Original Research Article

Ecotoxicological Assessment of locally refined diesel and kerosene on *Aspergillus niger* in Rivers State

ABSTRACT

Aim: To evaluate the effect of locally refined diesel and kerosene on *Aspergillus niger* in three aquatic bodies.
Study Design: The study employs experimental examination and statistical analysis of the data and interpretation.
Place of Study: Fresh water, brackish water, and marine water samples were collected in sterile bottles from Ugama Ekede Stream, Ugama Ekede River and at the foot of the Atlantic ocean in Udun Ama all in Andoni Local Government Area Rivers State, using sterile sampling bottles. These samples were transported to the microbiological laboratory with ice pack within 24 hours for both isolation of test organisms and toxicity.
Methodology: Standard microbiological techniques were used; toxicity testing procedures were carried out by preparing locally refined diesel and kerosene at concentrations of 0%, 5%, 10%, 25%, and 50%, tested for durations of 0h, 24h, 48h, 72h, 96h. The cultures were incubated at 35^0C for 48 hours. LC_{50} was determined.
Results: The results specify that logarithm of mortality of *Aspergillus niger* increases with increased toxicants concentration and exposure time. The median lethal concentration (LC_{50}) of the locally refined diesel and kerosene increases in the following order: (Note: the higher the LC_{50}, the lower the toxic effect. *Aspergillus niger* in locally refined diesel in fresh water (47.77%) < *Aspergillus niger* in locally refined kerosene in fresh water (48.02%) < *Aspergillus niger* in locally refined diesel in brackish water (48.09%) < *Aspergillus niger* in locally refined kerosene in brackish water (48.14%) < *Aspergillus niger* in locally refined diesel in marine water (48.09%) < *Aspergillus niger* in locally refined kerosene in marine water (47.98%).
Conclusion: locally refined diesel in fresh water (LC_{50} = 47.77%) is the most toxic, having the lowest LC_{50} while locally refined kerosene in brackish water (LC_{50} = 48.14%) have the lowest toxicity effect. These results show that locally refined diesel and kerosene can inhibit the growth of *Aspergillus niger* in aquatic ecosystem.

Keywords: Locally refined diesel and kerosene; toxicity; Aspergillus niger; fresh water; brackish water; marine water; ecosystem.

1. INTRODUCTION

The ecosystem is painstaking as man's imperative asset which must be sheltered for his life sustainability. However the situation is like chalk and cheese where oil refinery and petrochemical plants function [1]. Petroleum currently, is Nigeria's and undeniably the world's most vital derived energy source [2]. Nigeria's economy is sustained by the petroleum industry [3]. Petroleum in its innate state is referred to as crude oil [4]. Crude oil is converted by petroleum refinery industry into auxiliary products such as liquefied petroleum gas, gasoline, aviation fuel, kerosene, diesel fuel, fuel oils, lubricating oils and feed stocks for petrochemical industry [5]. Crude oil is primarily either black or green but it can also be light yellow [6]. Crude oil is a multifaceted organic compound made up of a large array of hydrocarbons [7]. Crude oil is a vastly complex mixture of hydrocarbon containing 84% carbon, 14% hydrogen and non-hydrocarbons which include numerous inorganic elements in form of sulphur 1-3% (hydrogen sulphide, sulphides, disulfides, elemental sulphur), nitrogen less than 1% (basic compounds with amine groups), oxygen fewer than 1% (found in organic compounds such as Carbondioxide, phenols, ketones, carboxylic acids), metals fewer than 1% which comprises of nickel, iron, vanadium, copper, arsenic and salt fewer than 1% found in sodium chloride, magnesium

chloride, calcium chloride [8] and [9]. Crude oil has inconsistent amounts of low molecular weight compounds, which are evolved as gases under elevated pressure on earth's surface.

Locally refined diesel and kerosene are terms used to describe petroleum products gotten from crude way of oil refining that is without expertise technology. And these products are very common in the Niger Delta region especially in Rivers State where militancy and oil bunkery activities are on the increase. Locally refined kerosene is one of the most primarily used energy sources for households and bush burning by subsistence farmers in Nigeria [10]. Locally refined kerosene is one of the refined petroleum products gotten from crude oil by fractional distillation [11]. The number of carbon in kerosene (paraffin) ranges from 10-14 with boiling point of 1650- 2000 [12]. The local kerosene is produced locally by inexperienced personnel, with makeshift fractional distillation apparatus. Contrasting the usual industrially refined kerosene, locally refined kerosene production is governed by irregularity, lack of proficiency and inappropriate processing (i.e., the simple distillation process) resulting in coloured poor quality product which poses severe risk to consumers and equipment. The simple distillation process involves the use of crude oil, wood fire which serves as source of heat energy, galvanized pipes (of about one inch are connected to the metal drum as conductors) immersed in a water bath as condenser [10]. The first distillation product is collected as petrol, then kerosene and lastly diesel, the rest is disposed off as waste. Thus, these mixtures of petrol or diesel in kerosene or diesel in petrol are the resulting by-products. Because it is not fractionalized appropriately, these coloured by-products are relatively hazardous. Industrially refined kerosene is a lucid liquid fuel with a mixture of hydrocarbon containing 6 - 16 carbon atoms in length. It is a middle distillate of petroleum refining process, defined as the fraction of crude oil with boiling points between 145 and 30000C. It is a multifaceted constituents of joined and straight chained compounds; 55.2%, paraffin, 40.9% naphthalene and 3.99% aromatics [13]. Industrially refined diesel has its colour varies starting from calories to chocolate [14] and Its constituents are 75% alkenes or saturated hydrocarbon and 25% of aromatic compounds (including naphthalene and alkybenzenes), obtained from core distillate fraction between 200°C and 350°C at atmospheric pressure, resulting in a mixture of carbon chains that naturally contain between 8 and 21 carbon atom per molecule at some point in petroleum separation.

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are commonly considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergilli* are ubiquitous in nature. They are in nature widely distributed, and have been observed to in abroad range of habitats because they can colonize a wide variety of substrates. [15]. *Aspergillus niger* is a haploid filamentous fungi and is a very essential microorganism in the field of biology. The fungi is most usually found in mesophilic environments such as decaying vegetation or soil, plants and enclosed air environment [15]. This fungus play an important role in degrading petroleum hydrocarbons by producing competent enzymes because of their aggressive growth, greater biomass production and broad hyphal growth in the soil, fungus proffer potential for biodegradation technology [16]. The high surface -to-cell ratio of this fungus makes them improved degraders under certain niches [16] and it can particularly handle breaking down a few of the biggest molecules present in nature [17].

2. MATERIAL AND METHODS

Fresh water effluent was collected in sterile bottles from Ugama Ekede Stream, while brackish water was collected from Ugama Ekede River, marine water effluent was collected in sterile sampling bottles at the foot of the Atlantic ocean in Udun Ama all in Andoni Local Government Area Rivers State. These samples were transported to the microbiological laboratory with ice pack within 24 hours for both isolation of test organisms and toxicity. All samples collections were carried out at weekly interval for a period of 4 months.

Microbiological Analysis

Petroleum Utilizing Bacteria (PUB)

Total hydrocarbon degrading bacteria for each effluent were enumerated using spread plate method. An aliquot (0.1 ml) of the dilution of 10^{-3} were aseptically transferred unto properly dried mineral salt agar plates containing antifungi (Fluconazole) to inhibit fungi growth, in duplicate, spread evenly using bent glass rod and incubate at 37□C for 7days. After incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates. Further identifications were carried out on the Discrete colonies on the plate (Institute of petroleum studies Rivers State).Total

Heterotrophic petroleum utilizing bacteria counts for each sample were calculated using the formula by [18] below:

Bacterial population (log10 cfu/ml) = Number of colonies x Dilution factor /Volume plated (size of inoculum)

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Petroleum Utilizing Fungi (PUF)

The total hydrocarbon degrading fungi in each of water samples were enumerated using spread plate method. An aliquot (0.1 ml) of the dilution of 10^{-3} dilution was aseptically transferred unto properly dried mineral salt Agar plates containing antibiotic (Tetracycline and Penicillin) to inhibit bacterial growth, in duplicate, spread evenly using bent rod and incubated at 37□C for 7days, pure culture of fungal isolates were counted and sub-cultured unto Potato Dextrose Agar slant in bijou bottles for preservation and identification (Institute of Petroleum studies Rivers State). Total heterotrophic petroleum utilizing fungi counts for each water samples were calculated using the formula by [18]

Fungal population (log10 cfu/ml) = Number of spores x Dilution factor /Volume plated (size of inoculum)

Identification of Fungus

Macroscopic: Examination of growth was done by observing the colonial morphology, color of colony, texture, shape and surface appearance.

Microscopy: This was done by using the wet prep (needle mount) and slide culture characteristics like sexual and asexual reproduction structures like the conidial head, sporangia, the vegetative mycellia, septate or non septate hyphae [18].

Toxicity Test procedures

The toxicity tests were done as described by [19]. Setting up thirty 250ml conical flask aseptically covered with cotton wool. The test was carried out in five (5) separate conical flask containing appropriately autoclaved water samples from fresh, marine and brackish water from the habitat of the organism separately. In each of the conical flask, the four toxicant concentrations (5%, 10%, 25%, 50%) were added separately. while the control consists of fresh, marine and brackish water without toxicants respectively. One millilitre (1ml) of broth containing the test organism was added to each toxicant concentration in the conical flask containing (5%, 10%, 25%, 50%, and control respectively). Then an aliquot (0.1ml) from each of the concentrations of the effluent were then plated out using spread plate technique on Potato dextrose agar immediately after inoculation as zero (0) hour, inoculation and spreading continues after 24, 48, 72 and 96hours respectively and was incubated for 48 hours at room temperature (37± 2ºC). After which the colonies on the plates were counted [19].

The Percentage Log Survival and Log Mortality of *Aspergillus niger* in local refined Diesel and Kerosene

The percentage log survival and Mortality of *Aspergillus niger* in local refined diesel and kerosene used in the study was calculated using the formular adopted by Williamson and Johnson [20]; Nrior, ***et al*** [21]. The percentage log survival of the bacterial isolates in the local diesel was calculated by obtaining the log of the count in each toxicant concentration (log C), dividing by the log of the count in the zero toxicant concentration (log c) and multiplying by 100 (equation i).

Percentage log mortality was obtained by subtracting percentage log survival of test toxicant from 100 (equation ii)

Thus:

$$\%\ \log\ \text{survival}\ = \frac{\log C}{\log\ c} x\ 100 \qquad \text{(i)}$$

$$\%\ \log\ \text{mortality}\ = 100\ -\ \%\ \log\ \text{survival} \qquad \text{(ii)}$$

Median Lethal Concentration (LC_{50}) of the pollution bio-monitors

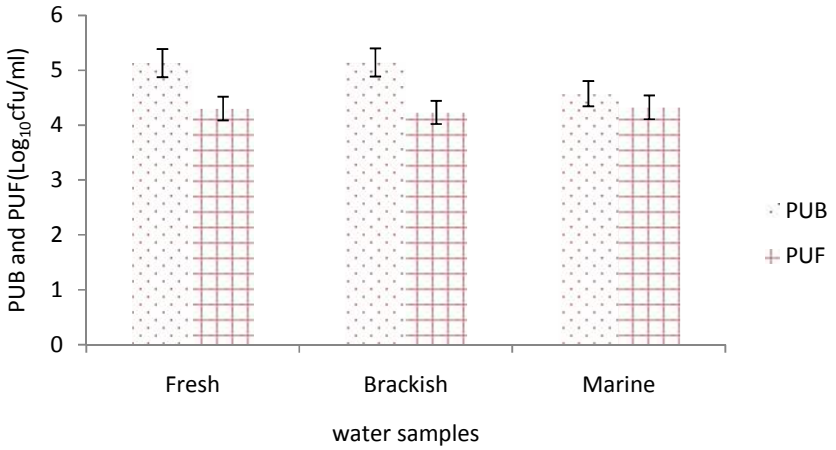
The Median Lethal Concentration (LC_{50}) was computed from mean % log mortality and sum of dose difference using standard statistical analysis using the formula (equation iii) below:

$$LC_{50} = LC_{100} - \frac{\text{Sum of Dose diff. X Mean \% log Mortality}}{\text{\% Control}} \quad \text{(iii)}$$

3. RESULTS AND DISCUSSION

The total petroleum utilizing bacterial and fungal counts in the three aquatic bodies as presented in fig. 1. shows fresh water and brackish water having the highest bacterial population. Salinity in marine water, water current, turbidity and nutrient distribution may be a major determining factor why microbial population was not as high in marine water compare to fresh and brackish water [15] and [22].

The percentage log survival of *Aspergillus niger* exposed to locally refined diesel and kerosene toxicants in fresh, brackish and marine water are revealed in tables 1 and 2 respectively.



Key: Pub= petroleum utilizing bacteria, Puf= petroleum utilizing fungi

Fig.1: Variations in petroleum utilizing bacteria and petroleum utilizing fungi in tri-aquatic environment

Table 1: Effect of different concentration of locally refined Diesel in Fresh, Brackish and Marine water on *Aspergillus niger* population (Percentage Log survival) during 96hours of exposure period.

	Treatments		
Conc. Of diesel	F+D+Asp	B+D+Asp	M+D+Asp
0(Control)	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
5%	94.79 ± 2.11^{a}	95.45 ± 1.28^{a}	95.25 ± 1.80^{a}
10%	96.66 ± 1.43^{ab}	97.82 ± 0.87^{b}	97.18 ± 1.10^{b}
25%	95.76 ± 1.74^{ab}	96.22 ± 1.48^{a}	96.85 ± 1.50^{ab}
50%	97.62 ± 1.24^{b}	98.01 ± 1.00^{b}	97.82 ± 1.69^{b}

*Means with the same superscript across the column shows no significant difference at ($p\geq0.05$)

Key: F-fresh water, B-brackish water, M-marine water, D-Diesel, Asp-*Aspergillus niger*

In (table 1), There was no significant difference in the population of *Aspergillus niger* in 5% and 50% diesel concentration in fresh, brackish, and marine water samples respectively. The higher the concentration of diesel, the higher the population of *Aspergillus niger.* This agrees with the fact that *Aspergillus* utilizes large volume of petroleum products [23].

Table 2: Effect of different concentration of locally refined Kerosene in Fresh, Brackish and Marine water on *Aspergillus niger* population (Percentage Log survival) during 96hours of exposure period.

	Treatments		
Conc. Of kerosene	F+K+Asp	B+K+Asp	M+K+Asp
0(Control)	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}	100.00 ± 0.00^{c}
5%	95.44 ± 2.24^{a}	96.02 ± 2.59^{a}	95.30 ± 2.21^{a}
10%	97.24 ± 1.11^{ab}	96.15 ± 1.75^{b}	96.59 ± 2.07^{ab}
25%	95.95 ± 2.59^{ab}	96.02 ± 2.11^{a}	96.14 ± 2.82^{ab}
50%	98.20 ± 1.03^{bc}	98.46 ± 0.73^{bc}	98.01 ± 0.66^{bc}

*Means with the same superscript across the column shows no significant difference at ($p\geq0.05$)

Key: F-fresh water, B-brackish water, M-marine water, K-kerosene, Asp-Aspergillus niger

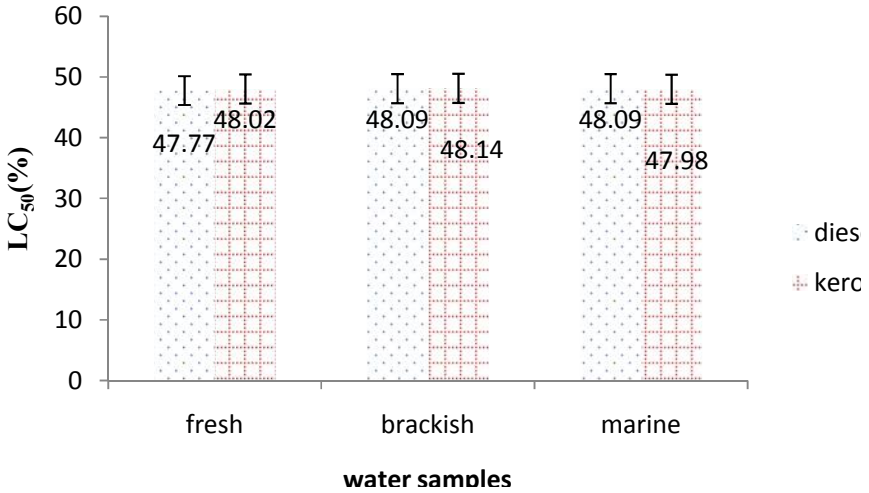


Fig.2: Lethal Toxicity of locally refined diesel and kerosene on *Aspergillus niger* in fresh water, brackish water and marine water.

Aspergillus niger population (table 2), tend to increase with an increased kerosene concentration, this was also reported by [23], during a research on fungi degradation of petroleum compounds from soil and Tarball. At 25% concentration of kerosene, percentage log survival of *Aspergillus niger* became stable across the table showing that the organism has met one of the basic requirements as bioremediation agents, this was reported by [24] and [25]. The lethal toxicity of *Aspergillus niger* as shown in (Fig. 2), shows *Aspergillus niger* in diesel in fresh water with the highest toxic effect. From the results of the experiment, *Aspergillus niger*, demonstrated high capability to biodegrade diesel and kerosene, this could be as a result of its aggressive mycelium which can infiltrate insoluble substances such as crude oil and hence increase the surface area available for microbial attack [26] and [27]. *Aspergillus* has been reported severally as hydrocarbon utilizer [28], [29], and [30]. The fungus have high tolerance to the toxicity of hydrocarbons due to its physiology such as extensive hyphal growth, production of extracellular enzymes which allow for digestion of energy sources, high

surface-to-cell ratio and adaptation to such variations in the environment and have the mechanism for the elimination of spilled oil from the environment [31], [32], [16] and [17].

The median lethal concentration (LC_{50}) which serves as the indices for monitoring toxicity [22], of the locally refined diesel and kerosene increases in the following order: The higher the LC_{50}, the lower the toxic effect; LC_{50} of *Aspergillus niger* in locally refined diesel and kerosene in the three aquatic environment is as follows; 47.77%, 48.02%, 48.09%, 48.14%, 48.09%, 47.98%.

4. CONCLUSION

Locally refined diesel in fresh water (LC_{50} = 47.77%) is the most toxic, having the lowest LC_{50} while locally refined kerosene in brackish water (LC_{50} = 48.14%) have the lowest toxicity effect. These results show that locally refined diesel and kerosene can inhibit the growth of *Aspergillus niger* in aquatic ecosystem although the organism can degrade petroleum products at specific concentrations. *Aspergillus niger* is used in the industrial production of citric acid the main ingredient in soda, gluconic acid, and several useful enzymes and also plays a role in dissolving heavy metal sulfide. It is therefore recommended that strict measures be taking to checkmate oil bunkery and spillages in Nigeria particularly in Rivers State so as to preserve this economically important microorganism.
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