Microbiology Research Journal International

32(1): 47-60, 2022; Article no.MRJI.85941
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Ecotoxicological Evaluation of Artisanal Effluents on *Bacillus, Enterobacter, Amorphotheca, Cladosporium* and *Penicillium* Species in Brackish Water

Samuel A. Wemedo [a], Janet O. Williams [a], Renner R. Nrior [a] and Deinbo G. Bagshaw [a*]

[a] Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, P.M.B 5080, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors.. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2022/v32i130367

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85941

Original Research Article

Received 04 February 2022
Accepted 09 April 2022
Published 18 May 2022

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. Each of the test tubes was inoculated with one milliliter (1ml) of the test organism. Five sets of concentrations were prepared for the five test organisms (*Bacillus subtillis MW808817, Enterobacter ludwigiiMW767009, Amorphotheca resinae*

---

*Corresponding author: E-mail: deinbo.bagshaw@external.totalenergies.com;

EU040230, Cladosporium cladosporioides MW793722 and *Penicillium chrysogenum MN184857*). The organisms were exposed to the pollutant for duration of 0, 4, 8, 12 and 24 hours and plated out using spread plate technique. The cultures were incubated for 24 hours 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.

Keywords: Ecotoxicological evaluation; effluents; percentage mortality; median lethal concentration.

1. 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. Microorganisms are most tolerant of pristine natural environmental conditions and most sensitive to even the lowest concentrations of environmental pollutants [1]. Therefore, results obtained from microbial toxicological studies are very important in developing site-specific environmental quality monitoring protocols and risks assessment. It could also be used in the development of early warning detection strategies for polluted sites [1]. Environmental quality monitoring goal is relevant in conducting remediation activities and enable an area to be recognized as free of major environmental hazards [2]. Revegetation of contaminated sites and diverse microbial community composition are frequently used to indicate remediation in toxicity evaluation, [3].

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 [4]. Test organisms in toxicity studies elicit different responses or sensitivity to the pollutants. Factors such as exposure period, dose or concentration of the pollutant (s), the organism`s susceptibility to the pollutant, and the age of the organism, all impact on the toxicity response of the organisms [5]. Organisms at periods of active cell differentiation are usually more adversely affected [6]. The concentration of the pollutant and exposure period are key factors in toxicological studies. Chemicals can produce toxic effects if administered in certain concentrations. Low-dose exposures may have biological impact or generate adverse health effect if the exposure is prolonged or happens during a critical window of development [7]. Petroleum hydrocarbons are considered persistent in the environment and are harmful contaminants that can have toxic impact on an ecosystem. Crude oil discharges into water bodies results in the disruption of aquatic life including microbial communities. Fractions of the crude oil may be naturally attenuated, while other fractions may persist for longer period. Some fractions of the hydrocarbons, at low concentrations, may stimulate microbial activities, while others may present toxic effects on the microbes. Prolonged exposure period, even at low concentrations, can result in toxic effect [8]. 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 [4]. 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 [4]. 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. Most of toxicity test approaches use eukaryotes such as, zooplankton, benthic macroinvertebrates, and fishes as test species. However, due to the relevance of microbial activities in biogeochemical cycling, the impacts of pollutants on microbial life in aquatic environment is receiving greater attention. Microbial populations respond to environmental pollution in diverse ways [9]. Barnhart and Vestal [10] in their study, used two systems of measurement of microbial activity to determine the impacts of toxicants on microbes. The systems were analyzed for suitability as test protocols and adaptability to field applications. The first system involved measuring the integration of 14C-labeled acetate into microbial lipids and microbial glucosidase activity. Activities were measured per unit biomass, determined as lipid phosphate. Both methods were useful in detecting toxicity. The systems showed almost the same sensitivities in testing the impacts of metals, though the acetate integration system was more sensitive in detecting the toxicity of organic compounds. The integration system was applied to determine the impact of a point source contamination on the microbial population [1].

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 [4]. Accumulation of hydrocarbons in the membrane lipid bilayer of cells, may affect both the integrity and functional capabilities, which may lead to disruption of the cell membrane and eventually result in death [5]. The application of bacterial bioassays has gained considerable acceptance in recent times. It presents advantages such as ease of processing, economy of space, ease of cultivation, low cost and free of any ethical questions [4]. Their relevance in bioassays is based on growth inhibition, mutagenic potentials and the inhibition of physiological and enzymatic activities [5]. Nitrobacter is one of the preferred toxicity test organisms because of some of its inherent advantages such as, obligate autotrophy, sensitivity to various toxicants and its prevalence in wastewater environments, [11].

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 [4]. The toxic effects of chemicals on microbes range from mild irritations to death and between these two extremes there are growth inhibitions, fertility reduction, and the impairment of enzyme activities [5]. The chemicals may occur naturally in the environment or are produced and released from industrial and other anthropological activities. The organisms in a toxicological survey in an environment range from multicellular to microscopic unicellular organisms [10].

For clarity on the impact of environmental pollution, it is essential to complement chemical analysis with bioassays that provide evidence on biological effects. The toxic effects of contaminants can be determined using living organisms or cells, [4].

The biological response of organisms to contaminants gives an indication on the toxicity of the chemicals present in the environment. Apart from exhibiting sensitivity only to the bioavailable portion of contaminants, bioassays also provide information on the combined impact of chemicals and to spot the chemicals, which are poisonous only after bioactivation, [12].

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.

2. 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) from $\times10^5$ test tube was plated out on triplicate nutrient agar plates for total heterotrophic bacteria (THB) and $\times10^3$ 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, 10g ; $MgSO_4.7H_2O$, 0.42g ; KCl, 0.29g ; KH_2PO_4, 0.53g ; K_2HPO_4, 0.50g ; NH_4NO_3, 0.42g and 15g of agar in 1litre of distilled water was used for the cultivation of hydrocarbon utilizers, using vapour transfer technique [13]. 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 35^0C 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 [14], 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 minute. 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 microlitres for 35 cycles.

2.4 Experimental Design for Toxicity Evaluation

The experimental set up was modified from Obire and Nrior [15]. 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 as shown in Table 1. Into the test tubes with different effluent concentrations were added 1ml of microbial broth. Aliquots (0.1ml) 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 24h. Colonies were counted in the triplicate plates and mean recorded.

Table 1. Experimental design for toxicity test, Obire and Nrior (17) modified

SN	Concentration (%)	Vol. of Diluent (ml)	Vol. of Effluent (ml)	Final Vol. (ml)	Microbial Broth (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

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 (0h)							
% 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
4h							
% 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
12h							
% 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
24h							
% 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

The percentage (%) log survival and percentage (%) log mortality were calculated according to Nrior et al (12). The median lethal concentration, LC_{50} was calculated using the formular:

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

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 [16].

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

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 (14).

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

= 100 - % log survival in each toxicant concentration, Nrior et al [13]

3. RESULTS AND DISCUSSION

Molecular identification method showed *Bacillus subtilis MW802817, Enterococcus ludwigii MW767009, Amorphotheca resinae EU040230, Cladosporium cladosporioides MW793722* and *Penicillium chrysogenum MN184857* as the dominant hydrocarbon utilizers in the study samples and are used as the test organisms for toxicity evaluation. The percentage (%) log survival and percentage (%) log mortality of the test organisms at different concentrations of hydrocarbon effluents at 0, 4, 8, 12, 24h exposure time in brackish water are shown in Tables 2-6.

3.1 Lethal Toxicity of Hydrocarbon Effluents on Microbial Isolates

The lethal toxicity of hydrocarbon effluents on the test organisms, *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, 24h exposure time in brackish water were shown in Figs. 1-5.

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, 24h exposure time in brackish water are shown in Tables 7-11.

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 [14] 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. This was also reported by [5] in their study on the influence of exposure time on phenol toxicity to refinery wastewater bacteria. The study area and the Niger Delta region generally, have a long history of petroleum activities which has induced genetic response in the indigenous microbes to utilize hydrocarbons and, in some cases, withstand hydrocarbon pollutants. It also corroborates the report of Serrano et al [15] in a study on the evaluation of soil biological activity after a diesel fuel spill. Serrano et al [15] 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 24h 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 toxic to the microbial population in the study area but not as toxic as the washing bleach used in the report of Obire and Nrior [16]. The study of Obire and Nrior [17] reported that chlorine as low as 10ppm caused up to 95% mortality of *Pseudomonas aerogenes* and *Mucor racemosus* in four hours of exposure.

Odokuma and Nrior [11] 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 [18], Nrior and Obire [15], Williams and Odokuma [19] and the data gathered from this study that exposure period clearly affects the biological responses of the biota to the pollutants. The implication will be a stagnation or reduction in the natural roles of these organisms in biogeochemical cycles to maintain the balance of nature. The introduction of pollutants into the environment will result in shift from the natural roles of the organisms to existential needs. Garcia et al [9] reported in their study that organisms could trade-off natural roles for stress tolerance traits in response to climate change.

For comparative analysis on the tolerance of the toxicity test organisms, the results of this study indicated that the bacteria used 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 [19], Obire and Nrior [15] and Okpokwasili and Odokuma [20]. From the findings of this study, the 0.00% log mortality of the fungal test organisms at 12.5% effluent concentration implies the complete obliteration of the ecosystem services and functions of these organisms in the microbial community structure of the study area. *Amorphotheca resinae* has been reported to have high affinity for kerosine and could be used for the remediation of kerosine polluted sites and production of biosurfactants [21].

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

Conc (%) **Control**	**1.625** 100	**3.25** 100	**6.5** 100	**12.5** 100	**25** 100	**50** 100	**75** 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 (%) Control	1.625 100	3.25 100	6.5 100	12.5 100	25 100	50 100	75 100
Start (0h)							
% 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
4h							
% 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
8h							
% 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
12h							
% 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
24h							
% 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 (0h)							
% 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
4h							
% 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
8h							
% 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
12h							
% 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
24h							
% 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 (0h)							
% 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
4h							
% 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
8h							
% 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
24h							
% 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

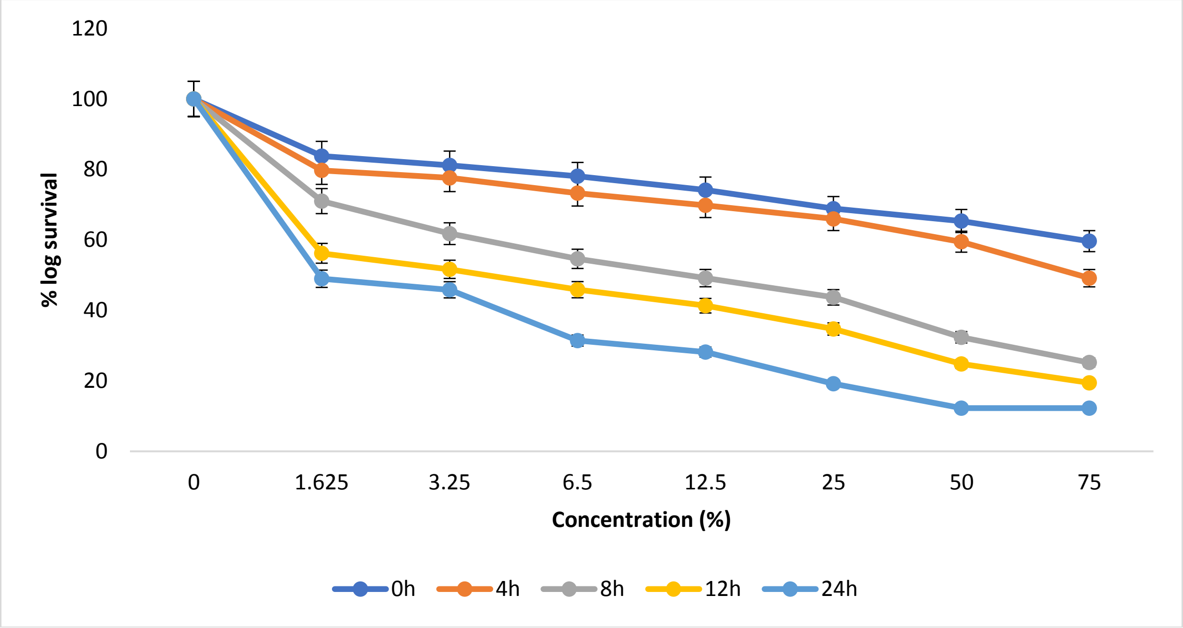

Fig. 1. Lethal toxicity of hydrocarbon effluents on *Bacillus subtilis* MW802817 in brackish water

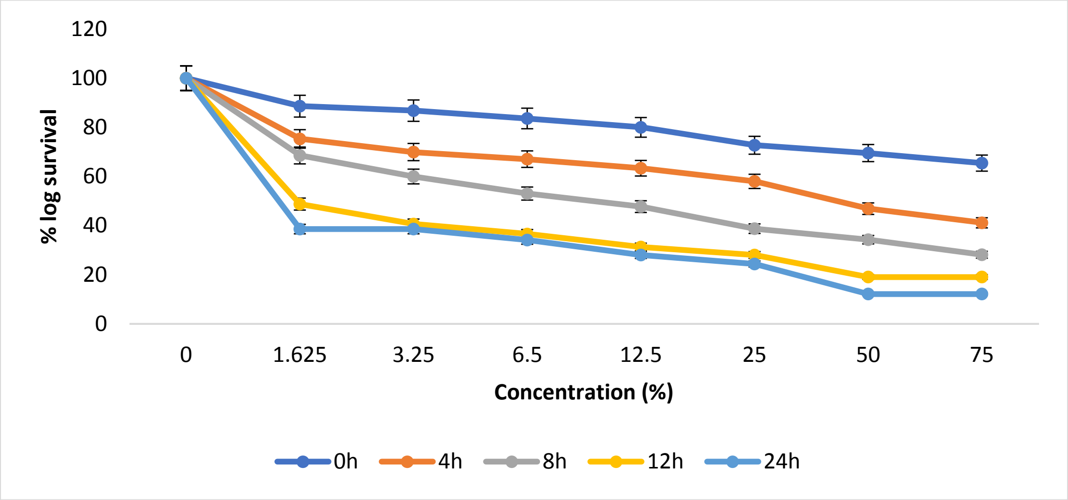

Fig. 2. Lethal toxicity of hydrocarbon effluents on *Enterobacter ludwigii* MW767009 in brackish water

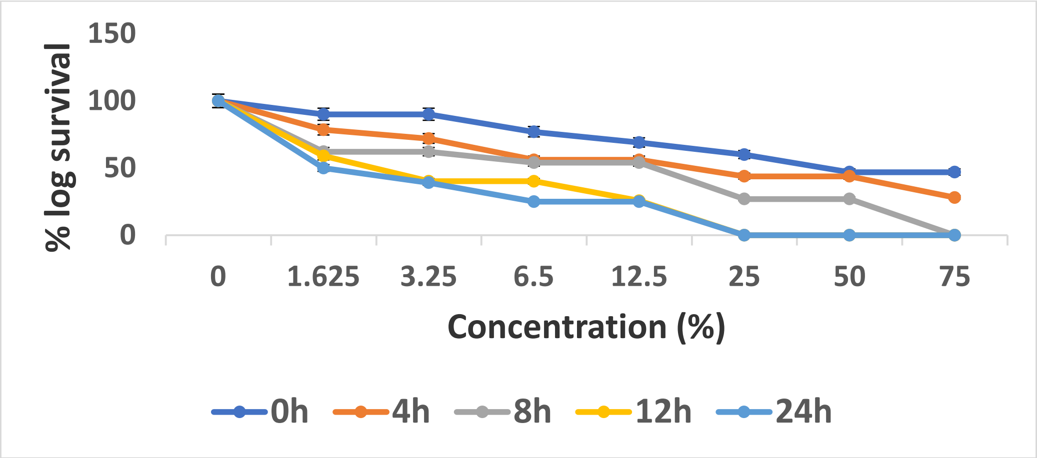

Fig. 3. Lethal toxicity of hydrocarbon effluents on *Amorphotheca resinae EU040230* in brackish water

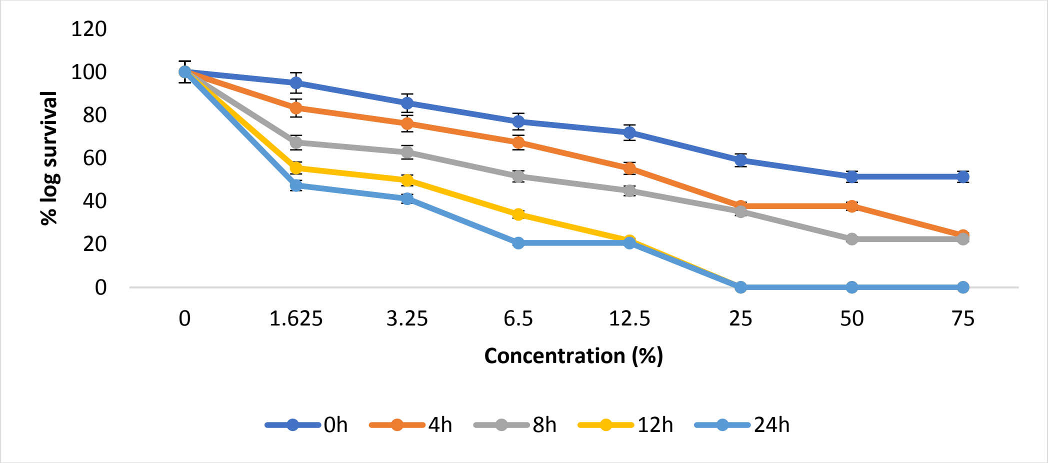

Fig. 4. Lethal toxicity of hydrocarbon effluents on *Cladosporium cladosporioides MW793722* in brackish water

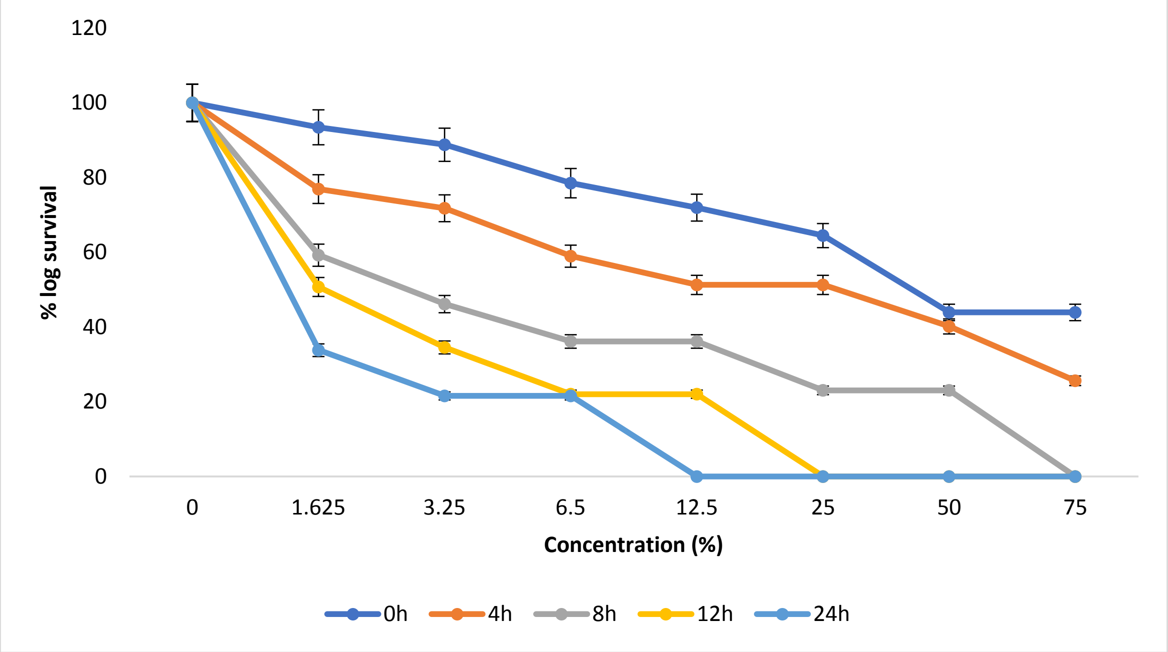

Fig. 5. Lethal toxicity of hydrocarbon effluents on *Penicillium chrysogenum* MN184857 in brackish water

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

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

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

$$= 75 - 44.069$$

$$= 30.931$$

$$LC_{50} = 30.93\%$$

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, [22].

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

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

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

= 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

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

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

= 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

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

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

$$= 75 - 57.23$$

$$= 17.77$$

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

4. CONCLUSION

From the findings of this study, the 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 [23]. 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.

5. RECOMMENDATION

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brandt KK, Jorgensen NOG, Nielsen TH, Winding A. Microbial Community-level toxicity Testing of Linear Alkylbenzene Sulfonates in Aquatic Microcosms. FEMS Microbiology Ecology. 2004;49(2): 229-241.
2. Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK. Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review. Journal of Toxicology. 2018;101-116.
3. Nydahl AC, King CK, Wasley J, Jolley DF, Robinson SA. Toxicity of Fuel-contaminated Soil to Antarctic Moss and Terrestrial Algae. Environmental Toxicology and Chemistry. 2015;34: 2004-2012.
4. Logar RM, Vodovnik M. The Applications of Microbes in Environmental Monitoring. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 2007;577-585.
5. Nweke CO, Okpokwasili GC. Influence of Exposure Time on Phenol Toxicity to Refinery Waste Water Bacteria. Journal of Environmental Chemistry and Ecotoxicology. 2010; 2(2):020-027.
6. Choi MK, Son S, Hong M, Choi MS, Kwon JY, Lee J. Maintenance of Membrane Integrity and Permeability Depends on a Patched-Related Protein in Caenorhabditis elegans. Genetics. 2016;202(4):1411-1420.
7. Duran R, Caumette P, Urriza GSM, Bordenave S. Effects of Heavy Fuel Oil on the Bacterial Community Structure of a Pristine Microbial Mat. Applied and Environmental Microbiology. 2007;73: 6089-6097.
8. Mahaffey WR, Pritchard PH, Bourquin AW. Effects of kepone on Growth and Respiration of Several Estuarine Bacteria. Applied Environmental Microbiology. 1982; 43(6):1419-1424.
9. Garcia MO, Templer PH, Sorensen PO, Demott RS, Groffman PM, Bhatnagar JM. Soil Microbes Trade-off Biogeochemical Cycling for Stress Tolerance Traits in Response to Year-Round Climate Change. Frontiers in Microbiology. 2020; 11:616.
10. Barnhart LH, Vestal JR. Effects of Environmental Toxicants on Metabolic Activity of Natural Microbial Communities. Applied and Environmental Microbiology. 1983;46:970-977.
11. Odokuma LO, Nrior RR. Ecotoxicological Evaluation of Industrial Degreaser on Nitrobacter Sp. ISCEST Current Studies in Comparative Education, Science and Technology. 2015;2(2):356-365.
12. Van Beelen P. A Review on the Application of Microbial Toxicity Tests for Deriving Sediment Quality Guidelines. Chemosphere. 2003;53(8): 795- 808.
13. Abu GO, Dike PO. A Study of Natural Attenuation Processes involved in a Microcosm Model of a Crude Oil-Impacted Wetland Sediment in the Niger Delta. Bioresource Technology. 2008;99:4761-4767.
14. Serrano A, Tejada M, Gallego M, Gonzalez JL. Evaluation of Soil Biological Activity after a Diesel Fuel Spill. Science of the Total Environment. 2009;407:4056–4061.
15. Obire O, Nrior RR. Effect of Concentrate Detergent on Pseudomonas fluoerescens and Mucor racemosus. Current Studies in Comparative Education, Science and Technology. 2014;1:189-199.
16. Nrior RR, Ngeribara NN, Baraol RP, Amadi LO. Ecotoxicity of Local and Industrial Refined Kerosene on Key Environmental Monitor; Nitrobacter Sp. In Tri-aquatic system in Nigeria. International Research Journal of Public and Environmental Health. 2017;4(9):199-204.
17. Okpokwasili GC, Odokuma LO. Tolearance of Nitrobacter sp. to Toxicity of Hydrocarbon Fuels. Journal of Petroleum Science and Engineering. 1996;16:89-93.
18. Williams JO, Odokuma LO. Modelling Physicochemical Fate of a Simulated Oil Spill in Brackish Surface Water of Niger Delta, Nigeria. International Journal of Current Research and Academic Review. 2014;2(19):141-152.

19. Okpokwasili GC, Odokuma LO. Tolerance of Nitrobacter Toxicity of some Nigerian crude oils. Bull Environ Contam. Toxicol. 1994;53(3):388-395.
20. Muriel JM, Bruque JM, Olias JM, Sanches AJ Production of Biosurfactants by Cladosporium resinae. Biotechnology Letters. 1996 ;18(3):235-240.
21. Nrior RR, Obire O. Toxicity of Domestic Washing Bleach (calcium hypochloride) and Detergent on Escherichia coli. Current Studies in Comparative Education. Science and Technology. 2015;2(1):124-135.
22. GESAMP Standard for Toxicity Ranking of Chemicals/Effluents in Marine Environment; 2002.
23. Holt JG, Krieg NR, Sneath PHA, Stanley E, Williams ST (eds). Bergey`s Manual of Determinative Bacteriology 9th ed. Williams and Wilkins Baltimore, Maryland, U.S.A;1994.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/85941