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Microbes in Environmental Changes: Changes in the Chemical and Microbiological Characteristics of Agricultural Soil of Urhonigbe Forest Reserve, Edo State, Nigeria as a Consequence of E&P Activities in OML 4

Onwuachi-Iheagwara, P.N.[1]; Iheagwara, B.I.[2]; Oloro, J.[1]; Iheagwara, P.[3] and Ogofure, G.[4]

[1]Department of Petroleum Engineering, Delta State University, Oleh, Delta State, Nigeria
[2]Dept. of Biological Science, Kwame Nkrumah University of Science Technology, Kumasi, Ghana,
[3]Department of Computer Science, Afe Babalola University Ado-Ekiti, Ekiti State, Nigeria
[4]Department of Microbiology, University of Benin, Ugbowo, Benin City, Nigeria
Corresponding E-mail: **pniheagwara@gmail.com**

Abstract
Soils reveal a diverse array of information. Hydrocarbon exploitation effects the environment. This research investigates change in soils in a rural community due to its proximity to an E&P site. 6 soils samples and 3 composite hydrocarbon-impacted drill cutting samples from Oben village and OML 4 respectively were studied. The authors investigated the relationship between changes in microbial diversity, population and soils properties in Oben village due to petroleum prospecting activities in OML 4 (approximately 5 Km away). OML 4 was excised out of the Urhonigbe Forest Reserve in 1974 due to its vast hydrocarbon deposits. Changes were occasioned by introduction of hydrocarbon into the soil during drilling, exploration and exploitation activities. Much is known about changes in an ecosystem due to massive major oil spills; but minute chronic release of hydrocarbon within legally sanctioned limits is disregarded and often overlooked. Although operational discharges may be considered small in volume, they are repetitive and chronic. The prevailing regulatory approach is incapable of capturing fundamental issues involved in contemporary, continuous, routine, release of hydrocarbon or its products into the environment due to mundane procedures. This article documents changes in the soil of Urhonigbe Forest Reserve due to several years of E & P operations in OML 4. Findings were compared to 2008 Environmental Impact Assessment (EIA) report to show effects on Oben village that proximate OML 4. Although, no major oil spill has occurred, the study revealed that area proximate to E & P activities were affected. Microbiological testing revealed reduction in total heterotrophic bacterial isolates count in agricultural soils of the Reserve and OML4's hydrocarbon-impacted soil as $7.0\pm0.30 \times 10^6$ cfu/g and $2.23\pm0.15 \times 10^2$ cfu/g, respectively as against $1.4 \times 10^8 \pm 2.6 \times 10^{10}$ cfu/g soil , $2.5 \times 10^7 \pm 2.2 \times 10^8$ cfu/g soil during wet season and dry season respectively. Understanding the subtle and pernicious effect of E & P operations on neighbouring rural communities calls for re-examination of environmental laws; without major oil spills, E & P operations change soil in proximate locations.

Keywords: *bacterial diversity, contaminated soil, minute chronic contamination, Urhonigbe Forest Reserve, Effect of release of hydrocarbon with legally sanctioned limits.*

Introduction

Microbes are everywhere. Their abundance and diversity is indicative of the nature of any environment where they exist (Cline, 1998; Hidayat et al., 2012, Hamawand et al., 2013, CREDC, 2019). Can their presence or absence be used to map changes in the chemical and microbiological characteristics of soil with time? And can this knowledge be used to warn Scientist, Engineers and Environmentalist of impeding environmental dangers in advance?.

It is contended that the introduction of hydrocarbon in the environment imprints on the environment changes that can be mapped over time. During the drilling operations and extraction processes there were changes in the soil medium (Human Rights Watch, 1999). This simplifies the complex microbial community by the elimination of the more sensitive species; with disproportion increase in the number of tolerant microbes. As microbes exist within well defined boundary, dependent on several physical- chemical parameters (such as nutrient availability, pH, and e.t.c.), changes in microbes' population and density therefore, can be used to track minute changes in the environment. This offer stakeholders and responsible parties the advantage of early detection.

Environmental monitoring is expensive. Methodologies such as remote sensing and aerial survey are not very effective for small contamination (Moroni et al., 2019). These tools are also, often beyond the monetary reach of most developing countries. Aerial survey is also effective only in macroscopic contamination; but most macroscopic chronic contamination started as a microscopic contamination. Often the political will is not available as environment deterioration due to chronic but continuous contamination is often a slow gradual process and the government or administration saddled with the restoration may not be the leadership that turned a blind eye during its creation. Within the compensation framework, the polluter pays principle is

often not available to affected parties in some developing economies (Luppi et al., 2012). Often when environmental degradation occurs, a blame game ensues. This is daunting in countries with weak intuitions or where corruption is high and cost of litigation prohibitory (Briggs David, 2003). In the Ogoni catastrophe in Nigeria, for example, government institutions and most observers (Baghebo et al., 2012) have accredited the blame to the multinational oil companies for failure to strictly adhere to environmental laws in Nigeria (National Oil Spill Detection and Response Agency Act 2006 among others) but the Multinational oil companies alleged that majority of the oil spillage occurring in the Niger Delta are caused by third party interference. Despite the desire for the petrol-dollar a balance must be struck between the needs for proper control, oil sector integrity and its development (Moffat et al., 1995). This is evident from the experience of Oloibiri, the first site of oil discovery in Nigeria in 1956, which is now a ghost town (Odisu, 2015).

However, this paper urges the stakeholders to look beyond the blame game and focus on the development of an easy inexpensive methodology to detect and rectify the environmental degradation at the microscopic stage before it blossom into macroscopic level.

A rapid and cost effective tool is required for the identification of chronic contamination site. It is proposed that small minute dose of contaminant(s) over time, affect not only the immediate environment (on which the pollution occurs) but also environments in proximate to it. It is also proposed that microbes can serve as an adaptable, easy-to-use, inexpensive tool to detect and rectify the environmental degradation at the microscopic stage. For this study Urhonigbe Forest Reserve was considered because it offers a natural rural forest reserve abut a major E & P extraction site with a unique history of no major oil spills. It offers a rare opportunity to study two contrasting environs in close proximity and enable our hypothesis to be challenged. Did the drilling and extraction activities in OML 4 negatively impact Oben village? Even without major spills? Was there a significant change in the soils in parts of the Urhonigbe Forest Reserve closest to the Oil mining lease 4 as a consequence of these activities? It is believed this would allow a better understanding of the relationship between soil microbes, soil ecosystem balance and contamination in the Urhonigbe Forest Reserve.

Study Area Description

The Urhonigbe Forest Reserve lies within the lowland tropical rain forest with high rains fall of approximately 2480 mm/pa. There are two seasons; the wet season spreads from April to October and dry season, from November to March. A major industrial take of the Urhonigbe Forest Reserve was the Oben flow station

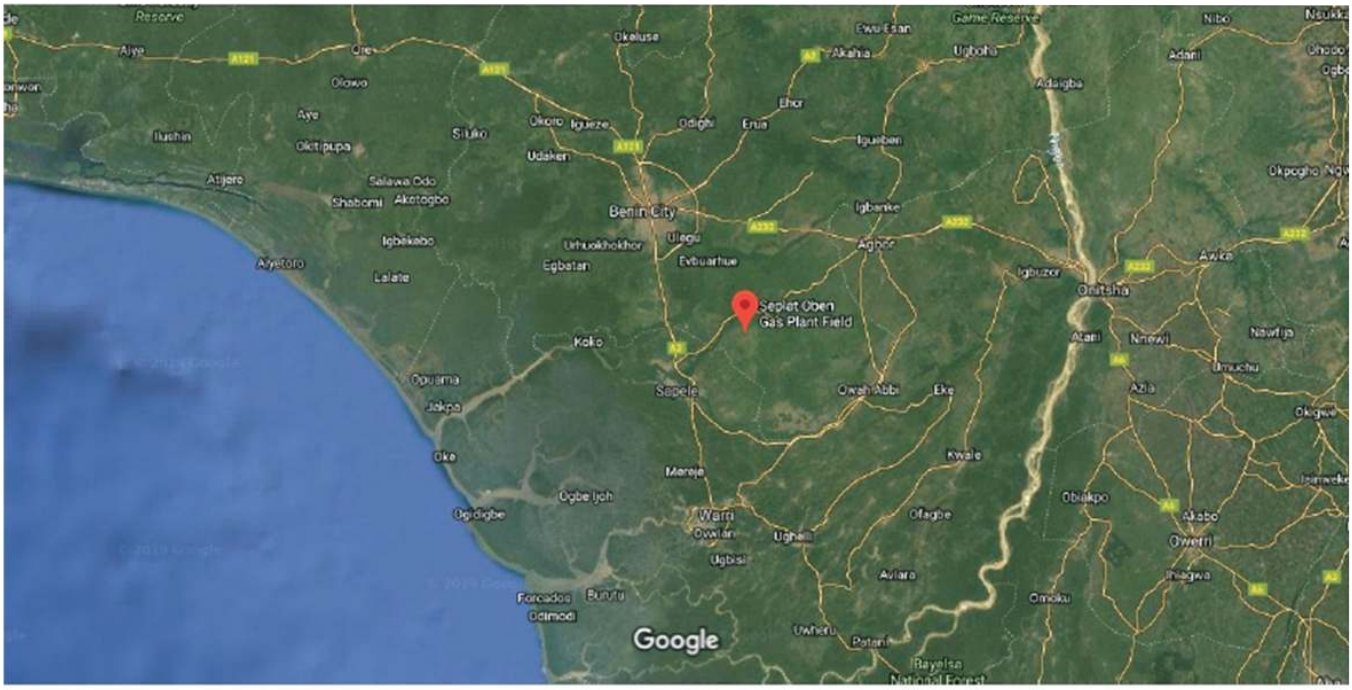

Fig. 1: Map of Edo State, Nigeria showing Oben field. ©: *Google Map*

and gas plant.

Oben Oil Field (figure 1) was discovered in 1972 and came on stream in 1974. By 1985 oil production was peaking at 40 Mbopd. A total of 32 wells drilled encountered hydrocarbon. Oben Oil field is approximately 50 ft above mean sea level. It is located in OML 4 in Edo State, Nigeria. According to the operator of the Oben field, SEPLAT, [http://seplatpetroleum.com/operations/omls-4-38-41/], it is the main producing field in this block; it covers an area of 267 km^2. OML 4 is 78 km NE of Warri, Delta State.

Facilities on the Block include a 60,000 bopd capacity flow station, a 465 MMscfd capacity non-associated gas processing plant and an associated gas compressor station with five 10 MMscfd associated gas ('AG') compressors. These facilities are housed in an area that was once premium agricultural land (figure 2). Oil exports are via the Oben Amukpe pipeline to the Amukpe facilities from the Oben flow station, to either the Forcados terminal or Warri refinery. The license is held by SEPLAT and was renewed in 2018 for a further 20 years. It shall be due for renewal on 21 October 2038.

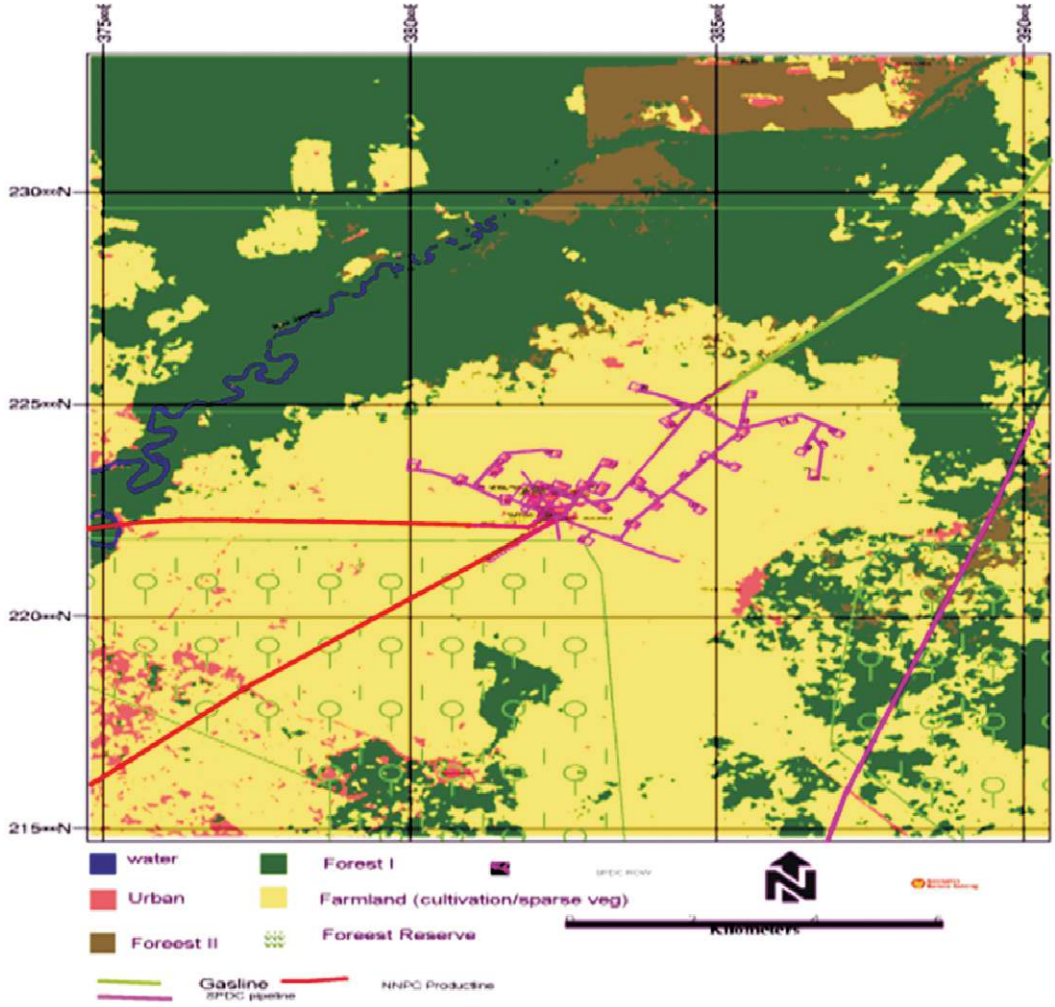

Fig. 2: Map showing Oben (OML 4) and the Urhonigbe Forest Reserve. ©: *Shell, 2008*

Methods / Techniques

Collections of samples

Loose free soil samples were collected from 6 diverse locations in the Urhonigbe Forest Reserve and 3 composite drill cuttings samples from Oben Oil Field figure 3). The loose free soil samples from the Forest Reserve were dark brown or black in color. The soils samples were collected at a depth of 10-15 cm from the surface, after the removal of the organic debris and leaves associated with the topsoil. The total weight of

soil samples collected was 4.00 kg. The soils were collected from each site with a sterile trowel, on 20th August 2018. The composite drill cuttings were dark brown to black; some samples were with slight show of hydrocarbon.

After collection, all samples were bagged, numbered and preserved to prevent contamination. They were sent to the University's laboratory for analysis in accordance with established guideline and procedures (Ugbe, 2012).

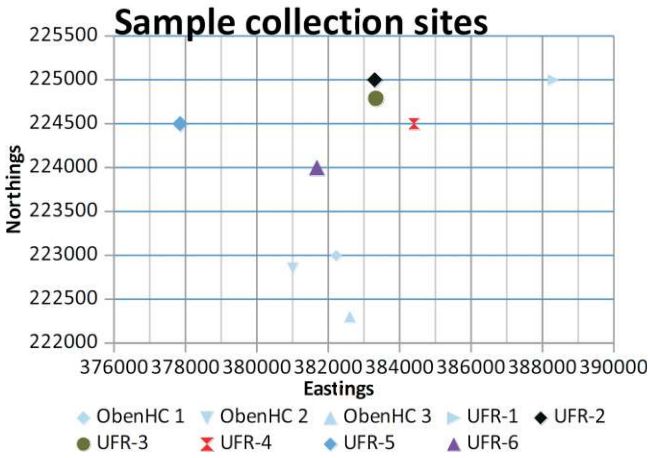

Fig. 3: Sample collection sites in Oben oil field (OML 4) and Oben village, Urhonigbe Forest Reserve

Examination of Samples

The samples were subjected to physical, chemical and microbiological examinations. Some examinations were conducted on the field while others were in the University's laboratories.

Physical and Chemical Tests

Physical and chemical tests were performed using standard laboratory equipment; the beach top model 3505 Digital P^H meter was used for p^H determination, electrical conductivity (EC) was measured in soil to water suspension with a Model DDS-307 conductivity meter with an accuracy of ± 1.0%. The results were expressed in micro-Siemens (µS) per cm.

Isolation and Characterization of Bacteria

Isolation of bacteria and fungi were undertaken using standard best practices (Kayode-Isola et al., 2008). 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, salmonella Shigella agar and Simon citrate agar. The isolates were gram stained, and other biochemical tests oxidase, catalase, and indole test performed.

The fungal isolates were viewed after wet mount and staining with Lactophenol blue, under the microscope. Specialized structures such as hyphae and conidia were observed and were correlated with standard texts.

Evaluation of the population of bacteria and fungi in the samples were by serial dilution and standard plate count. The cultures were purified following subculture of the isolates into differential cum selective medium and biochemical tests were carried out on the isolates. 100 ml stock of the samples were prepared into 200 ml flask and serially diluted into tubes of 9 ml of distilled water, twice. One (01) milliliter of the sample was plated onto nutrient agar supplemented with chloramphenicol and another with potato dextrose agar supplemented with fluconazole. The plates were incubated for 24 hours at a temperature of 28±2 °C. The fungi cultures were incubated in a humidified environment in the laboratory for 4-72 hours at a temperature of 30°C. Fungal isolates were characterized and identified following methods stipulated by Barnett and Hunter (1986).

The isolates were counted and enumeration using the formula by Willey (2008).

$$\textbf{Estimated population} = \frac{\text{Number of colonies X dilution}}{\text{Volume of inoculums}} \quad ....(1)$$

Results/Discussion

Physical and Chemical Tests

Soil texture at Oben was sandy to loamy sands. P^H values of the soil were acidic. In 2008, the soil P^H of the soil in Urhonigbe Forest Reserve was noted as "strongly to moderately acidic" with mean values of 4.78 (dry season) and 4.94 (wet season). In 2018, the mean P^H value of 4.48 was observed in the dry season; no visitation of the location was made during the rainy months. Four visitations occurred during the dry months. The organic matter values were high with an average of 2.45 in 2018; the average in 2008 was 2.67 (Shell, 2008) Exchangeable cations were low. At face value, not much had changed in the Reserve, but can microbial study reveal otherwise?

Microbiological Examinations

When observations from 2018-2019 samples (Tables 1

and 2) were compared to historical data from the 2008 EIA report, the changes in the soils in areas in close proximity to the flow station are apparent. Results of total heterotrophic bacterial count revealed that the rural agricultural soils (7.0±0.30 x10^6 cfu/g) in the Reserve were healthier than the impacted soil from OML 4 (2.23±0.15 x 10^2 cfu/g) today but their condition had deteriorated when compared to historical values.

Table 1: Cultural morphological & biological characterization of bacterial isolates from agricultural and hydrocarbon impacted soils

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
Gram stain	-	+	-	-	-
Cell type	Rod	Rod	Rod	Curve	Rod
Arrangement	Pair	Single	Single	Single	Single
Catalase	-	+	+	+	+
Bile esculin	-	-	-	-	-
Oxidase	+	+	-	+	-
Indole	+	-	+	-	-
Urease	-	+	-	+	+
Citrate	+	+	+	-	-
Lactose	-	+	+	+	+
Surcose	+		-	+	+
Maltose	+		-	+	+
Glucose	+	-	+	+	+
VP	+	+		-	
Mannitol	-		+	-	+
Spore	-	+	-	-	+
Probable isolate	*Psedomonas sp Psedomonas aerubinosa ?*	*Bacillus sp Bacillus subtilis ?*	*Escherichia sp Escherichia coil ?*	*Staphyloccu sp Staphyloccus aureus ?*	*Bacillus sp Bacillus cereus ?*

The investigation in 2018 revealed that bacterial isolates from rural village were predominately *Bacillus sp. Staphylococcus sp. Pseudomonas sp.;* the predominant fungal isolate was *Penicillium sp*. From the hydrocarbon- impacted soil and drill cuttings, isolates were *Pseudomonas sp.*, *Bacillus sp.* and *Aspergillus, Fusarium* (fungal isolates). The hydrocarbon- impacted samples (Table 3a) were observed to exhibit lower bacterial and fungal population compared to the agricultural soil samples from the Oben village (Table 3b). The *Pseudomonas sp.* was isolated in both the agricultural soil and hydrocarbon-impacted soil by phenotypic identification. It is known that *Pseudomonas sp* is common in hydrocarbon contaminated soils.

The 2008 heterotrophic bacterial counts for the environment revealed seasonal variation during the wet and dry season. In the wet season value ranges from 1.4 x 10^8 to 2.6 x 10^{10}cfu/g soil and from 2.5 x 10^7 to 2.2 x 10^8 cfu/g soil during the dry season. The bacterial population of the 2008 was dominated by *Bacillus sp, Mocrococcus sp, Klebsiella sp, Staphylococcus sp, Proteus sp* and *Escherichia coli*. Population of hydrocarbon utilizers were very low (*Bacillus a*nd *Pseudomonas* species) when present in soil samples. Often hydrocarbon utilizers were not present this

Table 2a: Heterotrophic bacteria of drill cuttings from Oben (OML 4) Field 2018

S/N	Sample No.	Heterotrophic Count (cfu/g. soil)	Season	Predominant bacterial genera
1	ObenHC 1	2.23 x 10^2	dry	*Pseudomonas sp., Bacillus sp.*
2	ObenHC 2	2.08 x 10^2	dry	*Pseudomonas sp., Bacillus sp.*
3	ObenHC 3	1.98 x 10^2	dry	*Pseudomonas sp., Bacillus sp..*

Table 2 b: Heterotrophic bacteria of the Oben village, Urhonigbe Forest Reserve 2018

S/N	Sample No.	Heterotrophic Count (cfu/g. soil)	Season	Predominant bacterial genera
4	UFR-1	7.00×10^7	dry	*Bacillus sp.*, *Escherichia coli*, *Pseudomonas sp.*, and *Staphylococcus sp.*
5	UFR-2	7.4×10^7	dry	*Bacillus sp.*, *Staphylococcus sp.*
6	UFR-3	7.2×10^7	dry	*Pseudomonas sp.*, and *Staphylococcus sp.*
7	UFR-4	7.3×10^7	dry	*Bacillus sp.*
8	UFR-5	7.3×10^7	dry	*Pseudomonas sp.*, and *Staphylococcus sp.*
9	UFR-6	6.7×10^7	dry	*Bacillus sp.*,

showed that the soil was not contaminated. Indeed the Environmental Impact Assessment report of 2008, (Shell, 2008) stated "The total hydrocarbon (THC) values are low and below the 50 mg/ kg …...The low THC values maybe due to lack of oil spill incidences in the area.", The 2008 THC values were 2.40 to 25.61 mg/kg with an average of 9.82 mg./kg. This bespoke of the cleaner and more hydrocarbon-free nature of the soil in 2008.

The fungal counts were lower than the bacterial counts and also showed seasonal variation. Seasonal variation is to be expected as there is a positive correlation between water supply and microbial population (Zogg, 1997 and Bell, 1982). Predominant fungal isolates were *Mucor sp*, *Penicillium sp*, *Aspergillus sp* and *Cladosporun sp* with some of the *Penicillium* and *Mucor* species as petroleum degraders. However, it is of great significance that *Pseudomonas sp.* and *Bacillus sp.* were detected from the impacted soil samples. *Pseudomonas aeruginosa* has been described as having bioremediation abilities (Marchak 1995, Kayode-Isola et al., 2008, Lúcia, 2009). From the research of Modupe et al., (2018), the increased presence of *Pseudomonas sp.* in Oben Oil field should also serve as a warning. In 2008, the environmental impact assessment there was a low percentage of hydrocarbon utilizers (0.01 -0.90%) in the soil samples. (Shell, 2008), its increased population is indicative of a more favorable environment for their survival.

The predominant fungal isolates identified in the 2018 samples were *Fusarium, Penicillium*, and *Aspergillus;* but in 2008 these were *Mucor sp*, *Penicillium sp*, *Aspergillus sp* and *Cladosporun sp,* some of the *Penicillium* and *Mucor* species are petroleum degraders (Usmani et al., 2006, Syed et al., 1998, Hidayat et al 2012).This is also a silent warning of a changing environment.

Conclusion

In this investigation, the bacterial isolates obtained from samples of agricultural soils collected in 2018, were *Bacillus sp., Escherichia coli, Pseudomonas sp.*, and *Staphylococcus sp.* and from crude oil impacted soil *Pseudomonas sp.* and *Bacillus sp.*. In 2008, the bacterial diversity obviously was more profuse. The 2008 soil was dominated by *Bacillus sp*, *Mocrococcus sp*, *Klebsiella sp*, *Staphylococcus sp, Proteus sp* and *Escherichia coli.* It has been observed over and over again that microbial species unable to thrive in a changed environment are often wiped off or reduced in population. The agricultural soil of 2008 would be home to a more profuse diversity of microbes than the hydrocarbon-impacted soil of the OML 4 and the 2018 agricultural soil in the Reserve. Hence without *any* major spillage, the soils of 2018 Urhonigbe Forest Reserve were affected because of its close proximity to the exploration activities in OML 4.

There is an inherent global implication in this, as actions taken in any place may have unforeseen implication for other areas and its environs. Microbes are fundamental

to life on earth... They are key players in the soil carbon sequestration but often absent or poorly represented in hydrocarbon contaminated soils. Oil exploitation often involves deforestation with its negative effects. This can led to changes in weather patterns, soil texture etc. (Watts, 2001; Watts 2005; Okoh et al, 2006; Nwaugo et al., 2007; Zetter 2011).Trees, plants and microbes are central players in the hydrologic, nitrogen and carbon cycles. Soil organic carbon is the basis of soil fertility and a critical part of the natural carbon cycle. It has been estimated that the world's soils hold around twice the amount of carbon found in the atmosphere and in vegetation. There is an urgent need to preserve our soils.

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Declaration of Interest Statement

The authors declare that there is no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

List of abbreviations and acronyms

Cfu/g	Colony forming unit per gram
Cfu/ml	Colony forming unit per milliliter
GPS	Global Positioning System

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