Microbial Diversity of a Remote Aviation Fuel Contaminated Sediment of a Lentic Ecosystem in Ibeno, Nigeria

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Abstract

Environmental pollution from Oil & Gas Exploration & Production (O&G E&P) activities remains one of the major problems in the oil-producing communities of Nigeria. This results from improper oily wastes disposal as well as incessant oil spills in the region. The operator's lack of responsible business practices in wastes management and the over-dependence of the economy on oil and gas earnings, in the most part, exacerbate the problems of environmental pollution. Government's lack of political will power which treats issues on oil pollution with levity and long period of neglect of these polluted sites leave the environment ecologically destabilized. Studies to ascertain the ecological status of remote aviation fuel-contaminated sediment of a lentic ecosystem in Inua Eyet Ikot village, Ibeno, Nigeria, have been carried out using conventional microbiological culture-dependent methods. This methodology is known to reveal only <1% of the microbial diversity present. These results were therefore considered inaccurate and grossly misleading. In this present study, sediment samples from this lentic ecosystem in Ibeno, Nigeria previously polluted by Aviation fuel in 2001 (about 14 years ago) were collected and analyzed to assess its prokaryotic diversity using both the conventional culture-dependent as well as culture-independent (16S metagenomic) techniques involving community DNA extraction, DNA sequencing and bioinformatics analyses. The culture-dependent techniques revealed the presence of only six genera of bacteria and no archaea was detected. The 16S metagenomic methods revealed that the sediment of the polluted lentic ecosystem harbors a much more diverse population of bacteria and archaea. These results corroborate the 'great plate count anomaly' principle and demonstrate that the use of 16S metagenomic tools will redefine the actual ecological status of the environment.

Keywords: Aviation fuel polluted site; Bioinformatics analyses; Community DNA extraction; Culture-dependent techniques; DNA sequencing; Lentic ecosystem; Prokaryotic diversity

Introduction

The Nigerian economy relies heavily on the revenue derived from the oil sector, as they provide 70% of government revenue and about 95% of foreign exchange earnings [1]. The nonchalant attitude of the oil operating companies in their improper ways of oily wastes disposal, intermittent oil spillages [2,3], relatively underdeveloped technology for spill prevention and response measures, and insufficient local capacity and political will power of government to stem the spate of incessant oil spill incidents in Nigeria [4], all result in increased risks of oil pollution and environmental degradation. Oil and gas reserves in Nigeria are concentrated in the Niger Delta region of the country, and have attracted the presence of multinational oil companies to the area [5,6].

The Niger Delta region consists of nine states [7] with Akwa Ibom State as the highest oil-producing state and thus attracts the highest financial allocation from the federal government [8]. Ibeno Local Government Area hosts the operational base of Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of Exxon Mobil and Qua Iboe Terminal (QIT) [9].

Thousands of barrels of oil have been spilt into the environment through oil pipelines bursts and oil tanker accidents in the country since the inception of oil activities, partly as a result of lack of regular maintenance of the oil installations e.g., pipelines replacements after they out-lived their installed lifespan and partly because of sabotage and other causes like oil tanker accidents. Notably, some of these facilities have been in use for decades without replacement [10]. For example, in 2004, an abandoned oil well at Oloibiri, Bayelsa State released about 20,000 barrels of oil into the environment [11] and evidence proved that the well had been leaking for many years without response from the operating company [12]. In January 1998, 40,000 barrels of crude oil from Idoho production platform of Exxon Mobil was spilt into the environment and caused severe damage to the entire Nigerian coastline [13]. Between 1997 and 2001, about 2,097 oil spill incidents were recorded in Nigeria [10]. Oil spill cases occur daily and in most cases more than once in a day in the Niger Delta region of Nigeria.

On 8th August 2001, Exxon Mobil's aviation fuel pipeline ruptured releasing about 1000 barrels of aviation fuel into a lentic ecosystem in Inua Eyet Ikot village, Ibeno, Akwa Ibom State. Dispersant was used as the only remedial measure to contain the spill. Microbial diversity of the spill site is monitored using the culture-dependent methods [14,15] which is known to reveal only <1% of the microbial population [16]. These results are unrealistic and therefore do not present the true ecological status of the site. Fourteen years after the spill, the ecological integrity of the site is questionable and appropriate remediation action can not be carried out.

Studies report that culture-dependent techniques only account for a small subset, approximately <1% of the total microbial diversity present in the environment [17]. According to [18], it is possible to detect a wider variety of microbial diversity using metagenomic tools [19,20] than by using culture-dependent methods. According to the

'great plate count anomaly', it is observed that most microbes cannot be grown under laboratory conditions; some may actually be nonviable, while others may be viable but non-culturable (VBNC) [21]. This study on the microbial diversity of a remote aviation fuel-contaminated sediment of a lentic ecosystem in Ibeno, Nigeria is aimed at identifying the microbial communities that inhabit the sediment of a lentic ecosystem polluted by aviation fuel about 14 years ago using both the culture-dependent and culture-independent (metagenomic) approaches. This is the first time, to the best of our knowledge, that metagenomic tools is used to determine the ecological integrity of any environmental media in the Niger Delta region.

Materials and Methods

Site description

The lentic ecosystem in Inua Eyet Ikot, Ibeno is located on longitude 04°32.647' N, and latitude 007°59.95' E. The site is located off QIT-Jetty Road in Ibeno, Akwa Ibom State, Nigeria.

Sediment sample collection

Sediment samples from this remote aviation fuel-contaminated lentic ecosystem were collected at different points using a hand-held auger into sterile polythene bags and taken to the laboratory. The samples were composited and a composite sediment sample used for the analyses.

Culture methods

Serial dilution: Ten-fold serial dilutions of the sediment samples were made as described by the American Public Health Association [22,23].

Inoculation and incubation: One milliliter of appropriate ten-fold serial dilutions of the sediment samples were inoculated onto nutrient agar (Oxoid CM 314), malt extract agar (Oxoid) and sabouraud dextrose agar plates in triplicates using pour plate methods [22,23] and spread plates methods [24,25]. The hydrocarbon utilizing bacterial counts (HUB) were enumerated by the spread plate technique using oil-mineral salt medium (MSM) [26]. The media were supplemented with cycloheximide (100 μg/ml and benomyl (50 μg/ml) to prevent fungal growth (Eka and Forgathy, 1972). The crude oil used was sterilized by filtering through Millipore filter (0.45 μm pore size) and stored in sterile bottles. Inoculated plates were incubated at 28 ± 2°C for 18-24 hr and 48-72 hr for the enumeration of total heterotrophic bacteria and fungi, respectively. Visible discrete colonies in incubated plates were counted and plates with population densities that ranged from 30 and 300 colonies were multiplied by the reciprocal of the dilution factor and expressed as colony forming units per gram (cfu/g) of sediment samples.

Maintenance of pure culture: Discrete colonies were purified by repeated sub-cultures unto appropriate agar media. Pure cultures were preserved on nutrient agar slants and stored in the refrigerator (4°C ± 2°C) and at ambient temperature (28°C ± 2°C) for further tests.

Characterization and identification of microbial isolates: Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques and biochemical tests including carbohydrate utilization [27]. Identification of the bacterial isolates was accomplished by comparing the characteristics of the cultures with that of known taxa as in ref. [28]. Characterization and identification of fungal isolates was carried out as in ref. [29,30].

16S Metagenomic (culture-independent) methods

DNA extraction and purification: Community/metagenomic DNA was extracted from the sediment sample using the Zymo® soil DNA extraction Kit (Model D 6001, Zymo Research, USA) following the manufacturer's instructions [31]. The purity as well as the quantity of the extracted DNA was examined by recording its UV absorption spectrum using NanoDrop spectrophotometer and running the DNA in 1% agarose gel electrophoresis.

DNA amplification and sequencing: The extracted DNA was amplified by Polymerase chain reaction (PCR) using primers pairs 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'-ATTACCGCGGCTGCTGG - 3' as forward and reverse primers, respectively. Successful amplification of DNA was verified by electrophoresis. The nucleotide sequence of the purified products was determined by Next Generation Sequencing (NGS) technique in an automated PCR cycle-Genome Sequencer™ MiSeq (Illuminar). Analysis and alignment was performed using Vector NTI Suite 9 (InforMax, Inc; USA). Overall bioinformatics analysis was done using NCBI-BLAST-2.2.24 and CLC BioGenomics workbench v7.5.1.

Results and Discussion

Results of culture-dependent techniques

Microbial counts: Microbial growths were found on nutrient agar medium at dilutions 10^{-1} to 10^{-3}, blood agar medium at dilutions 10^{-1} and 10^{-2}, Mineral salt medium at dilution 10^{-1} and 10^{-2}, mannitol salt agar medium at dilutions 10^{-1} and 10^{-4}, and on MaConkey agar medium at dilution 10^{-1}. The rest of the media had no visible growth (Table 1a).

Microbial counts of the aviation fuel-polluted sediment of the lentic ecosystem using the culture-dependent techniques are as presented on (Table 1b) as the microbial counts.

The characteristics of the prokaryotic organisms isolated from the sediment samples from the remote aviation fuel-contaminated site using conventional microbiological culture-dependent methods are presented on Table 2. They were classified into the different taxonomic groups as shown on Table 3.

Microbial diversity using Metagenomic (culture-independent) tools

Summary of sequencing statistics and classification rate: The sequencing statistics showing total reads and reads passing quality filtering used for the 16S Metagenomic analyses are as shown on Table 4 and the summary of the classification rate are shown on Table 5.

Figure 1 shows the summary of the classification rate of the prokaryotic organisms present in the aviation fuel-contaminated lentic sediment by taxonomic levels.

The 16S Metagenomic analyses of the sediment from a remote aviation fuel-contaminated lentic ecosystem shows that the prokaryotic composition were classified into bacteria with 17,100 reads (91.0%), archaea with 697 reads (3.71%) and 995 reads (5.29%) were unclassified at the kingdom level, suspected to belong to the third kingdom (domain), the eukaryota (Figure 2).

This 16S metagenomic tool was able to identify the organisms into the different domains; the bacteria, archaea and the other unclassified, suspected to be the third domain, the eukaryota. The results of this 16S metagenomic report also identified and thus classified all the prokaryotic organisms present at this remote aviation fuel-contaminated lentic

Culture Medium	Dilution Factor				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Nutrient Agar	90	31	10	NG	-
Blood Agar	23	9	NG	-	-
Thiosulphate Citrate Bile Salt (TCBS)	NG	-	-	-	-
Starch Nitrate Agar (SNA)	2	NG	-	-	-
Mineral Salt Medium (MSM)	31	19	NG	-	-
Manitol Salt Agar (MSA)	1	NG	-	-	-
MacConkay Agar (MCA)	6	NG	-	-	-
Basal Salt Medium (BSM)	NG	-	-	-	-
Salmonella-Shigella Medium (SSA)	NG	-	-	-	-
Sulphate Solubilizing Bacterial Agar (SSBA)	NG	-	-	-	-
Phosphate Solubilizing Bacterial Agar (PSBA)	NG	-	-	-	-
Eosin Methylene Blue Agar (EMBA)	NG	-	-	-	-

Table 1a: Counts of microbial cells using culture-dependent techniques. NG: No growth.

Culture medium	Microbial counts (cfu/g)
Nutrient Agar (NA)	3.1×10^3
Mineral Salt Medium (MSM)	3.1×10^2

Table 1b: Viable counts of microbial cells using culture-dependent techniques.

sediment into their various taxonomic groups including phylum, class, order, family, genus and species.

Prokaryotic organisms belonging to 27 Phyla were detected using this 16S metagenomic tool. Table 6 shows that the top 7 of 27 phyla (with the number of reads) belonged to the Phyla *Firmicutes* (9.65%), *Proteobacteria* (49.74%), *Actinobacteria* (5.58%), *Chloroflexi* (4.99%), *Acidobacteria* (3.94%), *Bacteroidetes* (2.89%), and *Euryarchaeota* (2.87%). At this level, 11.24% were unclassified. Figure 3 shows the top 5 of 27 phyla (70.05%) while the unclassified and others make up the remaining 15.90% and 14.05%, respectively.

A total of 57 classes of prokaryotic organisms were detected from the remote aviation fuel-contaminated lentic sediment. The top 7 of 57 classes (Table 7) were identified to be *Betaproteobacteria* (17.01%), *Deltaproteobacteria* (11.61%), *Gammaproteobacteria* (10.21%), *Alphaproteobacteria* (9.63%), *Clostridia* (6.81%), *Actinobacteria* (5.09%) and *Anaerolineae* (4.44%) (Figure 4).

A total of 109 orders of prokaryotic organisms were identified from the remote aviation fuel-contaminated lentic sediment in Ibeno, Nigeria. Table 8 presents the reads and percent of total reads of the top 7 of 109 orders while Figure 5 gives the orders as *Actinomycetales* (4.59%), *Clostridiales* (4.66%), *Pseudomonadales* (4.89%), *Rhizobiales* (5.63%), *Rhodocyclales* (16.26%), *Anaerolineales* (4.31%) and *Burkholderiales* (3.85%).

The 16S metagenomic result of the remote aviation fuel-contaminated lentic sediment identified and thus classified all the procaryotic organisms present into a total of 224 families. Table 9 presents the identities of the top 7 of the 224 families as *Rhodocyclaceae* (10.92%), *Pseudomonadaceae* (4.58%), *Anaerolinaceae* (4.31%), *Comamonadaceae* (2.93%), *Clostridiaceae* (2.79%), *Acidobacteriaceae* (2.29%), and *Hyphomicrobiaceae* (2.12%). A total of 19.86% were unclassified.

A total of 498 genera of prokaryotic organisms were identified from the remote aviation fuel-contaminated lentic sediment at Ibeno, Nigeria. The identities of the top 7 of the 498 genera were presented in Table 10 as *Zoogloea* (5.58%), *Pseudomonas* (3.83%), *Longilinea* (1.96%), *Edaphobacter* (1.86%), *Clostridium* (1.64%), *Geobacter*

(1.60%), and *Candidatus blochmannia* (1.60%). The eighth most predominant group was unclassified (26.06%).

Using the 16S metagenomic tools, a total of 718 prokaryotic species were identified to be present in the remote aviation fuel-contaminated lentic sediment at Ibeno, Nigeria. The seven most predominant species (Table 11) were identified to be *Zoogloea resiniphila* (5.52%), *Longilinea arvoryzae* (1.93%), *Edaphobacter modestus* (1.86%), *Candidatus blochmannia rufipes* (1.58%), *Peptoniphilus coxii* (1.02%), *Uliginosibacterium gangwonense* (1.00%) and *Chondromyces pediculatus* (0.94%). Of this 718 species, 50.43% of the species were considered unclassified.

In summary, the prokaryotic composition of the aviation fuel-contaminated lentic site was so diverse that 718 different species, 498 genera, 224 families, 109 orders, 57 classes, 27 phyla and 2 domains (excluding those unclassified) (Table 12) were identified using the 16S metagenomic tools as compared to the results obtained using the culture-dependent techniques. It is important to note that the pie chart shows only all classifications above 3.5% abundance while the "other" category in the pie chart is the sum of all classifications with less than 3.5% abundance.

Comparatively, the culture-dependent method of microbial identification showed limited microbial diversity right from the kingdom level to the species level than the culture-independent techniques (Table 12).

Ribosomal sequences are present in all organisms, and serve as phylogenetic markers which allow organisms to be distinguished on all phylogenetic levels. Metagenomics investigation to ascertain the true composition of the microbial community present in the sediment sample revealed complex consortia of prokaryotes which have been extensively documented in hydrocarbon-contaminated environments as well as during oil degradation [32]. Based on the quality of the metagenomic data set and the read length of the DNA fragments, the results showed that bacteria are the predominant group with 91.7% in the polluted sediment. This high degree of occurrence recorded by bacteria agrees with a study by ref. [33], which reports that by the evolution of enhanced hydrocarbon degradation which horizontal gene transfer is a major force, bacterial community structure changes and proliferation takes place in response to hydrocarbon pollution. Also, bacteria is said to utilize hydrocarbons as carbon and energy source more than any other microbial group. They have been shown to be more versatile than even fungi and therefore may play a greater role during biodegradation of hydrocarbons [34].

The 16S rRNA sequences detected in the investigated sediment samples, showed the highest similarities to those of the organisms *Zoogloea resiniphila* (5.52%), *Longilinea arvoryzae* (1.93%), *Edaphobacter modestus* (1.86%), *Candidatus blochmannia rufipes* (1.58%), *Peptoniphilus coxii* (1.02%), *Uliginosibacterium gangwonense* (1.00%), *Chondromyces pediculatus* (0.94%). Other members of the complex bacterial community identified from the remote aviation fuel-contaminated site included *Ralstonia* sp., *Nostocoida limicola* III strain Ben222, *N. moscoviensis*, *Ehrlichia* sp. HI-2000, *Waddlia* sp. G817, *Geobacter bremensis* strain Dfr1, *Arthrobacter uratoxydans* strain DSM 20647, *Enterococcus faecium*, *Mycobacterium scrofulaceum*, *Leptospira parva*, *Azovibrio* sp., *Bacillus sphaericus*, *Massilia timonae*, *Roseateles depolymerans* strain 61B2, *Rhodovulum* sp., *Modestobacter versicolor* strain CP153-2, *Streptomyces* sp., *Neochlamydia hartmannellae* strain A1Hsp, *Desulfovibrio idahonensis*, *D. fructosivorans* strain JJ, *Anabaenopsis circularis* NIES21, *Lactobacillus rhamnosus* strain PL60, *Candidate division* OP11 clone LGd8, *Alcanivorax* sp., *Saccharococcus*

Culture medium	Morphology	Gram reaction	Cell type	Urease	Citrate	Methyl red	Voges Proskaur	Motility	Starch hydrolysis	Oxidase	Catalase	Glucose	Lactose	Galactose	Sucrose	Maltose	Manitol	Hydrogen sulphide	Probable Organisms
NA	Irregular, flat, brown, and undulated colony	-	Rod	+	+	-	+	+	-	-	+	Ag	A	A	A	Ag	-	-	*Xanthomonas* sp.
NA	Regular, cream, and raised colony	+	Rod	-	+	+	-	+	-	-	+	Ag	-	A	Ag	A	-	-	*Bacillus subtilis*
NA	Regular, cream, and raised colony	+	Cocci	-	+	+	-	-	+	+	+	Ag	-	A	A	A	-	+	*Micrococcus* sp.
NA	Regular, cream and entire colony	+	Cocci	+	+	-	+	-	-	-	+	A	-	A	A	Ag	-		*Staphylococcus albus*
NA	Milky, viscous, and opaque colony. Odour present	-	Rod	+	+	-	+	+	-	-	+	Ag	Ag	Ag	Ag	Ag	-	+	*Proteus* sp.
BA	Irregular, flat, cream, and erose colony	+	Rod	-	+	-	+	+	-	+	-	Ag	-	A	Ag	A	-	+	*Lactobacillus* sp.
BA	Filamentous, flat, cream, lobate colony	+	Rod	+	+	-	+	+	-	+	+	Ag	-	A	A	A	-	-	*B. cereus*

Table 2: Characteristics of microbial isolates from sediment samples using culture-dependent techniques. NA: Nutrient Agar; BA: Blood Agar; A: Acid; Ag: Acid and gas.

Taxonomic Level	Frequency
Phylum	1
Class	3
Order	4
Family	5
Genus	5
Species	6

Table 3: Classification of isolates from Culture-Dependent methods into taxonomic groups.

Total Reads	Reads Passing Quality Filtering	% Reads Passing Quality Filtering
20,157	18,792	93.2%

Table 4: Sequencing statistics.

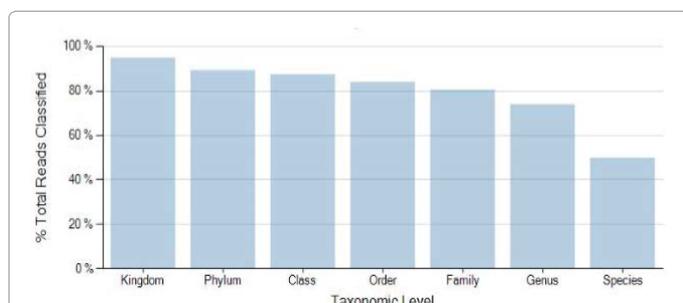| Taxonomic Level	Reads Classified to Taxonomic Level	% Total Reads Classified to Taxonomic Level
Kingdom	17,797	94.71%
Phylum	16,680	88.76%
Class	16,371	87.12%
Order	15,736	83.74%
Family	15,059	80.14%
Genus	13,894	73.94%
Species	9,316	49.57%

Table 5: Classification rate summary.

Figure 1: Summary of the classification rate by taxonomic levels.
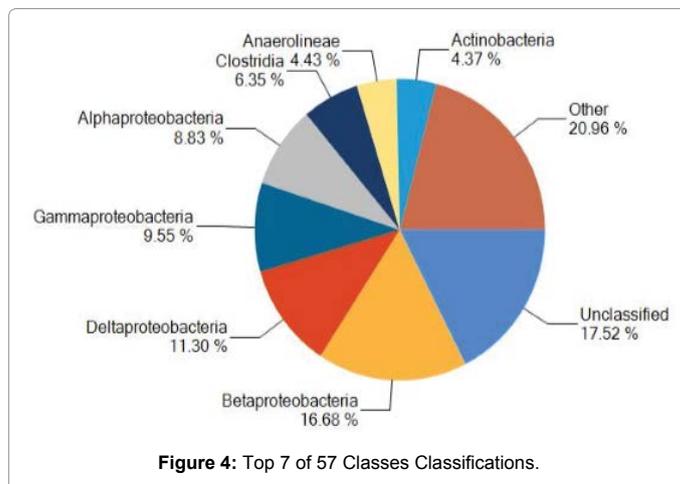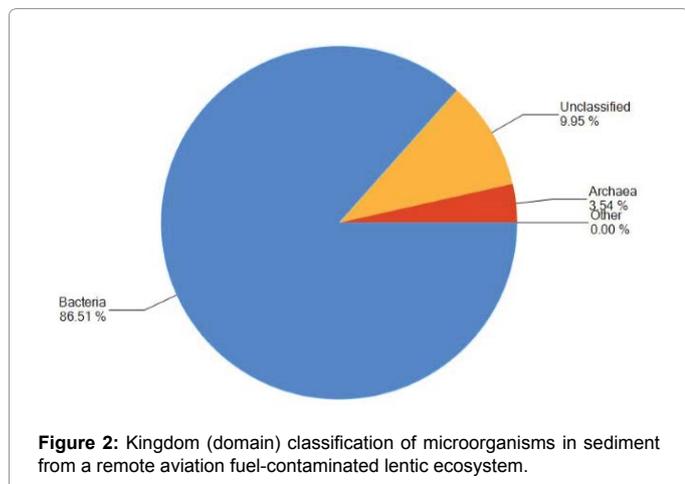
thermophiles strain 657, *Pseudoalteromonas* sp., *Pseudomonas* sp., *Shewanella benthica*, *Sphingomonas panni* strain C52, *Pseudomonas pseudoalcaligenes* strain M5-4, etc.

Uncultured bacteria were also detected by the 16S metagenomic methods to include the uncultured *Clostridiaceae* bacterium, uncultured *Holophaga* sp (AJ519667.1), uncultured Antarctic *cyanobacterium* clone FreP09 (AY541579.1), uncultured *Pedomicrobium* sp. clone Oversite 90 (AY647328.1), uncultured *Nitrospirae* bacterium clone AKYG1809 (AY921744.1), uncultured *Bacteroidetes* bacterium clone LiUU-9-73 (AY509370.1), and a host of others. This strongly implies that these group cannot be cultured which confirms the "great plate anomaly".

The 16S rRNA metagenomic analysis detected archaeal 16S rRNA sequences of *Methanococcus maripaludis* (AF005049.1), the uncultured *Methanosarcinaceae* archaeon (AJ879043.1), uncultured archaeon (AJ310855.1), anaerobic *methanogenic* archaeon (AJ244295.1), an unidentified archaeon (Y15393.1), *Methanococcus aeolicus* (DQ136171.1), uncultured *Methanosaeta* sp. etc.

Majority of the microbial cells revealed by the culture-independent methods were not encountered in the culture-dependent approach. This justifies the inefficiency of the conventional culture-dependent techniques. The use of 16S Metagenomics approaches will bypasses the need for microbial cultivation as majority of microbial species in any environment prove uncultivable in the laboratory mainly due to the lack of knowledge of the real conditions under which these bacteria grow in their natural environment [34]. Microbial identification by metagenomics approach therefore results in the discovery of unique and unrecognized microorganisms as well as complex microbial diversity in contaminated samples, thus revealing the true ecological status of any investigated environment. The very few (only 6) species of bacteria and no Archaean member revealed by the conventional culture-dependent methods obtained in this study is not surprising as the method identifies only the few microorganisms that can only be cultivated in the laboratory.

Crude oil and other petroleum hydrocarbons are not only found at the site of oil pollution, but are chemically heterogeneous and almost ubiquitous in the environment including most pristine soils and sediments [35] following seepage from natural deposits and biosynthesis by plants and microorganisms [36,37]. However, the effect of an oil spill is believed to cause the presence of a more diverse oil-degrading microbial community, ultimately leading to a microbial community that will be more efficient at degrading crude oil, should another accidental spill take place. The results from this study revealed efficient hydrocarbon-utilizers from this remote aviation

Figure 2: Kingdom (domain) classification of microorganisms in sediment from a remote aviation fuel-contaminated lentic ecosystem.

Classification	Number of Reads	% Total Reads
Proteobacteria	9,347	49.74%
Unclassified at Phylum level	2,112	11.24%
Firmicutes	1,813	9.65%
Actinobacteria	1,048	5.58%
Chloroflexi	937	4.99%
Acidobacteria	741	3.94%
Bacteroidetes	543	2.89%
Euryarchaeota	539	2.87%

Table 6: Top 7 of 27 Phyla classification.

Figure 4: Top 7 of 57 Classes Classifications.

Classification	Number of Reads	% Total Reads
Unclassified at Order level	3,056	16.26%
Rhodocyclales	2,053	10.92%
Rhizobiales	1,058	5.63%
Pseudomonadales	919	4.89%
Clostridiales	876	4.66%
Actinomycetales	862	4.59%
Anaerolineales	810	4.31%
Burkholderiales	724	3.85%

Table 8: Top 7 of 109 Orders classification.

Figure 3: Top 5 of 27 Phyla Classification.
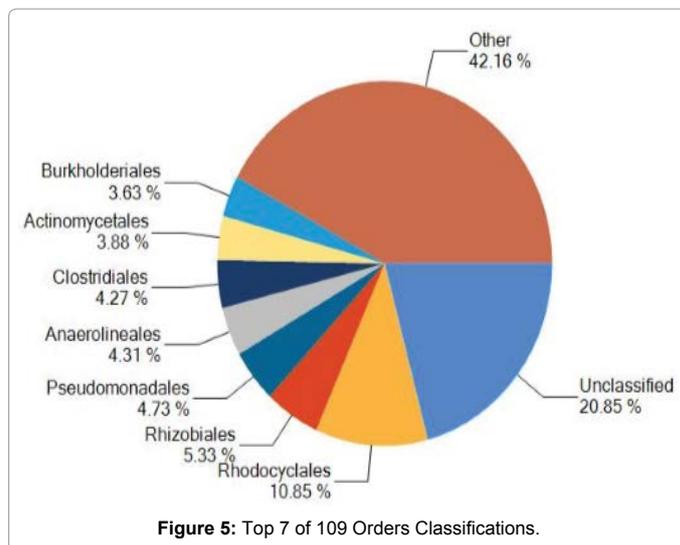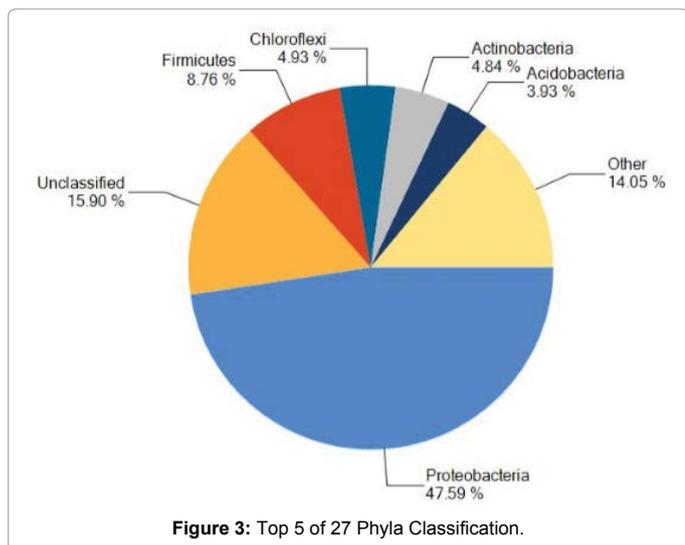
Classification	Number of Reads	% Total Reads
Betaproteobacteria	3,197	17.01%
Unclassified at Class level	2,421	12.88%
Deltaproteobacteria	2,181	11.61%
Gammaproteobacteria	1,919	10.21%
Alphaproteobacteria	1,810	9.63%
Clostridia	1,280	6.81%
Actinobacteria	956	5.09%
Anaerolineae	835	4.44%

Table 7: Top 7 of 57 Classes classification.

Figure 5: Top 7 of 109 Orders Classifications.

Classification	Number of Reads	% Total Reads
Unclassified at Family level	3,733	19.86%
Rhodocyclaceae	2,053	10.92%
Pseudomonadaceae	861	4.58%
Anaerolinaceae	810	4.31%
Comamonadaceae	551	2.93%
Clostridiaceae	525	2.79%
Acidobacteriaceae	431	2.29%
Hyphomicrobiaceae	399	2.12%

Table 9: Top 7 of 224 Families classification.

Classification	Number of Reads	% Total Reads
Unclassified at Genus level	4,898	26.06%
Zoogloea	1,049	5.58%
Pseudomonas	719	3.83%
Longilinea	368	1.96%
Edaphobacter	350	1.86%
Clostridium	308	1.64%
Geobacter	300	1.60%
Candidatus blochmannia	300	1.60%

Table 10: Top 7 of 498 Genera classification.

Classification	Number of Reads	% Total Reads
Unclassified at Species level	9,476	50.43%
Zoogloea resiniphila	1,037	5.52%
Longilinea arvoryzae	363	1.93%
Edaphobacter modestus	350	1.86%
Candidatus blochmannia rufipes	296	1.58%
Peptoniphilus coxii	192	1.02%
Uliginosibacterium gangwonense	188	1.00%
Chondromyces pediculatus	177	0.94%

Table 11: Top 7 of 718 Species classification.

Taxonomic level	Total Number of Classifications	
	16S Metagenomics tools	Culture-dependent methods
Kingdom	2	1
Phylum	27	2
Class	57	3
Order	109	4
Family	224	5
Genus	498	5
Species	718	6

Table 12: Summary of classification at taxonomic level.

fuel-polluted site; namely, species of *Bacillus, xanthomonas, Micrococcus, Staphylococcus, Arthrobacter, Nocardia, Pseudomonas, Azospirillum, Dechloromonas, Streptomyces, Sphingomonas, Ralstonia, Proteus*, among others as reported by ref. [34,38,39]. These group of microorganisms are commonly associated with oil-contaminated ecosystems due to their high osmophilic properties [40,41], implying that the microbial communities of an ecosystem can provide information on the health of that ecosystem.

Conclusions

In conclusion, as low as six species of bacteria with no archaeal representative were detected from the conventional culture-dependent techniques while the 16S metagenomic analysis detected a total of 718 microbial species comprising both bacterial and archaeal diversity. Comparing the efficiency of both approaches adopted, this study confirms and thus supports the "great plate count anomaly" as well as support the adoption of the 16S metagenomic and other complimentary OMICS tools to investigate the true ecological integrity of any investigated environment.

Acknowledgements

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