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Degradation Kinetics of Microbial Consortium Isolated from Diesel Oil Impacted Soil in Delta Park, University of Port Harcourt, Nigeria

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Authors' contributions

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

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ABSTRACT

Heavy-duty generators constitute a form of hydrocarbon pollution but enrich microorganisms to having degradative ability of hydrocarbons hence can be used for remediation. In this study a laboratory treatability (biodegradation screening) study was employed to investigate the hydrocarbon degradation competence of bacteria and fungi. Culture-dependent microbiological and physicochemical analyses was conducted on the soil samples obtained from the polluted site. The total aerobic heterotrophic bacterial and fungi counts increased from 5.0-7.5 (Log_{10} cfu/mL) between day 0-14 then reduced to 6.5 Log_{10} cfu/mL. The hydrocarbon-utilizing bacteria increased from 4.5-5.2 (Log_{10} cfu/mL). Percentage degradation hydrocarbons, attributed to fungi bacteria and bacteria/fungi consortia, were 42.3%, 54% and 70% respectively while the control had 6.0%. The total petroleum hydrocarbon (TPH) removal rate (K) was modelled using the first order kinetics: $y=-0.0398x+9.79$; $K=0.0398d^{-1}$. These results, correspond to a degradation efficiency of 70% and $t_{1/2}$ of 17 days for the bacterial/fungal consortium. The K values for the other setups were 0.019 d^{-1},

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0.0261 d^{-1} and 0.0022 d^{-1} with the corresponding degradation efficiencies of 42%, 54% and 6.01% and half-life of 37 days, 27 days and 315 days for fungi, bacteria and control respectively. This result indicates that the use of microbial consortia has high potentials in remediation of hydrocarbons and other pollutants of concern.

Keywords: Bioremediation; consortium; degradation kinetics; half-life; TPH.

1. INTRODUCTION

Crude oil drives the modest technology that defines the modern society we live in. It is used as a major energy source in both rural and urban areas. Despite its relevance to the modern society, petroleum hydrocarbon pollution poses a threat to the environment, plant, animals and humans [1]. Diesel oil is a key product of crude oil distillation. It consists mainly of polyaromatic hydrocarbons and alkanes of low molecular weight PAHs and low molecular weight [2]. The fate of the former compounds generally is of great concern to human health, since they are toxic to plants and carcinogenic to humans [3]. Uncontrolled releases of these compounds into soil around power houses are common as a result of inefficient control practices [4]. In such cases, diesel oil and its constituents might act as a recalcitrant and persistent soil pollutant. One of the consequences of diesel oil pollution is: decrease in the availability of oxygen, nutrients and microorganisms in the soil matrix [4].

Many autochthonous microorganisms in polluted soil are competent of degrading hydrocarbon compounds. Biodegradation of hydrocarbons in contaminated soils, which relies on the ability of bacteria, fungi and algae to degrade hydrocarbons components has been established as sustainable and efficient [5]. The ability of microorganisms to degrade hydrocarbons might not be unconnected with the fact that hydrocarbons are natural and neutral compounds [6]. In recent times hydrocarbon degradation has been a central research focus in real-world applications and it is the foundation upon which bioremediation is built [7]. The present study aims for the confirmation of hydrocarbon degrading ability of indigenous bacteria and fungi retracted from the contaminated soil and screening of efficient diesel degrading genera alongside the degradation kinetics.

2. MATERIALS AND METHODS

2.1 Study Area

University of Port Harcourt, Rivers State has three main campuses namely, the Abuja Park, Delta Park and Choba Park. One of the auxiliary power supplies is from the heavy-duty diesel engines, located opposite the Crab Parking lot, and adjacent to the Juhuel Fuel Station, Delta campus (Fig. 1). Fig. 2 illustrates the real-time Global positioning system on the location of the Delta Park and the generator house.

2.2 Sample Collection

The modified method of Karkush and Altaher [8] was employed in the collection of the soil samples in and around the diesel polluted site using a soil auger. Surface soil within 0-50 cm of the polluted site was sampled alongside with an unpolluted surface soil. The samples were packed in sterile containers and transported in an ice chest to the laboratory of the Department of Microbiology, University of Port Harcourt.

2.3 Physicochemical Analysis of the Soil

An electrochemical method was employed to determine the soil pH using the procedure [9]. The electrical conductivity was analysed using the Winlab conductivity device and according to APHA 2510B. Determination of soil nitrate was carried out by ultraviolet spectrometric screening method according to APHA 4500-NO^{3-} B. The Walkey-Black Method [9] was adopted to determine the total organic carbon starting with pulverisation of the soil samples. The ascorbic acid method (APHA 4500-PE) was employed in the determination of reactive phosphorus. The organic matter of the soil was calculated from the result of the total organic carbon. The formulas used for both parameters are shown below:

$$\% \text{ Organic C in soil (air-dry basis)} = \frac{(Blank\ titre - Sample\ titre) \times 0.195}{dry\ weight\ of\ soil} \quad (1)$$

$$\% \text{ Organic matter in soil} = \% \text{ Organic C} \times 1.729 \quad (2)$$

2.4 Chemical Analyses

Heavy metals studied was determined using Atomic Absorption spectrophotometer (AAS) as adopted by Ayotamuno et al. [10]. The values were expressed in mg/kg. Determination of TPH

was done according to Ofoegbu et al. [11] In summary, 10 g of soil samples were pulverised, treated and reacted with chloroform in a conical flask. Thereafter, the supernatant was reacted with Na_2SO4 and passed through a spectrophotometer set at 420 nm. The result was obtained from a standard graph. Gas chromatograph-flame ionization detector (GC-FID) was used to evaluate the stoichiometric value of residual polyaromatic hydrocarbons (PAH). Five grams of soil sample was extracted and weighed into a beaker and 25 L of diethyl ether were added under cork cover for 30 minutes. The extracts were passed through sodium sulphate and silica gel column to remove fatty acid. Aliquot of the extract was placed in the infrared spectrophotometer analyser as adopted by Orji et al. [12]. The percentage degradation was calculated using Eq. 3.

Percentage degradation (%) = [CTPH control – TPH treatment)/ TPH control] x 100 (3)

Where CTPH control is the concentration of total petroleum hydrocarbons of the control. TPH treatment = Total petroleum hydrocarbons of the treatment [13].

2.5 Microbiological Analyses

The total aerobic heterotrophic bacteria (TAHB) count was performed using nutrient agar and spread plate approach. Thinning-out of bacterial population was carried out using physiological saline. Duplicate plates of the media were prepared for every sample. Culture plates were incubated at room temperature (28 ± 2°C) for 48 h. Plates with counts of 30–300 colonies were chosen and the counts obtained were multiplied by the dilution factor. Total aerobic heterotrophic fungal (TAHF) count was carried out using Sabouroud dextrose agar, fortified with a portion of lactic acid. The medium was prepared according to manufacturer's instructions and sterilized at standard conditions, then distributed into the sterile disposable petri plates. About 0.1 L of sample was introduced into plates spread on already solidified and cooled media. The plates were observed for 3-7 days, observable growth was expressed as cfu/g Orji et al. [12].

Fig. 1. Map of the Delta campus, University of Port Harcourt, Rivers State. Constructed using the coordinates obtained from the site pre-visit and inventorization process

Source: Department of Geography and Environmental Management, University of Port Harcourt

Fig. 2. Google map of Delta campus, University of Port Harcourt, showing the location of the heavy-duty engines

2.5.1 Enumeration of hydrocarbon utilizing bacteria

The method of Agamuthu et al. [14] was employed for hydrocarbon utilizing bacteria (HUB) in the soil samples was enumerated using oil agar (OA) (1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO4, 0.01 g $FeSO_4.7H_2O$, 0.1 g NaCl, 20 g agar, 1 L used engine oil in 1000 L distilled water). The oil agar plates were fortified with nystatin (100 µg/100 L) and incubated at 30°C for 5 days before the colonies were counted. The bacterial colonies were randomly picked and pure culture was obtained by repeated sub-culturing on nutrient agar. The bacterial isolates were characterized using microscopic techniques (Gram staining) and biochemical tests.

2.5.2 Enumeration of hydrocarbon utilizing fungi

Soil samples was enumerated using oil agar (OA) on Bushnell Haas medium (1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO4, 0.01 g $FeSO_4.7H_2O$, 0.1 g NaCl, 20 g agar, 1 L used engine oil in 1000 L distilled water). The oil agar plates were fortified with chloramphenicol (62.5 µg/100 L) incubated at room temperature for 5 days before the colonies were counted. The method of Chukwura et al. [15] was employed in the identification of fungal isolates. The moulds were picked with a sterile needle and aseptically sub-cultured on an antibiotic fortified potatoes dextrose agar and then incubated at 28°C for 72 h. wet mounts were tested with the yeast for budding at x 40 with the light microscope. The fungal isolates were identified by morphological comparison.

2.6 Biodegradation Screening Experiment

Four conical flasks of 100 cm^3 were filled with 50 mL of sterilized Bushnell Haas media (BHM). A drop of stock solution of bacteria, fungi and mixed bacteria and fungi were separately introduced to three of the conical flasks containing the BHMT. The fourth conical flash which contain only the BHM served as a control set-up. The set-ups were then subjected to a shaker incubator at 150 rpm for four weeks at 28°C. The concentration of the TPH and biomass were monitored used spectrophotometric method with 0.5% McFarland standard. Three of the set-ups were inoculated with bacteria consortium, fungi consortium and bacteria-fungi consortia.

Aliquot were taken for analysis per 7 d. pH value was also investigated.

2.7 Fitting into a Degradation Model

2.7.1 Biodegradation kinetics

The parameters obtained in this study was plotted in an excel program and was fitted for a biodegradation kinetic model as explained by the first order kinetic [16]. The biodegradation rate constant for the first order kinetic equation is as presented in Eq. (4):

$$\ln C_t = \ln C_0 - kt \quad (4)$$

2.7.2 Biodegradation half-life time and percentage degradation

The biodegradation kinetics was calculated using the concentration of the contaminants of concern determined during the biodegradation. The biodegradation half-life ($t_{½}$) was extrapolated using the time required for contaminant concentration to reduce to half of their original concentration. The model was determined using the natural logarithm of the concentration of the pollutant, linear regression using the Mathematical software program. This model was used to obtain the predicted values for the progress of the degradation by using the inverse of decay constant (K).

$$t_{1/2} = \frac{0.6932}{k} = \frac{ln ln\ 2}{K} \quad (5)$$

$$\%D = \frac{THCo - THCt}{THCo} \times 100\% \quad (6)$$

2.8 Statistical Analysis

The data were statistically analyzed using the SPSS version 23. A two-way analysis of variance (ANOVA) was used to compare the biodegradation efficacy and their respective reaction kinetics of the consortium of bacterial and fungal isolates. The interpretation was done at $\alpha<0.05$.

3. RESULTS

3.1 Characteristics of Bacterial and Fungal Isolates Obtained from the Study Location

The baseline physicochemical characteristics of the different distances: P1 (0-100 cm), P2 (100-200 cm) and P3 (200-500 cm), polluted by heavy-duty diesel in the Delta Park, UNIPORT is shown in Table 1. The electrical conductivity, nitrate, phosphate decreases down the soil depth. The pH of the soil was 6.19 ± 0.009 at distant of 100 cm away from the site; 5.89±0.006 between 100-200 cm, while the value at the distance between 200-500 cm was 6.72±0.015. The biochemical characteristics of the bacteria obtained from the different depths of the polluted soil are represented in Tables 2 to 4. The tentative identity of the eight genera of bacteria in P1 are *Pseudomonas*, *Staphylococcus*, *Escherichia*, *Serratia*, *Aeromonas*, *Enterobacter*, *Micrococcus* and *Streptococcus*. Six bacterial identified in P2 were *Pseudomonas*, *Staphylococcus*, *Serratia*, *Enterobacter*, *Micrococcus* and *Streptococcus* while three bacterial genera *Pseudomonas*, *Serratia* and *Enterobacter* were tentatively identified at site P3

Table 1. Baseline properties of soil samples obtained from heavy-duty diesel generator engine sites in Delta Park, UNIPORT

Parameters(units)	Site P1(0)	Site P2 (200 cm)	Site P3 (500 cm)
pH	6.19 ± 0.009^{b}	5.89 ± 0.006^{a}	6.72 ± 0.015^{c}
Electrical conductivity (µs/cm)	48.29 ± 0.01^{c}	38.73 ± 0.14^{b}	16.11 ± 0.02^{a}
Nitrate (mg/kg)	1.39 ± 0.019^{c}	0.96 ± 0.15^{b}	0.43 ± 0.006^{a}
Phosphate (mg/kg)	13.69 ± 0.02^{c}	3.96 ± 0.017^{b}	0.87 ± 0.09^{a}
TPH (mg/kg)	19692.1 ± 4.1^{c}	833.7 ± 6.76^{b}	31.0 ± 4.16^{a}
PAH (mg/kg)	3.94^{b}	1.14^{a}	<0.1
Lead (mg/kg)	15.3 ± 0.009^{c}	12.75 ± 0.13^{b}	11.28 ± 0.01^{a}
Cadmium (mg/kg)	6.14 ± 0.01^{b}	3.67 ± 0.07^{c}	5.11 ± 0.06^{a}
Arsenic (mg/kg)	4.53 ± 0.012^{b}	4.65 ± 0.015^{c}	1.44 ± 0.032^{a}
Chromium (mg/kg)	9.31 ± 0.008^{b}	9.61 ± 0.009^{c}	9.11 ± 0.07^{a}

Concentrations/ Numeric values are triplicate Mean+Standard error; superscripts (alphabets) along the horizontal columns suggest correlational variance at p-values < 0.05. Hence, similar superscript suggests there is no significant difference, whereas, the reverse suggest significant difference. Also, superscripts a, b and c also indicate the proximity to α < 0.05

Table 2. Biochemical characteristics of bacterial isolates obtained from P1 (PT)

Test characteristics		Isolates							
		OAU1	**OAU2**	**OAU3**	**OAU4**	**OAU5**	**OAU6**	**OAU7**	**OAU8**
Shape		Rod	Cocci	Rod	Rod	Rod	Rod	Cocci	Cocci
Gram reaction		-	+	-	-	-	-	+	+
Catalase		+	+	+	+	-	-	+	+
Oxidase		+	+	-	+	-	-	+	-
Citrate			+	-	+		+	-	-
TSI	Slant	A	K	K	K	A	+	A	A
	Butt	A	A	A	K	B		K	A
	H_2S	-	+	-	-	I-	+	-	-
	Gas	+	-	+	-	-	+	+	-
Indole				+	-		-	-	-
MR		-	-	+	-	+	-	-	-
VP		-	+	-	-	-	+	-	+
Urease		+	+	-	-		-	-	+
Fructose		-	AG	A	AG	AG	+	-	
Lactose		-	-	AG	-	-	AG	--	AG
Maltose		AG	AG	-	AG	AG	AG	-	-
Manitol			+	A	AG	AG	A	AG	-
Sucrose				AG	A	AG	AG	AG	AG
Starch hydrolysis		-	+	-	-	+	-	-	-
Tentative Identity		*Pseudomonas*	*Staphylococcus*	*E. coli*	*Serratia*	*Aeromonas*	*Enterobacter*	*Micrococcus* sp.	*Streptococcus*

Table 3. Biochemical characteristics of bacterial isolates obtained from P2

Test characteristics		POAU1	POAU2	POAU4	POAU6	POAU7	POAU8
Shape		Rod	Cocci	Rod	Rod	Cocci	Cocci
Gram reaction		-	+	-	-	+	+
Catalase		+	+	+	-	+	+
Oxidase		+	+	+	-	+	-
Citrate			+	+	+	-	-
Tsi	Slant	A	K	K	+	A	A
	Butt	A	A	K		K	A
	H_2s	-	+	-	+	-	-
	Gas	+	-	-	+	+	-
Indole				-	-	-	-
Mr		-	-	-	-	-	-
Vp		-	+	-	+	-	+
Urease		+	+	-	-	-	+
Fructose		-	Ag	Ag	+	-	
Lactose		-	-	-	Ag	--	Ag
Maltose		Ag	Ag	Ag	Ag	-	-
Manitol			+	Ag	A	Ag	-
Sucrose				A	Ag	Ag	Ag
Starch hydrolysis		-	+	-	-	-	-
Tentative identity		*Pseudomonas*	*Staphylococcus*	*Serratia*	*Enterobacter*	*Micrococcus*	*Streptococcus*

Table 4. Biochemical characteristics of bacterial isolates obtained from P3 (PT)

Test characteristics		aOAU1	aOAU4	aOAU6
Shape		Rod	Rod	Rod
Gram reaction		-	-	-
Catalase		+	+	-
Oxidase		+	+	-
Citrate			+	+
TSI	Slant	A	K	+
	Butt	A	K	
	H_2S	-	-	+
	Gas	+	-	+
Indole			-	-
MR		-	-	-
VP		-	-	+
Urease		+	-	-
Fructose		-	AG	+
Lactose		-	-	AG
Maltose		AG	AG	AG
Manitol			AG	A
Sucrose			A	AG
Starch hydrolysis		-	-	-
Tentative Identity		*Pseudomonas*	*Serratia*	*Enterobacter*

Table 5. Macroscopic and microscopic characterization of fungal isolates obtained from the polluted soil

Isolates code	Macroscopy	Microscopy	Presumptive identification
FOAU1	Black dense growth	Septate cream erected hyphae with long conidiophores having black cluster conidia	*Aspergillus niger*
FOAU2; P2FOAU	Creamy tiny colony	Oval yeast cell with single budding	*Candida* sp.
FOAU3	Creamy velvet	Septate hyphae no conidia	*Microsporum* sp.
	Whiite, dense, thread-like growth, creamy on the reverse.	Non-septate, non-branching hyphae.	*Fusarium* sp.
FOAU4	Green, rough, clustered surface with milky and shiny crystals at the centre.	Septate hyphae with condiophore at the apical position.	*Penicillium* sp.

with no visible pollutant. Table 5 represents the fungal genera identified in the polluted soil: *Aspergillus*, *Candida*, *Microsporum*, *Fusarium* and *Penicillium*.

3.2 Biodegradation Screening

The result presented in the Table 6 shows the performance of different indigenous bacterial and fungal species during a biodegradation screening of diesel oil. *Pseudomonas* spp. and *Serratia* spp. were the best degraders followed by *Proteus* spp. and finally by *Micrococcus* spp., *Candida* spp. and *Fusarium* spp. *Staphylococcus* spp., *Aeromonas* spp. *Aspergillus* spp. did not show any evidence of degrading the diesel oil. As indicated in Fig. 3, the biomass, evaluated by McFarland standard, showed that bacterial count increased from 6.07 $\log_{10}$ cfu/L to 6.6 $\log_{10}$ cfu/L, the total fungal count during the degradation study increased from 5.2 $\log_{10}$ cfu/L to 5.7 $\log_{10}$ cfu/L and the consortium of bacteria and fungi increased from 5.3 $\log_{10}$ cfu/L to 7.3 $\log_{10}$ cfu/L on day 14 and fell to 6.5 $\log_{10}$ cfu/L on the 28th day. The biomass comparison of the THC, TFC, HUBC and HUFC is illustrated in Fig. 3. The variation in pH during the study revealed a change from pH 5.5 to 6.1 on the 6th day and again increased to the pH 7.4 on the 21st day and later declined to pH 6.0 with respect to the bacteria consortium. The fungal consortium was observed to decrease from pH 6.0 to pH 5.3 between the day 0 and 7, then declined to pH 5.5 on day 14 and increased again to pH 6.8 on day 21. For the consortia of bacteria and fungi, the pH increased from 7.8 to 8.1 on the 21st day. Fig. 3 illustrates the pH changes alongside the pH changes observed in the control setup. The control set up was without any inocula, and the dynamics of the pH was 7 on day 0, 6.5 on day 21 and 7.1 on day 28.

3.3 Percentage and Kinetic Model of Hydrocarbon Degradation

The loss in TPH from 19989.01 mg/L to 5993 mg/L using bacterial and fungi consortia was recorded. The bacterial consortia had a reduction from 19989.01 to 9514.66 mg/L and fungi consortia were from 19989.01 mg/L to 11533.7 mg/L. For bacterial and fungal consortia was 70% loss in TPH, bacterial consortia were 54% and fungal consortia 42.3%. First Order Kinetic Model fitted the degradation and loss of total petroleum hydrocarbon (Fig. 3). The goodness of fit and the Regression coefficient (R^2) revealed that the consortia of fungal isolates had an R^2 of 42.3% and rate constant of 0.019 (d^{-1}) while bacterial consortia had an R^2 of 54% and efficiency of 99.41% and a half-life of 27 days. The control experiment was observed to have a degradation of TPH of 6.01% and half-life of 315 days (Table 7).

4. DISCUSSION

The physicochemical characteristics result gotten from our investigation clearly shows that the study site was polluted. The downward trend of values with respect to electrical conductivity, nitrate, phosphate as was observed in his study resonate with the findings of Nwogu et al. [17]. Sharma and Reddy [18] reported that polluted soil environment always have a depleted nutrient condition especially nitrogen and phosphorus. This agrees with the physiochemical quality of the soil obtained from the polluted soil environment. The available phosphorus decreases because it becomes insoluble at a pH higher than 6.5 [19] as witnessed in the polluted sample. Besides, pH value of around 6.3 and 7.1 had a positive correlation to increase in biomass [20]. Microbial biochemical activity could also lead to nitrogen and phosphorus reduction since they are needed for the biodegradation of the hydrocarbon content in the oil [21]. The obvious increase of total organic carbon will prompt increase of microbial biomass with proportionate utilization of nitrogen and available phosphorus [22] to establish suitable C:N:P ratio. It is also possible according to Devatha et al. [22] that the organic carbon through chemical reaction forms volatile ammonium and nitrate ions and evaporating out of the soil afterwards. The reduction of these limiting nutrients will naturally select microorganisms that can tolerate nitrogen-phosphorus depletion and hydrocarbon degraders leading to structural change in microbial diversity and ecosystem services.

Microbial biomass as was observed in this study is higher in the unpolluted site in comparison to the polluted samples. This result is in agreement with Chikere and Fenibo [23]. The latter authors also reported the higher value of total heterotrophic bacteria in comparison to hydrocarbon utilizing bacteria just as this study confirmed. Similarly, total fungal count is higher than the hydrocarbon utilizing fungi. Crude oil pollution is reputed in changing the microbial composition and diversity [24]. Usually, the stable microbes in hydrocarbon polluted environment are either hydrocarbon degrading microbes or microorganisms that are tolerant to

hydrocarbon toxicity [25] as was noted in our degradation screening experiment. The best hydrocarbon degraders identified in this study are *Pseudomonas* spp. and *Serratia* spp. Other degradation study has also identified these two species of bacteria as expert degraders of petroleum hydrocarbons [26]. Likewise, fungal species that were found to degrade the diesel oil are *Candida* spp. and *Fusarium* spp., while *Aspergillus* spp. shows to be tolerant to the diesel oil. Other bacteria that have been found to degrade petroleum hydrocarbons are *Acetobacter* spp., *Flavobacterium* [26], *Acinetobacter* spp., *Bacillus* spp., *Micrococci* spp., *Streptomyces Trichoderma* spp. and *Penicillium* spp. [20]. Cellular factors that have made these microorganisms to be pronounced as eaxpert hydrocarbon degrader are the functional genes they possess, the biosurfactants and enzymes they produced [27].

Table 6. Diesel oil degradation potentials of bacteria, fungi and mixed consortia

	0	7	14	21	Isolate identity
BHM+OAU1	-	-	+	+	*Micrococcus* spp.
BHM+OAU2	-	+	+++	+++	*Pseudomonas* spp.
BHM+OAU3	-	-	-	-	*Staphylococcus* spp.
BHM+OAU4	-	+	++	++	*Proteus* spp.
BHM+OAU5	-	-	++	+++	*Serratia* spp.
BHM+OAU6	-	-	-	-	*Aeromonas* spp.
BHM+OAU7	-	-	-	-	*Staphylococcus* spp.
BHM+FOAU8	-	+	+	+	*Candida* spp.
BHM+FOAU9	-	-	-	-	*Aspergillus* spp.
BHM+FOAU10	-	-	+	+	*Fusarium* spp.

BHM = Bushnell Haas medium, OAU = Voucher ID for Bacterial isolates; FOAU = Voucher ID for fungal isolates
+++ = very highest

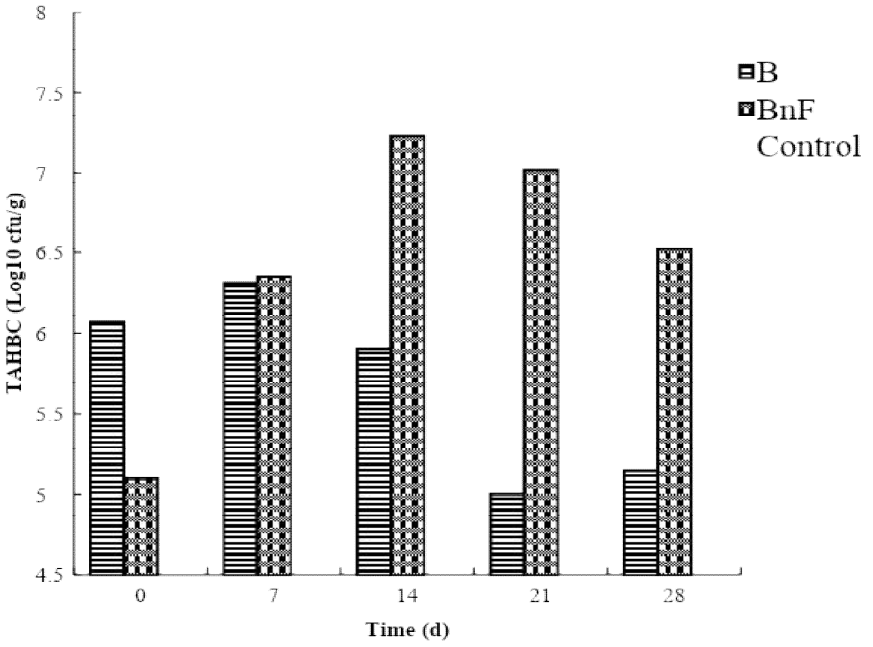

Fig. 3a. Total aerobic heterotrophic bacterial count during bioremediation of diesel polluted soil

Key: CT: Control, BnF: Consortia of bacteria and fungi; B: Bacterial consortia

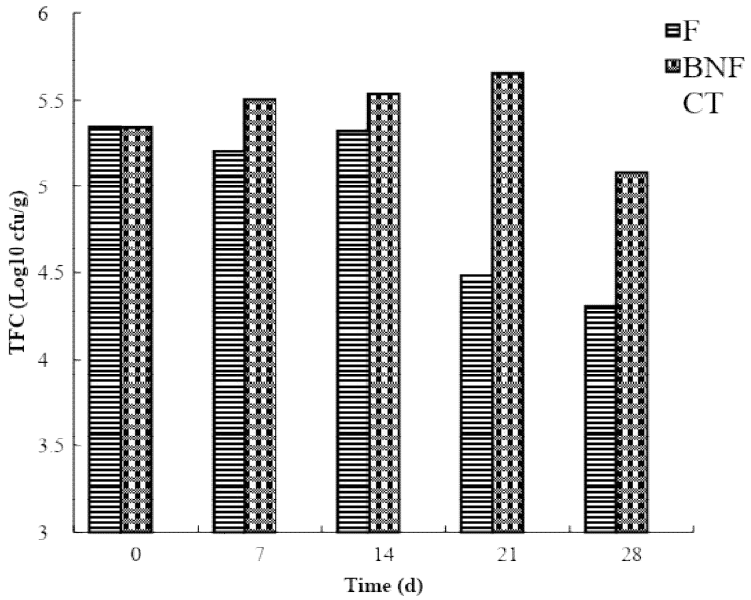

Fig. 3b. Total fungal count during remediation of diesel polluted soil
Key: CT: Control, BNF: Consortia of bacteria and fungi; F: Fungal consortia

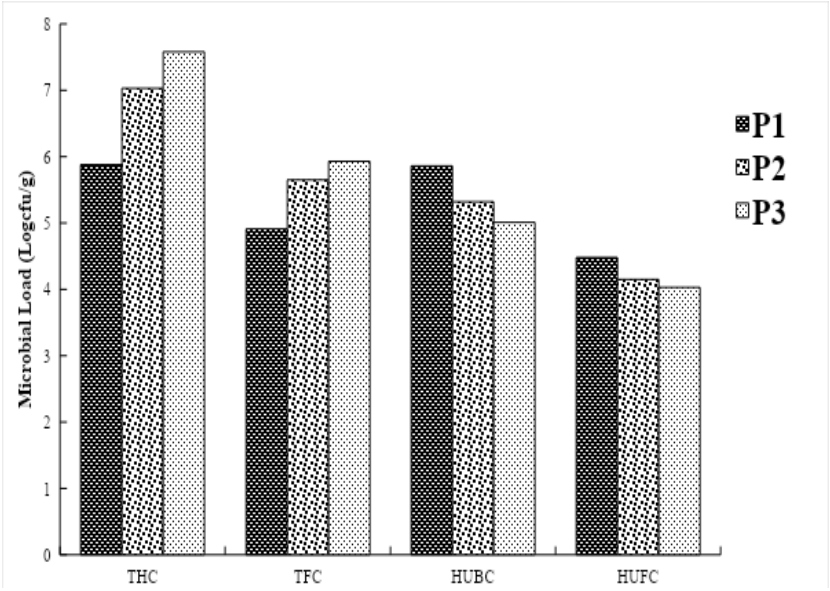

Fig. 3c. Microbial community profile of the soil samples obtained from the diesel polluted soil
THC = Total heterotrophic bacterial count; TFC = Total fungal count; HUBC = Hydrocarbon utilizing bacterial count; HUFC = Hydrocarbon utilizing fungal Count; P1 - Point 1; P2 - Point 2; P3 - Point 3

Table 7. Rate constants and regression coefficient of the diesel oil degradation

	R^2	Kinetic equation	$K(d^{-1})$	$t_{1/2}$ (d)	% D
Fungi	0.943	Y=-0.019x+9.9509	0.019	37	42.3
Bacteria	0.9941	Y=-0.0261x+9.9009	0.0261	27	54
Bacteria and Fungi	0.9342	Y=-0.039x+9.7922	0.0398	17	70
Control	0.9125	Y= -0.0022x+9.9097	0.0022	315	6.01

K = Degradation constant

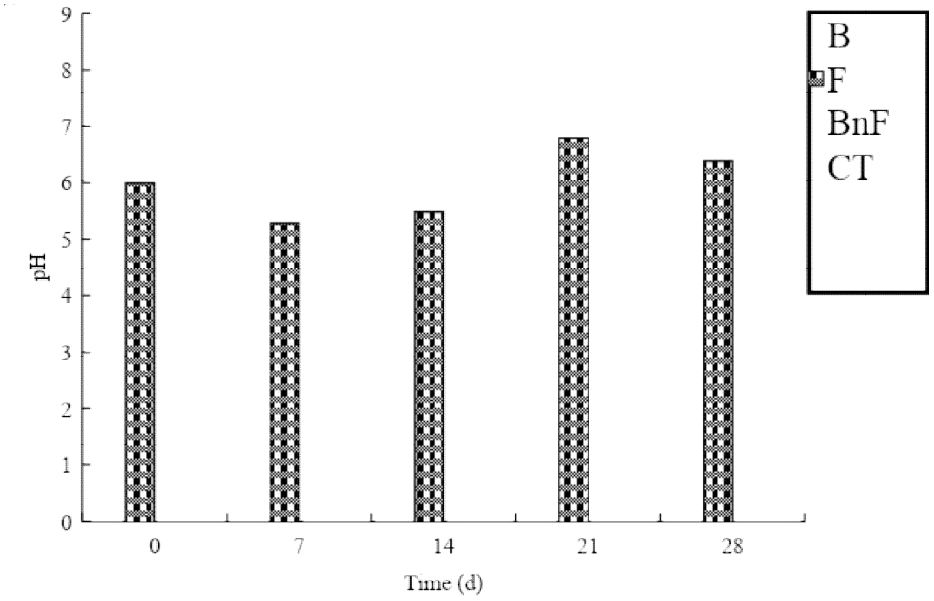

Fig. 3d. Changes in the pH of the experimental setup during the remediation of the diesel polluted soil

Key: CT: Control; BnF: Consortia of bacteria and fungi; F: Fungal consortia; B: Bacterial consortia

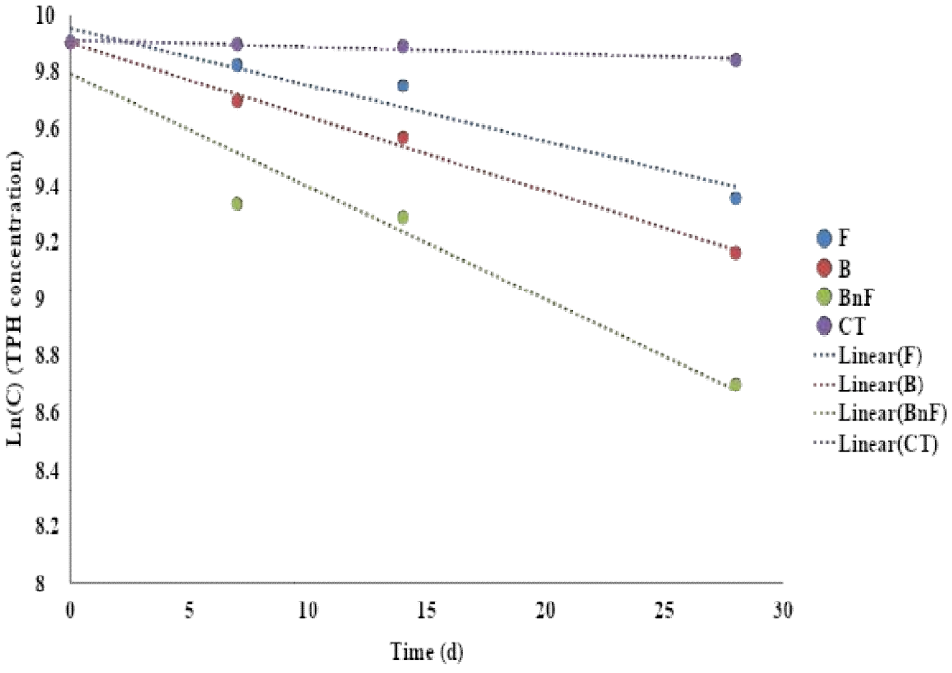

Fig. 4. Degradation kinetics for the bioremediation of diesel oil

Key: CT: Control; BnF: Consortia of bacteria and fungi; F: Fungal consortia; B: Bacteria consortia

The high percentage loss in TPH as witnessed in this study was attributed to bacterial and fungi consortia. The higher percentage been recorded in the consortia of bacteria and fungi had also been confirmed by other studies [28]. The reason could be as a result of co-metabolism. Fungi

presence in hydrocarbon degradation is well known for this phenomenon [29] mostly for the degradation of high molecular weight hydrocarbons [30]. The reliance on consortia for bioremediation study is key because it provides synergism that leads to depletion of pollutants for eco-recovery. The kinetics of biodegradation of hydrocarbons is solely relied on the speed of removal which involves half-life. The latter term is defined as the time taken for a compound to lose half of its quantity. The half-life of the control set-up was 315 days while the lowest half-life obtained in this study was 17 days. This shows that the indigenous microorganisms are good degraders of hydrocarbons. These results sound good but a half-life of 6.4 day has been recorded by Agarry and Latinwo [26] using the same first order model. Kinetic model is of utmost importance to the environmentalist as it provides in-depth information as regards predictive ability and cytotoxic impact control to biota. However, petroleum fractions may be difficult to predict accurately due to the variations in environmental proxies and adaptation to pollutants by microbial flora which could change from time to time. Speedy removal of hydrocarbons and associated metals is very important to man and his environment because these pollutants cause harm to the environment, plant, animals and to humans [31].

5. CONCLUSION

Application of bioaugmentation for bioremediation as shown in this study can be regarded to be efficient and cost effective. This study recorded the degradation of total petroleum hydrocarbon content (TPH) by 70% attributed to bacteria and fungi consortia with a half-life of 17 days. This result indicates that the use of microbial consortia has high potentials in remediation of hydrocarbons and other pollutants of concern. Culture dependent approach in the identification of hydrocarbon degrading bacteria is a huge limitation; hence, future studies should employ metagenomic study for in-depth insights associated with hydrocarbon polluted environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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