	79.46	79.46	100.0	100.0	100.0

Table 6: Lethal toxicity of effluent on *Penicillium chrysogenum MN184857* in brackish water

Conc (%)	1.625	3.25	6.5	12.5	25	50	75
Control	100	100	100	100	100	100	100
Start (0 h)							
% Log Survival	93.45	88.78	78.50	71.96	64.48	43.92	43.92
% Log Mortality	6.55	11.22	21.50	28.04	35.52	56.08	56.08
4 h							
% Log Survival	76.92	71.79	58.97	51.28	51.28	40.17	25.64
% Log Mortality	23.08	28.21	41.03	48.72	48.72	59.83	74.36
8 h							
% Log Survival	59.23	46.15	36.15	36.15	23.07	23.07	0.00
% Log Mortality	40.77	53.85	63.85	63.85	76.93	76.93	100.0
12h							
% Log Survival	50.73	34.55	22.05	22.05	0.00	0.00	0.00
% Log Mortality	49.27	65.45	77.95	77.95	100.0	100.0	100.0
24 h							
% Log Survival	33.81	21.58	21.58	0.00	0.00	0.00	0.00
% Log Mortality	66.19	78.42	78.42	100.0	100.0	100.0	100.0

3.1 Lethal toxicity of hydrocarbon effluents on microbial isolates

The lethal toxicity of hydrocarbon effluents on *Bacillus subtilis MW802817, Enterobacter ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* at different concentrations of effluents at 0, 4, 8, 12, 24 h exposure time in brackish water were shown in figures 1-5.

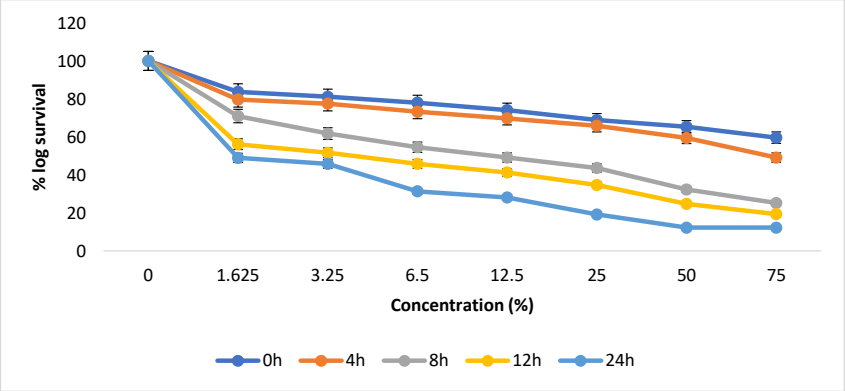

Fig 1: Lethal toxicity of hydrocarbon effluents on *Bacillus subtilis* MW802817 in brackish water

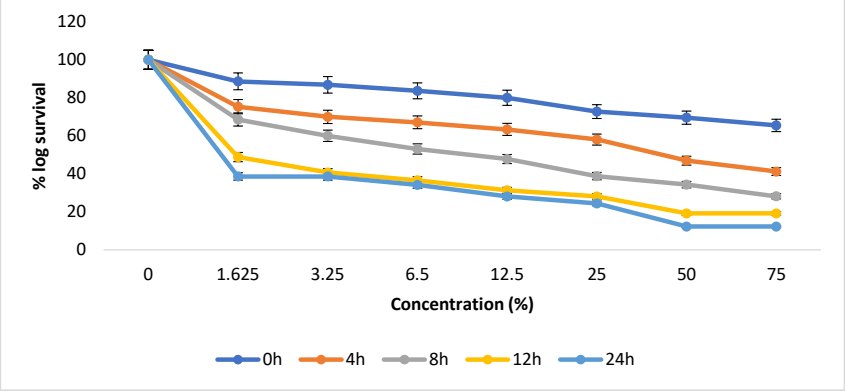

Fig 2: Lethal toxicity of hydrocarbon effluents on *Enterobacter ludwigii* MW767009 in brackish water

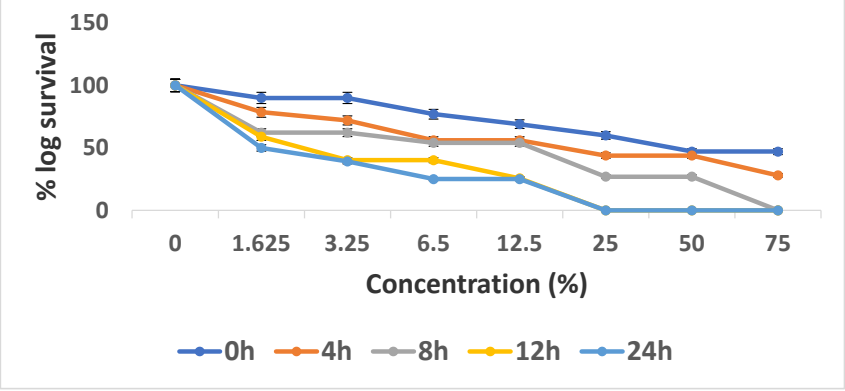

Fig 3: Lethal toxicity of hydrocarbon effluents on *Amorphotheca resinae EU040230* in brackish water

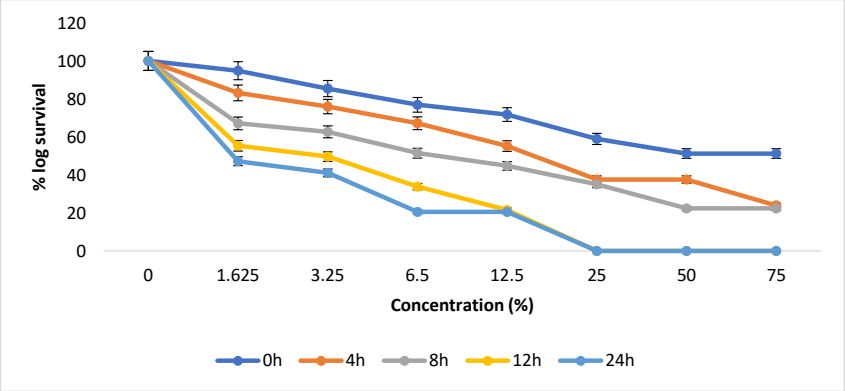

Fig 4: Lethal toxicity of hydrocarbon effluents on *Cladosporium cladosporioides MW793722* in brackish water

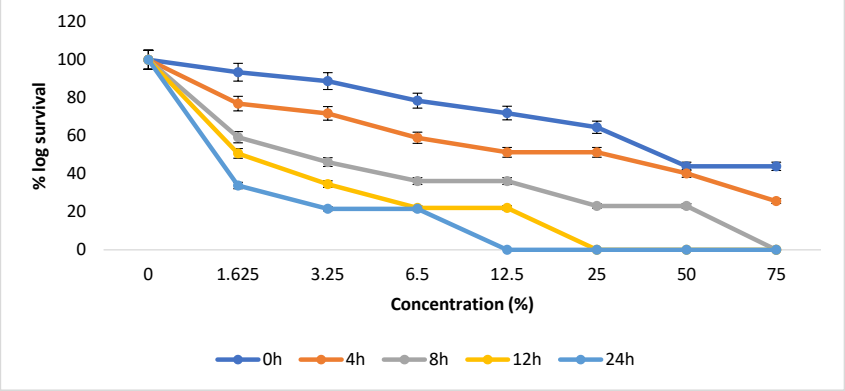

Fig 5: Lethal toxicity of hydrocarbon effluents on *Penicillium chrysogenum* MN184857 in brackish water

3.2 Median lethal toxicity (LC_{50}) of hydrocarbon effluents on microbial isolates

The median lethal toxicity, LC_{50} of hydrocarbon effluents on *Bacillus subtilis MW802817, Enterobacter ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* at different concentrations at 0, 4, 8, 12, 24 h exposure time in brackish water are shown in Tables 7-11.

Table 7: Median lethal concentration, LC_{50} for *Bacillus subtilis MW802817*

Dose	% Mortality	Mean % Mortality	Dose difference	Σ Dose diff × Mean % mortality
0%	-	-	-	-
1.625%	160.33	32.066	1.625	52.10725
3.25%	182.04	36.408	1.625	59.163
6.5%	216.76	43.352	3.25	140.894
12.5%	237.43	47.486	6	284.916
25%	267.63	53.526	12.5	669.075
50%	305.79	61.158	25	1,528.95
75%	334.36	66.872	25	1,671.8
				Σ = 4,406.90525

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\text{\% Control}}$$

$$= 75 - \frac{4406.90}{100}$$

= 75 – 44.069

= 30.931

LC_{50} = 30.93%

Table 8: Median lethal concentration, LC_{50} for *Enterobacter ludwigii MW767009*

Dose	% Mortality	Mean % Mortality	Dose Difference	Σ Dose diff × Mean % mortality
0%	-	-	-	-
1.625%	180.11	36.022	1.625	58.53575
3.25%	203.98	40.796	1.625	66.2935
6.5%	225.52	45.104	3.25	146.588
12.5%	249.54	49.908	6	299.448
25%	278.06	55.612	12.5	695.15
50%	317.98	63.596	25	1,589.9
75%	333.95	66.79	25	1,669.75
				Σ = 4,525.66525

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\text{\% Control}}$$

$$= 75 - \frac{4525.67}{100}$$

= 75 – 45.256

= 29.744

LC_{50} = 29.74%

Table 9: Median lethal concentration, LC_{50} for *Amorphotheca resinae EU040230*

Dose	% Mortality	Mean % Mortality	Dose Difference	Σ Dose diff × Mean % mortality
0%	-	-	-	-
1.625%	160.37	32.074	1.625	52.12025
3.25%	196.55	39.31	1.625	63.87875
6.5%	247.74	49.548	3.25	161.031
12.5%	270.24	50.048	6	300.288
25%	369.06	73.812	12.5	922.65
50%	382.06	76.412	25	1,910.3
75%	424.97	84.994	25	2,124.85
				Σ = 5,535.118

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\text{\% Control}}$$

$$= 75 - \frac{5{,}535.118}{100}$$

= 75 – 55.35

= 19.65

LC_{50} = 19.65%

Table 10: Median lethal concentration, LC_{50} for *Cladosporium cladosporioides MW793722.*

Dose	% Mortality	Mean % mortality	Dose Difference	Σ Dose diff × Mean % Mortality
0%	-	-	-	-
1.625%	152.12	30.424	1.625	49.439
3.25%	185.12	37.024	1.625	60.164
6.5%	250.04	50.008	3.25	162.526
12.5%	286.12	57.224	6	343.344
25%	368.36	73.672	12.5	920.9
50%	388.74	77.748	25	1,943.7
75%	402.34	80.468	25	2,011.7
				Σ = 5,491.773

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\text{\% Control}}$$

$$= 75 - \frac{5{,}491.77}{100}$$

= 75 – 54.92

= 20.08

LC_{50} = 20.08%

Table 11: Median lethal concentration, LC_{50} for *Penicillium chrysogenum MN184857.*

Dose	% Mortality	Mean % mortality	Dose Difference	Σ Dose diff × Mean % Mortality
0%	-	-	-	-
1.625%	185.86	37.172	1.625	60.4045
3.25%	237.15	47.43	1.625	77.07375
6.5%	282.75	56.55	3.25	183.7875
12.5%	318.56	63.712	6	382.272
25%	361.17	72.234	12.5	902.925
50%	392.84	78.568	25	1,964.2
75%	430.44	86.088	25	2,152.2
				Σ = 5,722.86275

$$LC_{50} = LC_{100} - \frac{\sum \text{Dose Difference x Mean \% Mortality}}{\text{\% Control}}$$

$$= 75 - \frac{5{,}722.86}{100}$$

$$= 75 - 57.23$$

$$= 17.77$$

$$LC_{50} = 17.77\%$$

The study showed the toxicological effect of petroleum releases into the environment on individual microbes. *Bacillus subtilis MW802817, Enterobacter ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* were identified as the dominant hydrocarbon utilizers in the study samples during the period of investigation from March 2020 to February 2021. These organisms were therefore, used for toxicity evaluation using fresh petroleum effluents. The study recorded the LC_{50} of the organisms to be 30.93, 29.74, 19.65, 20.08 and 17.77% respectively. These values when compared to GESAMP Standard for Toxicity Ranking of Chemicals/Effluents in Aquatic Environment (6) database, indicated that the effluent discharges were slightly toxic to the organisms. It indicates the age of the pollution as the organisms have acclimatized to the pollutants after a prolonged period of contact. The study area and the Niger Delta region generally, have a long history of petroleum activities. This has induced genetic response in the indigenous microbes to utilize hydrocarbons and, in some cases, withstand hydrocarbon pollutants. It corroborates the report of Serrano *et al* (7) in a study on the evaluation of soil biological activity after a diesel fuel spill. Serrano *et al* (7) reported that, there was an initial decrease in soil biological activity, as shown by the soil microbial biomass and soil enzymatic activities after a stress period of 18 days, but the microbial biomass and enzymatic activities gradually increased after the initial stress period and the germination activity of the soil was seen to recover 200 days after the spill. It could be deduced from the study that the initial high concentration of the contaminants had pronounced toxic effects on the soil biota, which gradually adapted to the pollutants after the initial stress period. From the findings of this study, the per centage log survival of *Bacillus subtilis MW802817* in the control surface water (without pollutant concentration) was 100%, but at 1.625% pollutant concentration, the % log survival of the organism was 88.77% (16.23% mortality) at the beginning of the study and decreased to 48.97% (51.03% log mortality) after 24h exposure in the same concentration. At 75% pollutant concentration at the beginning of the study, the % log survival of the organism was 59.64% (40.36% log mortality) and was decreased to 12.24% (87.76% log mortality) after

24 h of exposure to the pollutant concentration. Thus, from the results of this study, the percentage (%) log survival of the test organisms gradually decreased with increase in exposure time from 0-24h, while % log mortality increases. High percentage mortality was observed from 12–24-hour exposure time. This trend of gradual decrease in % log survival and increase in % log mortality as the pollutant concentration increases with exposure time were observed and reported in all the studies with the test organisms. It, therefore, implies that the hydrocarbon effluent used in this study was not as toxic as the washing bleach used in the report of Obire and Nrior (4). The study of Obire and Nrior (4) reported that chlorine as low as 10 ppm caused up to 95% mortality of *Pseudomonas aerogenes* and *Mucor racemosus* in four hours4 h of exposure.

Odokuma and Nrior (8) in their study on ecotoxicological evaluation of industrial degreaser on *Nitrobacter Sp*. reported that nitrite utilization decreased with increase in concentration and exposure period. This agreed with the reports of Okpokwasili and Odokuma (9), Nrior and Obire (10), Williams and Odokuma (11) and the data gathered from this study that exposure period clearly affects the biological responses of the biota to the pollutants.

For comparative analysis of the tolerance of the toxicity test organisms, the results of this study indicated that the bacteria used in the study were more tolerant of the pollutant than the fungi. At 75 % pollutant concentration (the highest concentration in the toxicity experimental design) and after 24 hours of exposure, the percentage log survival of *Bacillus subtilis MW802817* was 12.24 % and 87.76 % log mortality, while *Enterobacter ludwigii MW767009* had 12.19 % and 87.81 % log survival and log mortality respectively. Conversely, the fungi could not show any tolerance at higher pollutant concentrations and exposure time. The fungus, *Amorphotheca resinae EU040230,* exhibited 0.00 % log survival and 100% log mortality at 25 % pollutant concentration and 12 hours exposure time. Similarly, *Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* had 0.00% log survival and 100% log mortality at 25% pollutant concentration and 12 hours exposure time. *Penicillium chrysogenum MN184857* even had 0.00 %
log survival and 100 % log mortality at 12.5 % pollutant concentration after 24 hours exposure time. It clearly indicates that the fungi used in this study were more susceptible to the pollutants than the bacteria. The finding also re-emphasized the fact that pollutant concentration and exposure time affect the biological responses of the organisms. This is in agreement with the reports of Williams and Odokuma (11), Obire and Nrior (4) and Okpokwasili and Odokuma (9).

The results from this study also indicated that the median lethal concentrations, $LC_{50,}$ of *Bacillus subtilis MW802817, Enterobacter ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* were 30.93, 29.74, 19.65, 20.08 and 17.77 % respectively. It implies that the effluent was fairly toxic to the organisms when the respective LC_{50} were compared to the provisions of the GESAMP standard for toxicity ranking of chemicals/effluents in aquatic environment, (6).

4.0 CONCLUSION AND RECOMMENDATION

The effluent discharge was more toxic to *Penicilliun chrysogenum* MN184857 (LC_{50}) than the other test organisms. The ecotoxicological evaluation of the effluents from petroleum activities on the test organisms isolated from the study area showed that LC_{50} of the effluent was slightly toxic on the microbial population when the results obtained were compared to GESAMP Standard for Toxicity Ranking of Chemicals/Effluents in Aquatic Environment (6). The median lethal concentrations, $LC_{50,}$ of *Bacillus subtilis MW802817, Enterobacter ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857,* isolated from the study area, were 30.93, 29.74, 19.65, 20.08 and 17.77 %, respectively.

To prevent further decline in microbial population and composition, it is recommended that more stringent and regulatory measures be put in place to regulate the disposal of hydrocarbon effluents into the environment. This recommendation is made in view of the key roles some of the organisms play in vital biogeochemical cycles that sustain life on earth.

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