wjpmr, 2018,4(5), 48-55

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

ASSESMENT OF DIESEL OIL TOXICITY ON SOME HYDROCARBONOCLASTIC BACTERIA ISOLATED FROM IKO RIVER ESTUARY IN THE NIGER DELTA

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Article Received on 01/03/2018 **Article Revised on 22/03/2018** **Article Accepted on 12/04/2018**

ABSTRACT

This study investigated the toxicity of diesel oil on the diversity of hydrocarbonoclastic bacteria in Iko River estuarine ecosystems to ascertain reasons for poor natural attenuation of the ecosystems in the event of an oil spill. Water and sediment samples were collected from predetermined locations and subjected to microbiological analyses using standard methods. Bacterial identification results revealed taxonomic groups of hydrocarbonoclastic bacteria species including *Proteus mirabilis* strain IFSW, *Bacillus cereus* TESSWS$_3$, *Citrobacter amalonaticus* strain Y$_2$ESWS$_1$,*Citrobacter amalonaticus* Y$_1$FSW,*Enterobacter* sp.Y$_8$ESWS$_3$. Toxicity testing revealed that toxicant concentration, exposure time and bacteria type were key variables that mediated toxicity. A second-order polynomial regression model revealed significant (P<0.05, 0.01; R^2=0.9862) relationship between exposure time and bacterial survivability at 20% diesel oil. The study revealed that the higher the diesel concentration, the higher the toxicity. At 20% concentration of diesel oil, the growth of *Citrobacter amalonaticus* Y$_1$FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y$_8$ESWS$_3$ were retarded within 72 hrs and no acclimation period was observed in these isolates within 72 hrs. This could be attributed to the presence of low molecular weight compounds that are usually more toxic than long chain hydrocarbons, which are less soluble and bioavailable. *Citrobacter amalonaticus* strain Y$_2$ESWS$_1$ was tolerant to 80% of toxicant concentrations as was evidenced in its prolonged acclimation periods. In conclusion, this study has identified an uncommon hydrocarbonoclastic bacterium, *Citrobacter amalonaticus* strain Y$_2$ESWS$_1$, with commendable ability to tolerate reasonable concentrations of diesel oil. The bacterium is recommended for bio-augmentation in bioremediation technology of hydrocarbon-impacted estuarine ecosystems in the Niger Delta.

KEYWORDS: Toxicity, Diesel oil, Hydrocarbonoclastic bacteria.

INTRODUCTION

The tremendous increase in the production, refining and distribution of petroleum products are accompanied by increasing problems of environmental pollution. A major part of this problem results in the massive movement of its products from areas of high production to those of high consumption.[1] Polluting the tropical ecosystem has resulted in many consequences. Even though pollution can hardly be stopped, it can be controlled by bringing it to tolerable levels within the current environmental legislation. Drilling and extraction processes have dramatically increased, as well as oil product storage and transportation with the risk of accidents resulting in spillages and leakages. Accidents in the oil industry prevent the affected natural resources from being rationally exploited, because the productive processes are altered or the species habitat and the natural landscape aesthetics are directly modified.[2] The oil may be toxic to benthic organisms and subsequent biodegradation may be retarded both by lack of oxygen and surface area limitations.[3,4]

Microorganisms tolerant to this polluted environment oxidize the hydrocarbon by the actions of monooxygenases and dioxygenases converting them into other more soluble and unstable components.[5] Saturated and aromatic components, with one to five benzene rings are used by the microorganisms as energy sources.[6] Much of petroleum pollution can be eliminated by the activities of hydrocarbon-degrading microbial community especially hydrocarbonoclastic bacteria.[7] However, biodegradation of petroleum products by these hydrocarbonoclastic bacteria are limited by the resistant and toxic components of the oil itself, low water temperatures, scarcity of mineral nutrients, especially nitrogen and phosphorus, the exhaustion of dissolved oxygen and the scarcity of the hydrocarbon-degrading microorganisms.[8]

Although studies have been carried out on the toxicity of hydrocarbons on various aquatic resources,[9-13] more knowledge of the toxicity of diesel oil on the growth of hydrocarbonoclastic bacteria in aquatic ecosystem is

critical. This research is therefore intended to find out the possible toxic effect of the different levels of diesel pollution on the hydrocarbonoclastic bacteria in the estuarine water ecosystem.

2. MATERIALS AND METHODS

2.1. Sample Location

The Iko River estuary is located in Iko in Eastern Obolo LGA of Akwa Ibom State in the Niger Delta. The study location lies within latitude 7° 30' N and 7° 45' N and longitude 7° 30' E and 7° 30' E. The Iko river takes its rise from Qua Iboe River and drains into the Atlantic Ocean at the Bight of Bonny.[14] The Iko River estuary is formed by adjourning tributaries, creeks and channels. This provides an appropriate site for petroleum exploration and production activities, good fishing ground for artisan fishermen as well as breeding sites for diverse aquatic resources in the area.[14] The sampling stations established at the ecosystems were confirmed with a hand-held Garmin-eTrek-type (Garmin 760F) GPS.

2.2. Water and Sediment

Water samples were aseptically collected into clean 1 litre capacity plastic bottles. All containers were rinsed three times with water sample before collection.[15] All water samples were collected from the surface (10 – 25 cm). The intertidal sediment samples were obtained by scooping the top (1 - 5 cm depth) using a short core sampler.[16,17] The collected sediments were scooped from the bucket, mixed together to have a composite sample. All samples were collected in duplicates.

Samples were then placed in an ice-cooled chest and transported immediately to the Microbiology Laboratory for analysis. The mineral salt medium (MSM) of Zajic and Supplison[18] used consisted of dipotassium phosphate (0.8g/l), potassium dihydrophosphate (0.2g/l) ammonium chloride(0.4 g/l), magnesium sulphate (0.2 g/l), sodium chloride (0.1 g /l), ferrous sulphate (0.01 g/l).

Diesel oil was purchased from Mobil Filling Station, Murtala Mohammed High Way, Calabar. The product was collected in sterile container and stored at room temperature until when needed for study. The toxicant concentrations of 1%$^{v}/_{v}$, 5%$^{v}/_{v}$, 10%, 15%$^{v}/_{v}$ and 20%$^{v}/_{v}$ were employed according to the methods of Zajic and Supplison.[18]

2.3. Isolation and enumeration of the hydrocarbon-utilizing bacteria (HUB)

The vapour phase transfer method as described by Asitok *et al*.[19] was employed in this analysis. Hydrocarbon utilizing bacteria in the water and sediment sample were estimated by the viable plate count method using the surface spreading technique. After a ten-fold serial dilution of the water and sediment samples (10^{-1} to 10^{-4}), 0.1ml of the various dilutions were plated in triplicates into mineral salt medium supplemented with nystatin to inhibit fungal growth. After inoculating the medium plated with samples, sterile filter paper (Whatman no. 1) was aseptically placed on to the inside of the covers of the inverted Petri-dishes after saturation with 2.0 mls of filtered Bonny light crude oil and then sealed around with a masking tape. This was to ensure the supply of hydrocarbons by vapour phase transfer as the sole source of carbon and energy for growth of the organisms that developed on the agar surfaces.

The plates were incubated at room temperature (28±2°C) for 5 to 7 days before the colonies that developed were counted and expressed as colony forming units per milliliter (cfu ml^{-1}) of the water samples and colony forming unit per gram (cfu g^{-1}) of the sediment samples. Discrete colonies which developed were picked and purified by repeated sub-culturing and then stored on nutrient agar slants at 4 ^{0} C in a refrigerator for further studies.

2.4. Molecular characterization of hydrocarbon-utilizing bacteria

DNA extraction

DNA was extracted was from a 24 hour culture of microbial isolates in BHI broth and harvested by centrifugation at 14, 000 x g for 10 minutes. DNA extraction and purification was done using ZR fungal/bacterial DNA MiniPrep™50 Preps Model D6005 (Zymo Research, California, USA)

PCR amplifications were performed on a thermocycler (A & E Laboratories, UK Model Cyl-005-1.). The primer pairs used that amplified position 1492–1510 of bacteria 16S rRNA, were 27-F 5'-AGA GTT TGA TYM TGG CTC AG-3, and 1492-R 5′-TAC CTT GTT AYG ACT T-3.′ Amplifications were performed following an initial denaturation at 95°C for 5 min; 35 cycles of 96°C for 60 s, 55°C for 60 s followed by 72°C for 2 min; and a final period of extension at 72°C for 10 min.

Gel electrophoresis

Amplified products (10 µl) were separated using 2 % agarose gel electrophoresis in TAE buffer (40mM Tris-acetate, 2 mM EDTA [pH 8.3]) performed at 70 V for 1 hour. Gels were stained with 0.5 µg/ml of ethidium bromide for 45 min and destained with water for 20 min. Stained gels were examined under ultra-violet (UV) transilluminator in a photo documentation system (E-box). Major bands corresponding to the expected band size was considered in the analysis. A DNA ladder digest of 1 kb (Fermenters USA) was used as a molecular weight marker (Figure 1)

DNA Sequencing

DNA sequencing was performed by Sanger (dideoxy) sequencing technique to determine the nucleotide sequence of the specific microorganism isolated. This was done with Big dye kit. The labelled products were cleaned with the ZymoSeq clean-up kit. The cleaned products were injected with a 50 cm array, using POP7

into automated PCR cycle- Sanger Sequencer™ 3730/3730XL DNA Analyzers from Applied Biosystems.[20,21]

The result was obtained as nucleotides IN FASTA format. Identification of the specie present was done using the resultant nucleotides base pairs. This was performed by BLAST analysis by direct blasting on http://blast.ncbi.nlm.nih.gov. For every set of isolate, a read was BLASTED and the resultant top hits with minimum E-score for every BLAST result showing species name was used to name the specific organism.

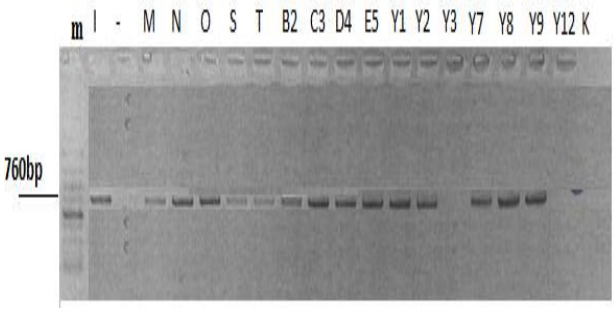

Figure 1: DNA ladder digest showing major bands corresponding to the expected band size.

2.5. Acclimation periods of bacterial growth

The bacterial isolates were subjected to acclimation to the various toxicant concentrations before being used for toxicity studies. The methods reported by Silva *et al*.[22] and Nseabasi and Antai[1] (modified) were employed. Twenty four (24) h broth culture of bacterial species were serially diluted to dilutions of 10^{-4} to tease out bacterial population. One (1 mL) from the dilution tube was introduced into 4.95, 47.5, 45, 42.5 and 40 mL of twenty MSM flasks pH 7.0.[18] Into the sets of 20 MSM flasks, 0.5, 2.5, 5, 7.5 and 10 mL to constitute 1, 5, 10, 15 and 20 % v/v respectively of diesel oil toxicant concentrations. Also one (1 mL) from the dilution tube was introduced into 4.95, 47.5, 45, 42.5 and 40 mL of twenty glucose mineral medium flasks to serve as control. The flasks were incubated at room temperature (28 ± 2^{o}C) for 24 h. At an interval of 8, 16, 24, 32, 40, 48, 56, 64 and 72 h bacterial flasks were pour plated on nutrient agar in triplicates and incubated at 28 ± 2^{o}C for 24 h and bacterial counts were recorded as colony forming unit per ml (CFU mL^{-1}).

2.6. Bacterial growth response to diesel oil toxicity

Diesel oil was used as the toxicant and the toxicant concentrations of 1, 5, 10, 15 and 20% v/v employed according to the modified methods reported by Nseabasi and Antai.[1] Twenty four (24) h broth culture of bacterial species were serially diluted to dilutions of 10^{-4} to tease out bacterial population. One (1 mL) from the dilution tube was introduced into 4.95, 47.5, 45, 42.5 and 40 mL of twenty MSM flasks at pH 7.0 [18]. Into the set of 20 MSM flasks 0.5, 2.5, 5, 7.5 and 10 mL to constitute 1, 5, 10, 15 and 20 % $^{v}/_{v}$ respectively of diesel oil toxicant concentrations. The flasks were incubated at room temperature (28 ± 2^{o} C) for 24 h. At an interval of 8, 16, 24, 32, 40, 48, 56, 64 and 72 h bacterial content of the flasks were pour plated on nutrient agar in triplicates and incubated at 28 ± 2^{o}C for 24 h and bacterial counts were recorded as colony forming unit per ml (CFU mL^{-1}).

Statistical analysis

The data collected were subjected to analysis using SPSS Excel 2007 to establish relationship between the microbial groups. Two factor analysis of variance was used to compare the mean counts among the physiological groups while a second order polynomial regression was used to determine the goodness of fit in the toxicity studies.

3. RESULT AND DISCUSSION

The acclimation periods of bacterial growth responses in glucose-minimal medium (control) is presented in Figure 2**.** The result in Figure 2 revealed that the acclimation period for the different bacterial isolates *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2 ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 was 8 h. Two-factor analysis of variance at all toxicant concentrations revealed that both the nature of the organism and the duration of exposure significantly ($p<0.05$) influence the growth of the organisms

The acclimation periods of bacteria *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 growth response to various concentration of diesel oil are presented in figures 3a-e**.** Figures 3a showed that at 1% concentration of diesel, *Citrobacter amalonaticus* strain Y_2ESWS_1 had acclimation period of 16 h while *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 had acclimation period of 8 h .

The second order polynomial regression analysis result of the plot of the mean log concentration of cells against the incubation time in 1% concentration of diesel showed R^2 values of 0.9907, 0.9297, 0.948 and 0.8967 for *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS_3 respectively indicating significant ability of the model to explain the events in 1% diesel concentration effect on the growth of the different bacterial isolates.

Figure 3b shows that at 5% concentration of diesel, *Citrobacter amalonaticus* strain Y_2ESWS_1 had a prolonged lag phase of 48 h after which there was a decline in the growth of the organism while *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS_3 had a lag phase of 8 h. The second order polynomial regression analysis of the plot of the mean log concentration of cells against the incubation time of 5% concentration of diesel showed R^2

values of 0.9913, 0.9637, 0.9736 and 0.9082 for *Citrobacter amalonaticus* Y_1FSW,*Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 respectively, indicating significant ability of the model to explain the events in 5% diesel concentration on the growth of the different bacterial isolates.

Figure 3c and 3d showed that at 10% and 15% concentration of diesel, *Citrobacter amalonaticus* strain Y_2ESWS_1 had acclimation period of 40 h while the growth of *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 were inhibited soon after their exposure to the toxicant. The second order polynomial regression analysis of the plot of the mean log concentration of cells against the incubation time of 10% concentration of diesel showed R^2 values of 0.9931, 0.9817, 0.9548 and 0.952 for *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS_3 respectively indicating significant ability of the model to explain the events in 10% diesel concentration effect on the growth of the different bacterial isolate.

The second-order polynomial regression analysis of the plot of the mean log concentration of cells against the incubation time of 15% concentration of diesel showed R^2 values of 0.9919, 0.9754, 0.9908 and 0.9724 for *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 respectively indicating significant ability of the model to explain the events in 15% diesel concentration as it effects the growth of the different bacterial isolate.

At 20% concentration of diesel *Proteus mirabilis* strain IFSW and *Citrobacter amalonaticus* strain Y_2ESWS_1 had acclimation period of 8 h and16 h respectively while there was no acclimation period in the growth of *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW (Figure 3e). The second-order polynomial regression analysis of the plot of the mean log concentration of cells against the incubation time of 20% concentration of diesel showed R^2 values of 0.9935, 0.9533, 0.9862 and 0.9839 for *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS_3 respectively indicating significant ability of the model to explain the events in 20% diesel concentration as it effect the growth of the different bacterial isolate.

Two-factor analysis of variance in all the toxicant concentrations revealed that both the nature of the organism and duration of exposure significantly (P<0.005) influenced the growth of the organisms.

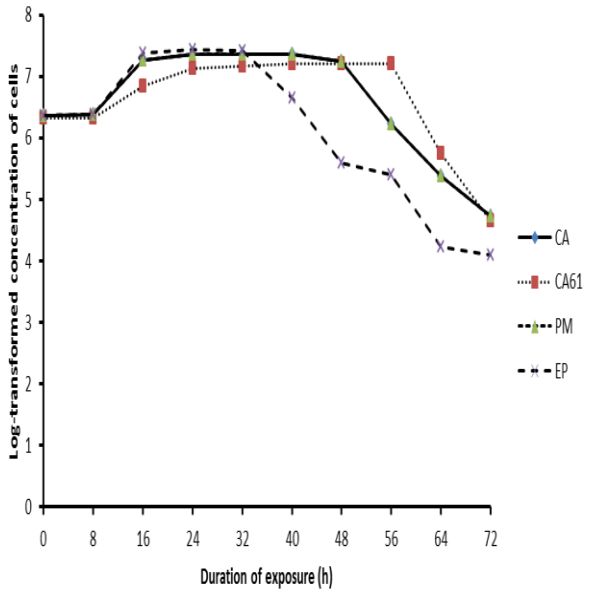

Fig. 2: Comparative acclimation periods of bacteria growth response to glucose-minimal medium (control). CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS_1; PM-, *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp. Y_8ESWS_3.

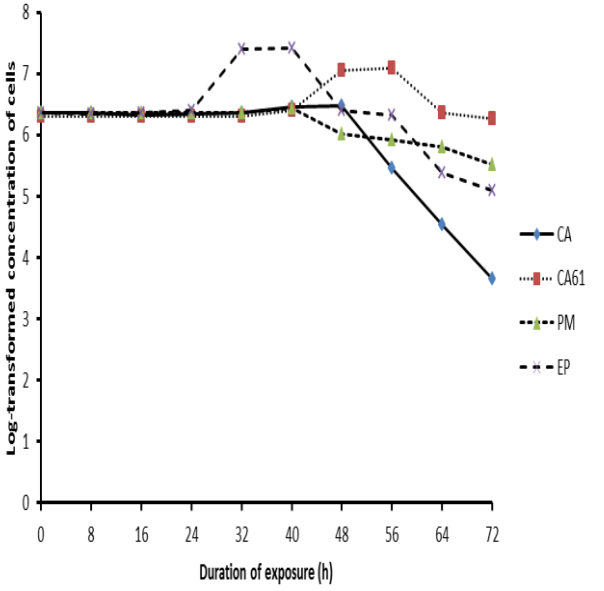

Fig. 3a: Comparative acclimation periods of bacteria growth response to 1% concentration of crude oil. CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS_1; PM- *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp.Y_8ESWS_3

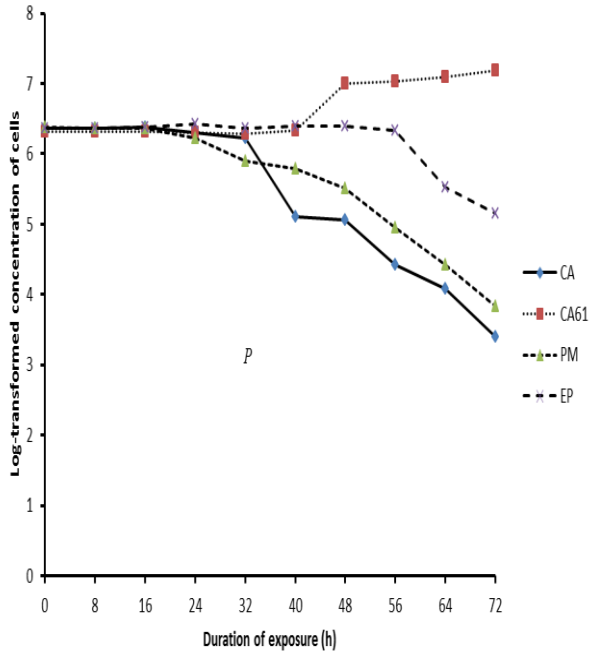

Fig. 3b. Comparative acclimation periods of bacterial growth response to 5% concentration of crude oil. CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS$_1$; PM- *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp. Y_8ESWS$_3$

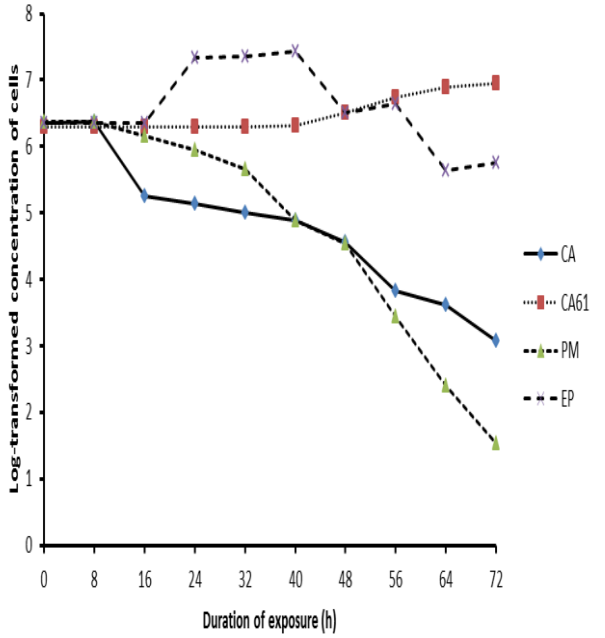

Fig. 3c. Comparative acclimation periods of bacterial growth response to 10% concentration of crude oil. CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS$_1$; PM- *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp.Y_8ESWS$_3$.

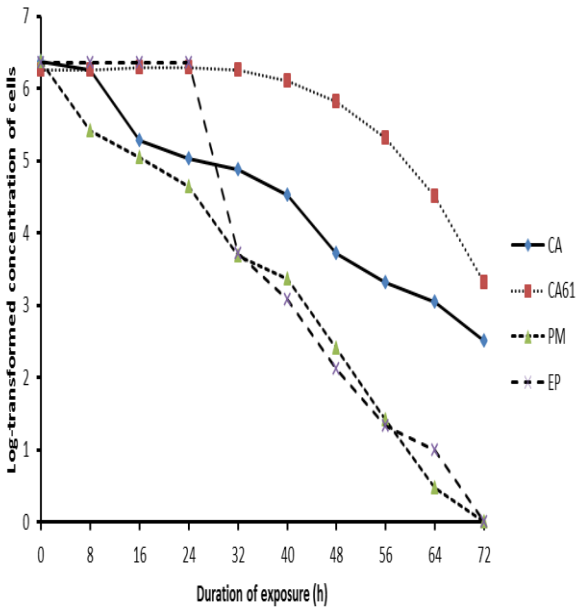

Fig. 3d. Comparative acclimation periods of bacteria growth response to 15% concentration of crude oil. CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS$_1$; PM- *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp. Y_8ESWS$_3$.

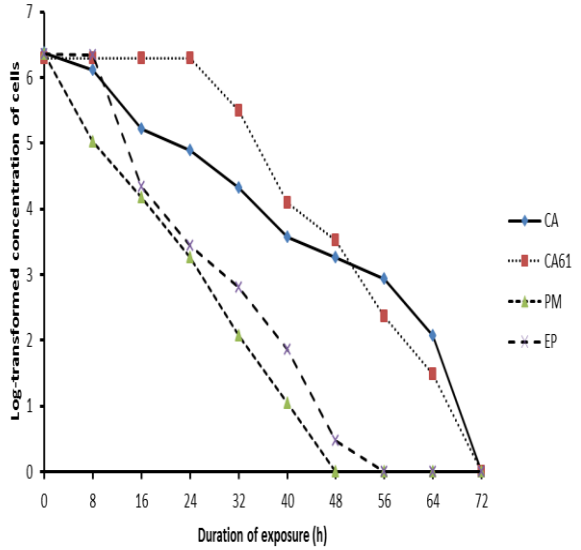

Fig. 3e.Comparative acclimation periods of bacteria growth response to 20% concentration of crude oil. CA- *Citrobacter amalonaticus* Y_1FSW; CA61- *Citrobacter amalonaticus* strain Y_2ESWS$_1$; PM- *Proteus mirabilis* strain IFSW; EP- *Enterobacter* sp. Y_8ESWS$_3$.

The toxicity of diesel oil to *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS$_1$, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS$_3$ is presented in figures 4 a,b,c,d**.** It was observed that the total viable count of bacterial isolates decreased with increase concentration of diesel oil and

increase exposure period. The result also revealed that the different concentrations of diesel were more toxic to *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS_3 than *Citrobacter amalonaticus* strain Y_2ESWS_1.

Figure 4a shows that 1 and 5 % toxicant concentrations, *Citrobacter amalonaticus* Y_1 FSW had a lag phase of 8 h same as control while there was no lag phase at 10,15 and 20% toxicant concentrations as the toxicant inhibited the growth of the bacterium . Figure 4b shows that at 1 and 5 %, *Citrobacter amalonaticus* strain Y_2ESWS_1 grew marginally until 48 h with log-transformed concentration of cells of 6.07 and 5.96 respectively, while for 10 and 15% the organism grew marginally until 32 h with log-transformed concentration of cells of 6.01 and 6.13 respectively before the toxicant inhibited the growth of the bacterium .

Figure 4c shows that at 1, 5 and 10 %, *Proteus mirabilis* strain IFSW had a lag phase of 8 h with log-transformed concentration of cells of 6.32,6,20 and 6, 08 respectively, there was no lag phase at 15 and 20% toxicant concentrations as the toxicant inhibited the growth of the bacterium soon after exposure.

Figure 4d shows that at 1, 5 and 10 %, there was lag phase until 8 h for *Enterobacter* sp.Y_8ESWS_3 and exponential growth of the bacterium reached peak log-transformed concentration of cells of 7.27, 7.11 and 6.67 respectively. However, there was a reduction of log-transformed concentration of cells after lag phase of 8 h in 15, and 20% toxicant concentrations.

A two-factor analysis of variance revealed that both the toxicant concentration and the duration of exposure significantly ($p< 0.05$) influence the growth of *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS_1, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS_3.

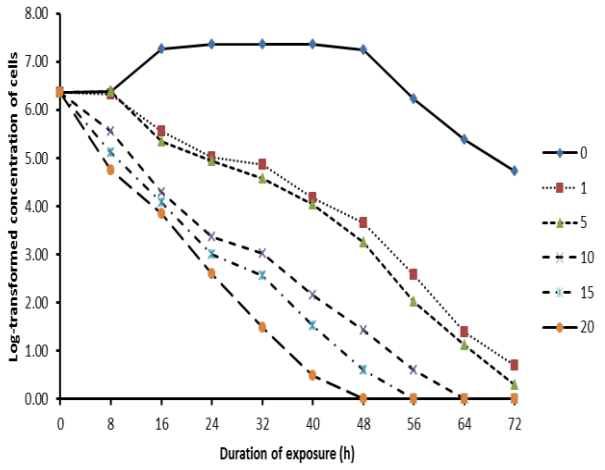

Fig. 4a: Periodic toxicity of diesel oil to *Citrobacter amalonaticus* Y_1FSW. Diesel oil concentrations are expressed as *per cent* (%).

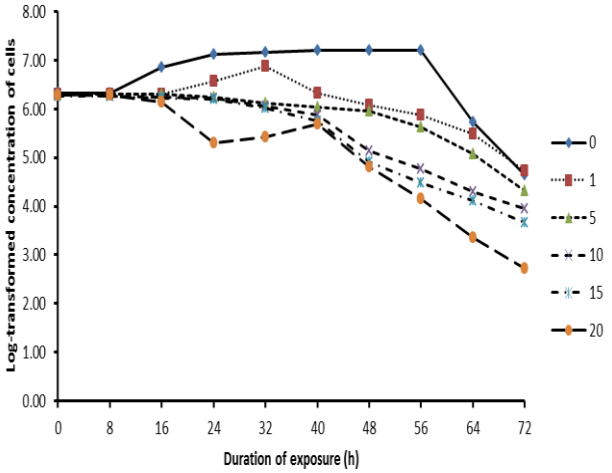

Fig. 4b: Periodic toxicity of diesel to *Citrobacter amalonaticus* strain Y_2ESWS_1. Diesel oil concentrations are expressed as *per cent* (%).

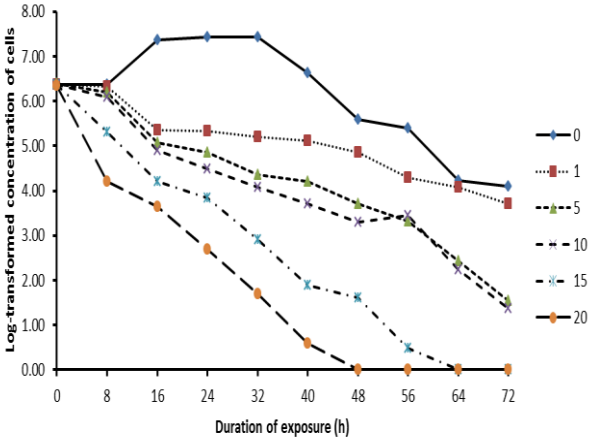

Fig. 4c: Periodic toxicity of diesel to *Proteus mirabilis* strain IFSW. Diesel concentrations are expressed as *per cent* (%).

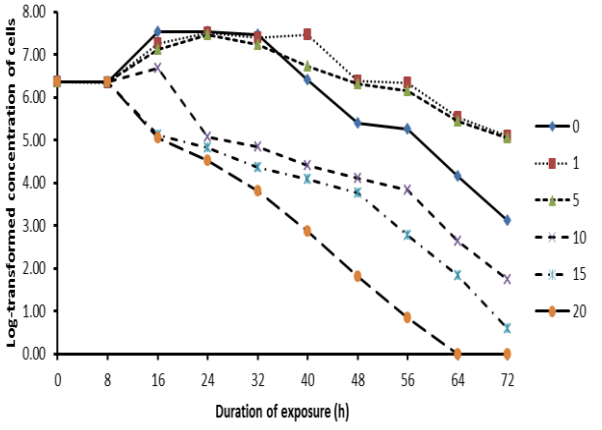

Fig. 4d: Periodic toxicity of diesel to *Enterobacter* sp.Y_8ESWS_3.Diesel concentrations are expressed as *per cent* (%).

Acclimation periods of bacterial growth response in diesel oil concentrations to *Citrobacter amalonaticus*

Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS$_1$, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS$_3$, revealed that there was acclimation for all the bacterial isolates at 1 and 5% concentration of diesel oil. The lag phase observed could be as a result of sudden alteration in the chemical or physical environment which can result in the adaptation of bacteria to the new environmental condition.[23,24] However at 10, 15 and 20% diesel oil concentration, there was no acclimation for *Citrobacter amalonaticus* Y_1FSW, and *Proteus mirabilis* strain IFSW as the toxicants inhibited the growth of the organisms between the hours of 0 and 8. This agrees with Nseabasi and Antai,[1] who reported that crude oil as well as other hydrocarbon spillage may result in damaging effect on associated microbial community because of their toxic properties. The statistical analysis revealed that the nature of the organism and the exposure time influences the toxicity on the growth of the organisms ($P<0.05$).

Toxicity of environmental contaminants is one of the primary reasons for remediation work. Though microorganisms are probably less sensitive to toxic pollutants than protozoa, microfauna and macrofauna but impacts would be expected.[25] The occurrence of chemical pollutants in the aquatic environment is of international concern due to their persistence, toxicity and bioaccumulation in the lipid tissue of aquatic biota.[26] These chemical pollutants can be accumulated in the reservoir, mostly water, sediment and biota,[27] with the sediment reservoir serving as sink from which the water and the biota are continuously polluted. The presence of pollutants can interact with natural biological systems and cause some direct or indirect consequences on both human and animal health.[28] Diesel oil is a complex mixture of alkanes and aromatic compounds that frequently are reported as soil contaminants. In the present study, the toxicity of diesel oil to *Citrobacter amalonaticus* Y_1FSW, *Citrobacter amalonaticus* strain Y_2ESWS$_1$, *Proteus mirabilis* strain IFSW and *Enterobacter* sp. Y_8ESWS$_3$ revealed that toxicity increased with exposure time leading to the reduction in the counts of the bacteria isolates. Bacteria activity was inhibited by the addition of diesel oil thus reduction in microbial growth was observed in the plating results. This result agrees with the findings of Abdul *et al.*[29] who reported that the effect of hydrocarbon on algae resulted in inhibited growth. However, Nayar *et al.*[30] observed an increased abundance of microbes after an oil spill. The bacteria population dynamics observed during diesel toxicity could be associated with the toxicity patterns of diesel in different concentrations when compared to the control.

This study has revealed that *Citrobacter amalonaticus* strain Y_2ESWS$_1$ was more tolerant to diesel oil than the other three organisms *Citrobacter amalonaticus* Y_1FSW, *Proteus mirabilis* strain IFSW and *Enterobacter* sp.Y_8ESWS$_3$ at higher concentrations (10, 15, and 20%) while *Citrobacter amalonaticus* Y_1FSW was more tolerant at 1 and 5% concentrations. The present study also reveals that the higher the concentration of diesel, the higher the toxicity. This finding agrees with the work of Dunstan *et al.*[31] who observed that low concentrations of low molecular weight hydrocarbons stimulated the growth of some microalgae, while high concentrations were toxic. This is also similar to the observations made by Shukor *et al.*[32] who asserted that degradation is generally unfavorable at concentrations higher than 1 or 1.5%. Statistical analysis showed that there was significant difference ($p< 0.05$) in the effect of diesel contamination on bacterial species. Therefore, *Citrobacter amalonaticus* strain Y_2ESWS$_1$ could be used in bioremediation of diesel pollution in Iko river ecosystem.

CONCLUSIONS

The study has shown that the toxicants used in this study can serve as a selective agent against certain microorganisms. The toxicity of diesel to bacterial test cultures was highly dependent on the concentration of the toxicants and on the microbial species at a particular concentration. Thus a longer biodegradation period or implementation of a bioaugmentation would have been a reliable solution for this problem (hydrocarbon contamination).

ACKNOWLEDGMENTS

We are grateful to Nigerian Institute of Medical Research, Yaba, Lagos for their contributions in the molecular studies as well as others who made various positive contributions to the work.

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