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RESEARCH ARTICLE

The effect of minute chronic release of hydrocarbon on soils of communities in proximity to oil fields

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Abstract

Objective: The study documents the effects of hydrocarbon exploitation (without major oil spills) on soils of a rural community, 5 Km from an E &P (exploration & production) site. **Method/Statistical analysis:** By 3 composite drill cuttings, 6 agricultural soil samples obtained from Oben oil field or oil mining lease 4 (OML 4) and Oben village, Nigeria were microbiologically examined. Water and crude oil samples were subjected to physical, chemical, and microbiological examinations. Pearson and Rosenberg model (1978) was used to describe changes in species richness and abundance from 2018 – 2008. The 2008 environmental impact assessment report (EIA) was the environmental baseline. **Finding:** Significant changes in microbe diversity and population was noticed from 2008 to 2018 although no oil spills occurred. Total heterotrophic bacterial count (TBC) revealed greater diversity in 2008 than in 2018. The average TBC for soil samples from rural community and E&P site were 7.0±0.30 x 106 cfu/g, and 2.23± 0.15×102 cfu/g, respectively, in contrast to 6.60 x 10 9 cfu/g in 2008. Percentages of hydrocarbon utilizing microbes increased; indicative of a more favorable environment. Chi-square test (chi-square critical value = 9.49 & p-value= 0.05) used to compare observed population data with the expected; showed changes were not random chance but due to E &P activities. The prevailing regulatory approach was incapable of capturing fundamental issues of contemporary continuous, routine release of contaminants. Need to understand the subtle and pernicious effect of E&P operations on neighboring communities. Environmental laws should be re-examined. **Applications**: Microbes can be used to monitor minute, chronic release of hydrocarbon, inexpensively. They are “markers” in environmental changes even before major pollution, contamination, spillage or devastation occurs.

Keywords: Acute contaminants on soils; contamination in nearby communities; oil field’s contaminants; contamination by proximity; chronic pollutants

1 Introduction

Microbes have been in existence for billions of years. They live in different environments. Their presence tells us much about the surrounding. They assist in degradation of pollutants and influence remediation of contaminated soils through complex metal-substrate-microbe interactions [1]. Their metabolic activities produce substances that can be used to map their existence and condition of the environment [2–4]. Their abundance and diversity therefore, are indicative of quality of environment; the more favorable the environment, the greater their population, (i.e. population density) [5–9]. Therefore, when condition changes, species of microbes, and their population densities change; this knowledge can be used to know minute changes in any site [10–12]. In this research, this hypothesis was tested.

Pollution is a consequence of industrialization. During manufacture of products, wastes are formed. When wastes accumulate; they cause pollution. Consequently, the environment is "degraded"; becomes less favorable to some microbes but more to others. This is because of change(s) in some chemical and physical parameter(s) in the soil or water. Approximately 80% of land mass worldwide is polluted by products of petroleum and or chemical [13].

Oil pollution may be accidental and may occur during exploration, drilling, and transportation of crude oil or refined products. Environmental contamination with crude oil is worldwide. [14] Recent examples of accidental pollution are the 03 December 1992, Aegean Sea tanker oil spill en route to Repsol refinery in A Coruña, Spain [15], off the coast of Spain and the 17 April 1992 KATINA P tanker, while transiting the Mozambique channel [16]. Both were as a result of unexpected rough weather; spilling 74,000 and 66,700 metric tons of crude oil respectively. Oil spills may be deliberate, as in acts of illegal dumping of spent or used oil and in oil sabotage. [17]. Oil sabotage occurs when individuals deliberately destroy oil installation or pipelines for monetary, political or military advantage. This was illustrated in The 1991 Gulf War oil spill which spewed an estimated 8 million barrels of oil into the Persian Gulf. Early reports from Iraqi forces claimed spill had been caused by the United States sinking of two oil tankers. Others believe it was the result of Iraqi forces opening valves of oil wells and pipelines as they retreated from Kuwait, with the apparent strategic goal to foil potential landing by US Marines. [16]. Unrest in the society also leads to oil sabotage [18].

Much has been documented on effect(s) of massive, enormous incidents of oil spills [19]. The implication of such incidents isn't in doubt. We do not seek to re-invent the wheel but rather to document effects of minute, chronic pollution on land. Media attention is riveted on large scale spills. Smaller incidents are often ignored and not reported. Minute but chronic spills are completely disregarded.

Prevailing regulatory approach is incapable of capturing fundamental issues of contemporary continuous, routine, low-dose exposures to contaminants that are within legally sanctioned limits [20]. This is more daunting in countries with weak intuitions where corruption is high and cost of litigation prohibitory [21].

According to the business dictionary, oil spill are the presence of significantly large amount or layers of crude or refined oil on soil or sea water [22]. This definition is limiting and implies that only large volume of pollutant is detrimental to the environment. Investigation by Abdulazeez (2007) [23] showed that pollutants are wide spread and due to "modern activities" of smoking cigarettes, fossil fuel burning, driving cars and use of fuels in industries. According to Frank Fischer (2000) [24], minute chronic pollution are virtually undetectable without scientific investigation. Not much evidence exists on effects of minute chronic oil spills. It is proposed that small minute dose over time, affect not only the immediate environment (on which pollution occurs) but also environments in proximate to it. To study this, effect(s) of E &P activities in Urhonigbe Forest Reserve, Edo State, Nigeria was considered (Figure 1).

The Reserve is a natural low tropical rain forest preserved for decades but in 1972, a portion was excised out, due to its vast hydrocarbon deposit. It was later known as Oben oil field or oil mining lease 4 (OML 4). In this research, soil, water and crude oil samples from Oben oil field and neighbouring rural community, Oben village, were studied.

2 Study area description

Urhonigbe forest reserve (5°56'17.52"; 5° 53' 19.93") lies within low tropical rain forest of Western Niger Delta Nigeria and is characterized by two prominent seasons, wet season (April- October) and dry season from November to March. In 1972, hydrocarbon was discovered in GPS 6° 0' 39/296" North, 5° 52' 3.718" East which covers a portion of the OML 4 and parts of Urhonigbe Forest Reserve showing River Jamieson, collection sites for the agricultural and composite soils and the pipeline reserve [25].

Production started in 1974 and by 1985, oil production was in excess of 40 Mbopd with a total of 32 wells drilled that encountered hydrocarbon. Oben is approximately 50 ft above mean sea level. Geologically, it lies in the Niger Delta basin. The Agbada, Akata and Benin formations chrono-stratigrahic units are identified in Quaternary sediment deposits. Hydro-geology consists of fine-medium grained sand aquifers (average thickness; 15 m); clay deposits (3.5-9.0 m). Static ground water level is

13.21 m-14.53 m. According to [25],static water level is low and ground water is portable with low hydrocarbon concentration of less than 0.03 ppm [25].

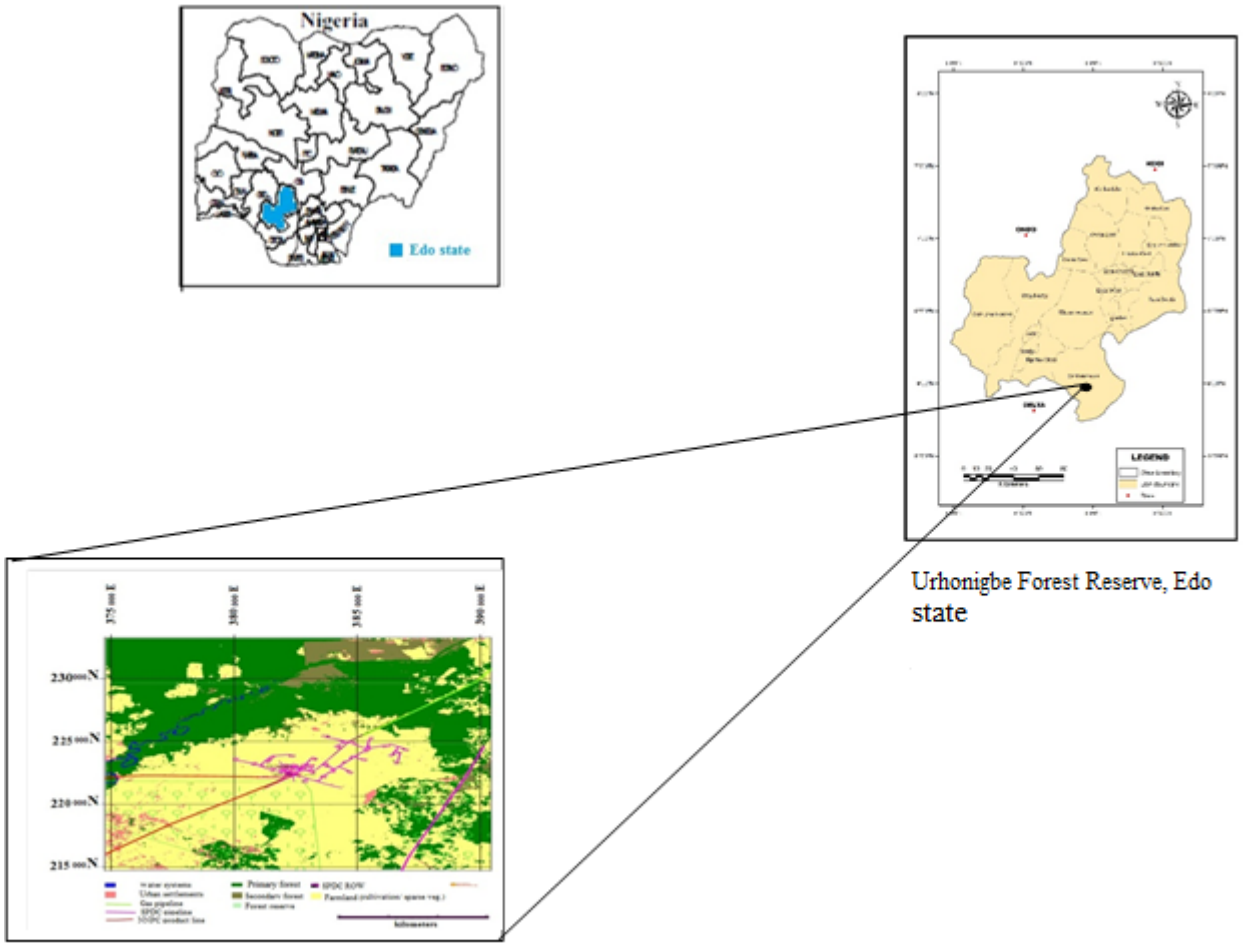

Fig 1. Map of Edo State, Nigeria, showing Urhonigbe Forest Reserve and OML 4. (Not drawn to scale)

3 Material and methods

It was proposed that environments in close proximity to oil drilling and production sites are negatively impacted and as a consequence, diversity of microbes in these places changes with time as petroleum activities take place proximate to them. Changes are believed to occur even without major oil spills. To test this, soil, composite drill cuttings, and water and crude oil samples were collected in 2018 from OML 4 and Oben village. These samples were analyzed in the University laboratory. Different methods are available to monitor natural and anthropogenic stress in organisms [26]. Pearson and Rosenberg model (1978) described changes in species richness, abundance and biomass [27]. An adaptation of Pearson and Rosenberg model was used and only changes in species richness and abundance were considered over a ten-year interval. This was done by comparing 2018 and 2008 microbiological data of the area. In 2008, an EIA was conducted in OML 4 and neighbouring village. The distance from sampling points in undisturbed sample sites to flow station in OML 4 is approximately 5 Km. All areas were originally in the Reserve and had the same geological and geographical settings and conditions prior to E&P activities. Any post-exploration condition must be as a consequence of such activities.

Thus, data used were from two major sources. (1) Data from EIA conducted in 2008. Under Nigerian law, EIA must be carried out before any major construction that may have environmental consequences. Conditions at the reserve, as of 2008 were assumed to be the environmental baseline. (2) Another set of data source were obtained in 2018 during excursion into

the Reserve. Comparative analyses of these two data sets would validate or reject the hypothesis, that environment in close proximity, to oil drilling and production sites are negatively impacted even without major oil spills.

3.1 Collections of samples and data

9 soil samples were collected and analyzed. This includes 3 composite hydrocarbon-contaminated soils (designated ObenHC 1-3) from OML 4 and 6 agricultural soils from Oben village designated UFR-1 –UFR-6. 2 water samples each were collected from River Jamieson (which transverses the Reserve) and a borrow pit in Oben village. Relationship between soil sample collections sites are shown in supplementary file. Soil collection sites were deliberately selected to approximate 2008 sampling points. Dark brown composite drill cuttings were collected from on-site surface dump site and preserved. Agricultural soils samples from Oben village were brown in color; slightly lighter in hue than composite drill cuttings. Soils samples were collected with sterile trowel at a depth of 10-15 cm from surface, after removal of organic debris and leaves associated with topsoil. Total weights of 4.00 kg of soil were collected from each site and preserved in sterile containers to prevent contamination. All soil samples were analyzed in the university's' laboratories for microbial activity in accordance with established guidelines and procedures[28]. Water samples were collected into sterile, transparent, glass jars with lids. Glass containers were earlier autoclaved at 121 degrees centigrade for 45 minutes.

3.1.1 Examination of samples

Samples were subjected to physical, chemical and microbiological examinations.

3.1.2 Physical and chemical tests

Several tests were undertaken as described by American Public Health Association, (APHA, 1989);[29]. The hydrogen ion concentration (pH) measurements were with a beach top model 3505 Digital pH meter. Electrical Conductivity (EC) was measured in the 1:1 soil to water suspension after pH measurement using Model DDS-307 conductivity meter with an accuracy of $\pm$ 1.0%. Results were expressed in micro-Siemens (μS) per cm. Dissolved oxygen (DO) measurement was with Dissolved oxygen meter 850081 DOK. The DO meter was also, used for TDS measurements with its additional probe. BOD was determined with h15421 dissolved oxygen and BOD bench top meter.

Soil texture was determined by visual examination, inspection and by grain size analysis. The sieve shaker (WQS vibrator) was used for grain size analysis. Udden[30] and Wentworth[31] classification with phi 0, 1, 2, 3 sizes 1,500 μm, 250, 125 which represented very coarse sand, coarse sand, medium sand and fine sand grain sizes scale were used.

3.1.3 Microbiological analysis

Microbiological analysis of samples included isolation, characterization and purification of isolates.

3.1.4 Isolation, characterization and purification

Isolation of bacteria and fungi in soil and water samples were by serial dilution and standard plate count. Cultures were purified following subculture of isolates into differential cum selective medium. 100 ml stock of samples were prepared into 200 ml flask and serially diluted into tubes of 9 ml of distilled water, twice. One (01) milliliter of sample was plated onto nutrient agar supplemented with chloramphenicol for bacterial isolates and another with potato dextrose agar supplemented with fluconazole for fungal isolates. Plates were incubated for 24 hours at 28$\pm$2°C.

Fungal cultures were incubated in a humidified environment in the laboratory for 48-72 hours at 30°C. Fungal isolates were characterized and identified following methods stipulated by Barnett and Hunter[32]. All isolates were counted and enumeration using the formula by Willey[33]. Using method described by Kayode-Isola et al.; 2008[34] pure cultures were extracted. Biochemical tests were carried out on isolates.

$$Estimated\ population = \frac{Number\ of\ colonies \times dilution}{Volume\ of\ inoculums} \quad (1)$$

Bacterial isolates were sub-cultured using streak plate method and pure colonies obtained onto favorable media of mannitol salt agar, *Bacillus* media, eosin methylene blue agar, *Pseudomonas cetrimide* agar, bile esculin agar, S*almonella Shigella* agar and Simon citrate agar. Isolates were gram stained, and other biochemical tests such as oxidase, catalase, and indole test were performed.

For identification of fungal isolates; isolates were stained with lactophenol blue and wet mounted. Fungal isolates were examined, under the microscope. Specialized structures such as hyphae and conidia were observed and were correlated with standard texts.

4 Results and discussion

Soil, water and crude oil samples from OML 4 and Oben village were examined establish effect(s) hydrocarbon exploitation activities in OML 4 on Oben village. Microbiological analysis of samples revealed several microbes of significance in crude oil and soil samples (Tables 1 and 2). From their characteristics, main fungal and bacterial isolates were identified. Diversity in crude oil samples was very limited. It is a harsh habitat for microbes[35]. This is probably due to its high toxicity and hydrophobicity. According to Latha and Kalaivani, (2012), presence of crude oil influences biodiversity and distribution of microorganisms in an environment[36]. Similar results was reported by Man et al. (2015)[37] who found *P. aeruginosa* the most dominant bacterial isolate in crude oil samples from China. Survival and growth of *P. aeruginosa* suggests of its ability to utilize components of crude oil as an energy source. This interpretation is in agreement with similar reports in other parts of the world.

Table 1. Biochemical characteristics of isolates from crude oil samples

Characteristics of fungal isolates			
Cultural	Black fluffy colonies with white edges		
Microscopic			
Nature of hyphae	Septate		
Type of spore	Conidiospore		
Colour of spore	Brown		
Appearance of special	Foot cells		
Isolate	*Aspergillus*		
Characteristics of bacterial isolates			
Cultural			
Elevation	Low convex	Low convex	Convex
Margin	Entire	Smooth	Smooth
Colour on MHA	Dark Cream	Cream	Cream
Morphological			
Gram stain	Negative	Positive	Positive
Cell type	Rod	Rod	Cocci
Arrangement	Single	Single	Chains
Spore staining	ND	Positive	ND
Biochemical			
Catalase	Positive	Positive	Positive
Indole	Negative	Negative	ND
Citrate	Positive	Positive	ND
Urease	Negative	Positive	ND
Oxidase	Positive	Negative	ND
Gr. Diff. Agar	PCA	BCA (Straw)	MSA, ORSAB
Identity	*Pseudomonas* sp.	*Bacillus* sp.	*Staphylococcus* sp.

Key: Gr. Diff. Agar =Growth on differential agar, PCA= *Pseudomonas cetrimide agar*, BCA (straw) = Straw colour on *Bacillus* cereus agar, MSA = Mannitol salt agar, ORSAB = Oxacillin resistant screening agar base

Table 2. Cultural, morphological and biochemical characterization of bacterial isolates from agricultural and composite soil samples

Characteristics	A	B	C	D	E
Shape	Round	Round	Spherical	Round	Spherical
Colour	Pale green	Cream	Milky	Milky	Milky
Margin	Entire	Rough	Entire	Entire	Entire
Opacity	Translucent	Opaque	Opaque	Opaque	Opaque
Elevation	Flat	Raised	Flat	Flat	Flat
Wet/ Dry	Wet	Dry	Wet	Wet	Wet
Grain stain	-	+	-	-	-
Cell type	Rod	Rod	Rod	Curve	Rod
Arrangement	Pair	Single	Single	Single	Single
Catalase	-	+	+	+	+
Bile esculin	-	-	-	-	-
Oxidase	+	+	-	+	-

Continued on next page

Table 2 continued

Indole	+	-	+	-	-
Urease	-	+	-	+	+
Citrate	+	+	+	-	-
Lactose	-	+		+	+
Sucrose	+		-	+	+
Maltose	+		-	+	+
Glucose	+	-	+	+	+
VP	+	+	-	-	
Mannitol	-		+	-	+
Spore	-	+	-	-	+
Probable isolate	*Pseudomonas* sp.	*Bacillus* sp.	*Escherichia* sp.	*Staphylococcus* sp.	*Bacillus* sp.

4.1 Agricultural soil samples and composite drill cutting samples

Soil samples were separated into two broad categories; composite soil samples with drill cuttings and agricultural soil samples from the Reserve (Table 3). Composite samples exhibited the less diversity (Pseudomonas sp., *Bacillus* sp.) and had an average microbial population of 2.01 X 10^2 cfu/g soil. Soil samples from the Reserve exhibited greater microbiological diversity (*Bacillus* sp., *B. subtilis*, *Escherichia coli*, *Pseudomonas* sp., and *Staphylococcus* sp.) with average population of 7.15 X 10^7 cfu/g soil.

Table 3. Total heterotrophicbacteria of soil samples and drilling cuttings from Oben Field (OML 4) and Oben village, Urhonigbe Forest Reserve

Soil samples and drilling cuttings from Oben Field (OML 4)				
S/N	Sample no.	Heterotrophic Count (cfu/g. soil)	season	Predominant bacterial genera
1	ObenHC 1	2.23 x102	dry	*Pseudomonas aeruginosa*, *Bacillus subtilis*.
2	ObenHC 2	2.08 x102	dry	*Pseudomonas aeruginosa*, *Bacillus subtilis*.
3	ObenHC 3	1.98 x102	dry	*Pseudomonas aeruginosa*, *Bacillus subtilis*.
Soil samples from Oben village, Urhonigbe Forest Reserve				
4	UFR-1	7.00 x 107	dry	*Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*
5	UFR-2	7.4 x 107	dry	*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*
6	UFR-3	7.2 x 107	dry	*Pseudomonas aeruginosa*, and *Staphylococcus aureus*
7	UFR-4	7.3 x 107	dry	*Bacillus cereus*, *B. subtilis*
8	UFR-5	7.3 x 107	dry	*Pseudomonas aeruginosa*, and *Staphylococcus aureus*
9	UFR-6	6.7 x 107	dry	*Bacillus cereus*, *B. subtilis*

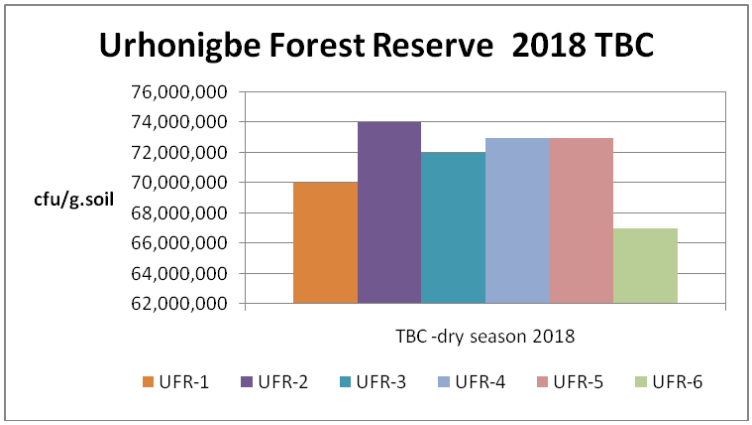

Fig 2. TBC in Urhonigbe Forest Reserve in 2018

It was observed that despite the close proximity of Oben village to OML 4, bacterial diversity obviously was more in agricultural soil from Oben village then in samples from OML 4. Although both were originally part of the Reserve, and had similar values in 2008, 10 years of E & P had changed the microbiological landscape in the two locations. It was surmised that during minute but chronic contamination (e.g. during drilling, transportation and other activities associated with the E & P industry) in OML 4, microbial species unable to thrive in the changed environment were wiped off or stunt in their development. As a consequence; agricultural soil from the Reserve was home to more profuse diversity of microbes than OML 4. There was also, greater microbial diversity in 2008 than in 2018. Yilei et al. (2020), observed change in microbial community due to presence of crude oil in the soil [14]

The average HBCs for agricultural soil samples from Oben village, crude oil and composite samples from OML 4 were: $7.0\pm0.30 \times 10^6$ cfu/g, $3.25\pm 1.35 \times 10^2$ cfu/ml and $2.23\pm 0.15\times10^2$ cfu/g, respectively. Agricultural soil samples from Oben village showed greater diversity than composite drilling cutting and soil samples from OML 4. (This is in contrast an average value of 6.60×10^9 cfu/g and microbes such as *Bacillus* spp., *Staphylococcus* spp, *Cladosporium* sp., *Mucor*, *Aspergillus Niger* sp. in 2008 [25])

Microbial abundance and diversity are indicators of soils' health. Healthier soils are home to greater number of microbes. On the average, samples from OML 4 exhibit lower bacterial and fungal population compared to agricultural soils samples from Oben village. From historical data [25], the Reserve was healthier in 2008 with its greater diversity as revealed by a comparison of 2008 and 2018 values. In 2018, bacterial isolates from Oben village, revealed *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., as predominant isolates and predominant fungal isolate was *Penicillium* sp., which is significant. From River Jameison water samples, *Mucor* sp., *Cladosporium* sp and *Candida* sp. were observed. From OML 4, prominent isolates were *Pseudomonas* sp. and *Bacillus* sp. (Table 3) and *Aspergillus*, *Fusarium* (fungal isolates). *Pseudomonas* sp. genera were one of the abundance and were isolated in soil and crude oil samples.

4.1.1 Physical and chemical tests

Soil texture at Oben was sandy to loamy sands with a soil P^H of 4.40. Subsurface materials were brownish top soil. Organic matter values were high and exchangeable cations low. From water samples, there were little observable changes. Average water surface temperatures were higher than in 2008. Temperatures were 31.0°C and 28.6°C for River Jameison and borrow pit, respectively [25]. This was considered insignificant as differences were 2.05°C and 1.97°C, respectively. Average dissolved solids (TDS) and conductivity were much higher in 2018.

Soil microbes contribute significantly to recycle of nutrients and energy; hence microbiological evaluation of microbes in 2008 and 2018 is crucial. Results revealed significance changes in THC from 2008 to 2018. HBC revealed greater diversity in 2008. In 2008 *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli* were more abundant in the dry season while *Mucor*, *Penicillium* sp. were more abundant during wet months. According to [25], there was practically no hydrocarbon utilizing bacteria in most samples analyzed in 2008; in most cases count were zero. Maximum hydrocarbon utilizing bacterial count in 2008 occurred during the dry season and was 5.0×10^5 cfu/g. soil. The hydrocarbon utilizing bacteria were *Bacillus* spp., *Micrococcus* spp., *Klebsiella* spp. However, in 2018, *Klebsiella* spp. was not detected in any samples. This could hint at critical changes in environmental condition not favourable to *Klebsiella* spp. It was also noted that samples with greatest TBC were from collection sites far from oilfield facilities (Figure 2).

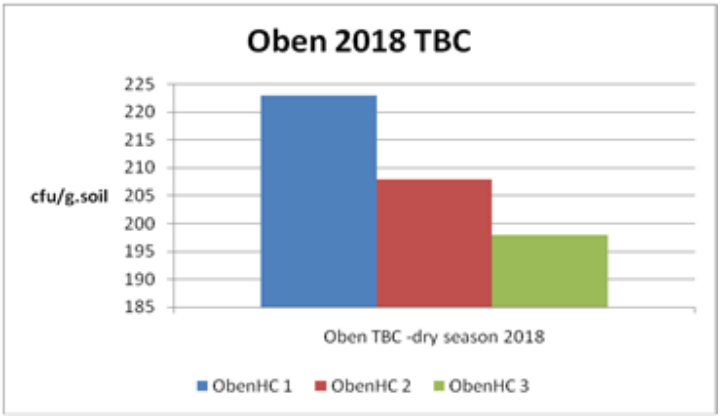

Fig 3. TBC in Oben sampling sites

Samples from OML 4 exhibited lower TBC (Figure 3). The stress (occasioned by introduction of hydrocarbon during drilling and production operations in OML 4 and consequent change in soil medium) simplify complex microbial community by elimination of more sensitive species and increase in number of tolerant microbes.

Microbes can be used as "markers" in environmental changes even before major pollution, contamination, spillage or devastation occurs. To validate this, two areas, OML 4 and Oben village were studied. OML 4 was carved out from the reserve; subjected to several years of petroleum explorations without major oil spillage, but effect(s) of hydrocarbon exploitation in OML 4 was evident in Oben Village, approximately 5 km away. Exploration and exploitation activities left "foot print" in Oben village and parts of the Reserve.

4.1.2 Statistical analysis

To determine categorical relationship between the two years (2008 and 2018) chi-square test was used. It compared observed population data with expected. It determines if changes were random chance variation or due to exploration/ exploitation activities. The expected value of 2.11 x 10^7 cfu/g. soil (average of dry season 2008) was used, with chi-square critical value of 9.49 and p-value of 0.05. Chi-square distribution and critical value were to accept or reject our hypothesis. It revealed observed frequency differed significantly from expected.

5 Conclusion

Subtle reduction in population and diversity in species over time were observed. In 2008, hydrocarbon utilizing bacteria were very in low proportion of TBC. They varied from nil to 5.0% during the dry season. By 2018, counts were higher and overall microbial diversity reduced. However, further research must be done to pinpoint culprits but exploration activities must be suspected [38–40]. Fungal isolates, *Fusarium, Penicillium*, and *Aspergillus* were identified in samples and are effective in heavy metal contamination treatment and remediation. Their increased presence may indicate contamination.

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Declaration of interest statement

The authors declare that there is no conflict of interest.

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