

# Use of 16S rDNA Sequencing to Determine Prokaryotic Diversity of a Remote Aviation Fuel-Polluted Lentic Ecosystem in Ibeno, Nigeria

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## Abstract

Ibeno, the operational base of Mobil Producing Nigeria Unlimited, a subsidiary of ExxonMobil, Nigeria remains one of the most impacted communities by oil and gas activities in the Niger Delta region of Nigeria. Lotic and lentic systems in the region which residents rely on, receive petroleum hydrocarbon inputs almost daily due to oil spills and oily wastes discharges from operators and bunkering activities. This research was carried out to determine the prokaryotic diversity in a remote aviation fuel-polluted lentic ecosystem after 16 years of pollution using metagenomic approaches. DNA extraction from the water samples was carried out using MoBio DNA extraction Kits following the manufacturer's instructions. Extracted DNA fragments were quantified using picogreen and by recording their UV absorption spectra using NanoDrop spectrophotometer. 16S rDNA sequencing was carried out on a Miseq Illumina sequencing platform and Quantitative Insight Into Microbial Ecology (QIIME) bioinformatics pipeline. Analyses revealed the dominance of bacterial and archaeal communities in both polluted and unpolluted water samples. The polluted sample had 93.83% bacterial and 3.43% archaeal population while the control sample revealed 58.05% bacterial and 39.69% archaeal population. Dominant bacterial phyla from the polluted samples were *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Cyanobacteria*, and *Chloroflexi* while dominant phyla in the unpolluted samples were *Proteobacteria*, *Firmicutes* and *Actinobacteria*. Dominant archaeal phyla from both polluted and unpolluted waters were Euryarchaeota and Crenarchaeota. The use of 16S rDNA metagenomic approach revealed a wide variety of bacterial and archaeal diversity from both polluted and control sites, thus revealing the true ecological status of both sites.

**Keywords:** 16S rRNA; DNA sequencing; Prokaryotic diversity

## Introduction

Since the inception of oil and gas exploration and production (O&G E&P) activities in Nigeria and in spite of the increasing revenue from these resources, the communities from which they flow continue to experience deprivation and environmental degradation due to daily inputs of petroleum hydrocarbon spills and oily wastes discharges [1]. Ibeno is one of the thirty-one (31) LGAs in Akwa Ibom State, Nigeria. It is the location of massive oil deposits, which have been extracted for decades by Mobil Producing Nigeria Unlimited (MPNU), a subsidiary of ExxonMobil Corporation and some marginal oilfield operators like Frontier Oil Ltd and Network Exploration and Production Nigeria Ltd [2,3]. Over the years, the rivers, streams, marine and forest which happened to be the major income sources for the majority of the rural dwellers in the region have become highly contaminated due to the O&G E&P activities [4].

The presence of petroleum hydrocarbon is considered one of the major factors that influence microbial diversity and succession in polluted water bodies [5]. Diverse groups of microorganisms naturally are capable of hydrocarbon degradation mostly as food due to the ubiquitous distribution of hydrocarbons in the environment from both natural and anthropogenic inputs [6,7]. Numerous genera of bacteria e.g., *Staphylococcus*, *Pseudomonas*, *Bacillus*, *Proteus*, *Micrococcus*, *Klebsiella*, *Enterobacter* [8]; fungi e.g., *Fusarium*; [9] and yeasts such as *Rhodotorula* have been isolated from contaminated aquatic ecosystems and their metabolic activities are strongly considered responsible for the removal of the hydrocarbons from the environment [6].

The gene that encode the small subunit ribosomal RNA is ubiquitous in prokaryotes [10] and serves in the classification of bacteria and archaea owing to its high degree of conservation and its fundamental function in living organisms [11]. It is important to note that several pieces of RNA are important for proper ribosome functions. This RNA

is not translated to proteins, the ribosomal RNA is the active component. Thus we refer to the "rRNA gene" or "rDNA" to designate the DNA in the genome that produces the ribosomal RNA. This study was designed to assess the prokaryotic diversity of a remote aviation fuel-contaminated lentic ecosystem after 16 years of aviation fuel pollution alongside a control lentic ecosystem with no history of aviation fuel pollution using 16S rRNA gene amplification and sequencing.

## Materials and Methods

### Site description and sample collection

Integrated sampling was carried out at an aviation fuel-polluted lentic ecosystem on longitude 04° 32.647' N, and latitude 007° 59.951' E and on longitude 04° 58.519' N, and latitude 007° 57.908' E as the control. Water samples were collected at different points in one-litre pre-washed plastic containers and taken to the laboratory in ice-packed cooler. Samples from individual site were composited and used for the analyses.

### Deoxyribonucleic Acid (DNA) extraction, sequencing and bioinformatics analysis

Community DNA of all the microorganisms was extracted using

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the MoBio DNA Extraction Kit. The eluted DNA was centrifuged at room temperature at  $10,000 \times g$  for 30 seconds. The supernatant was discarded leaving the DNA. The DNA was preserved for further analyses at  $-20^{\circ}$  to  $-80^{\circ}\text{C}$ . The concentration of the DNA was examined an ultra-violet absorbance spectrophotometry. DNA amplification involves the duplication of the DNA molecules with each strand serving as template for the duplication. Each strand of the DNA molecule serving as template was amplified by Polymerase Chain Reaction with the aid of 16S rRNA primers in a  $50 \mu\text{l}$  reaction mixture with the following programme: denaturation at  $94^{\circ}\text{C}$  for 3 min, and 30 cycles of  $94^{\circ}\text{C}$  for 20 sec, annealing at  $53^{\circ}\text{C}$  for 30 sec, and extension at  $68^{\circ}\text{C}$  for 5 min, with a final extension at  $68^{\circ}\text{C}$  for 10 min. The PCR products were by agarose gel electrophoresis. PCR products were sequenced using the Miseq Illumina platform. The 16S rRNA sequences were analyzed using the Quantitative Insight into Microbial Ecology (QIIME) version 1.8.0 (Figure 1).

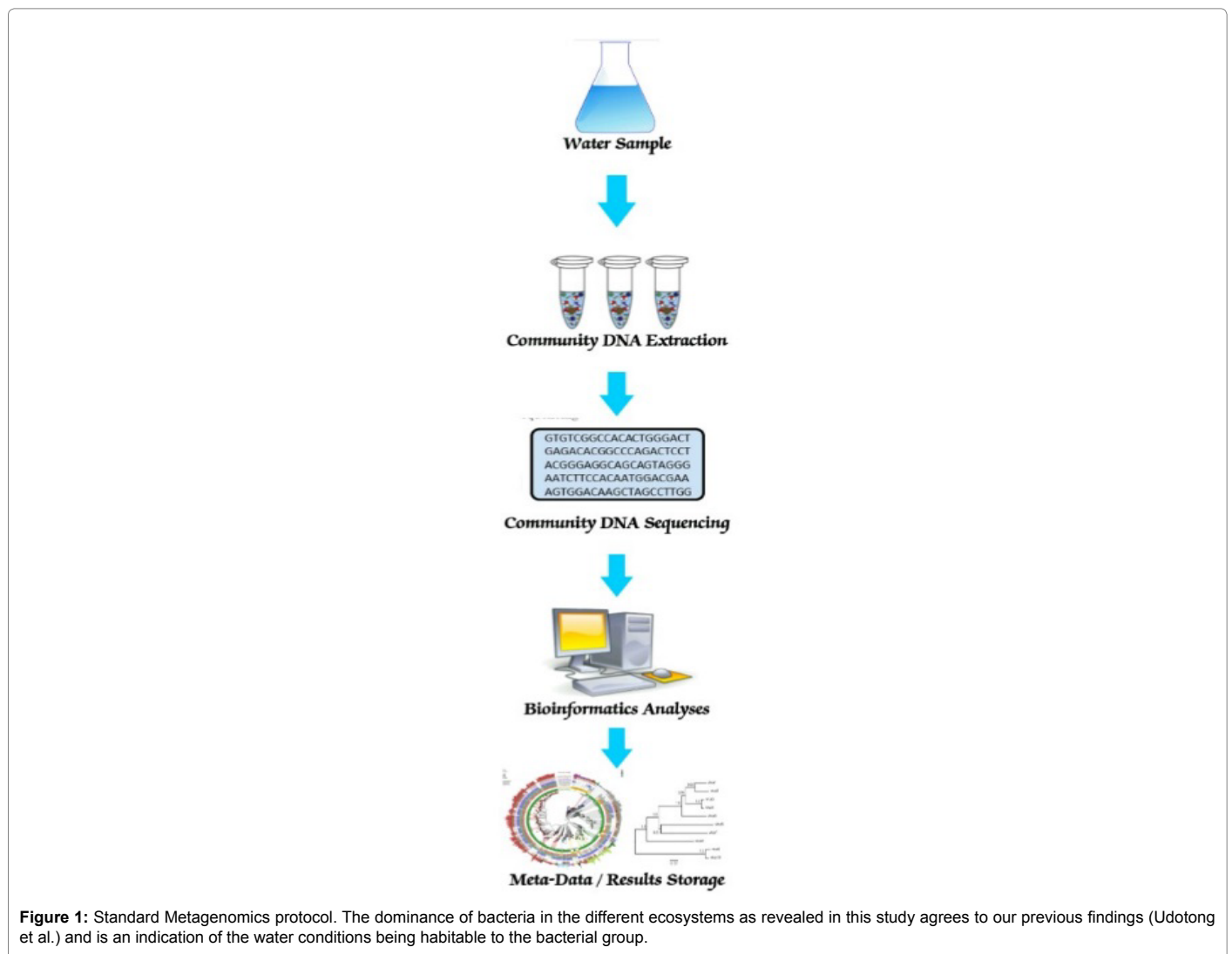
## Results and Discussion

Numerous sequences of bacteria and archaea were detected from samples of both water bodies using the 16S rDNA sequencing. The method revealed comparable results (Figure 2) indicating a higher bacterial population in the polluted water than that of the control water.

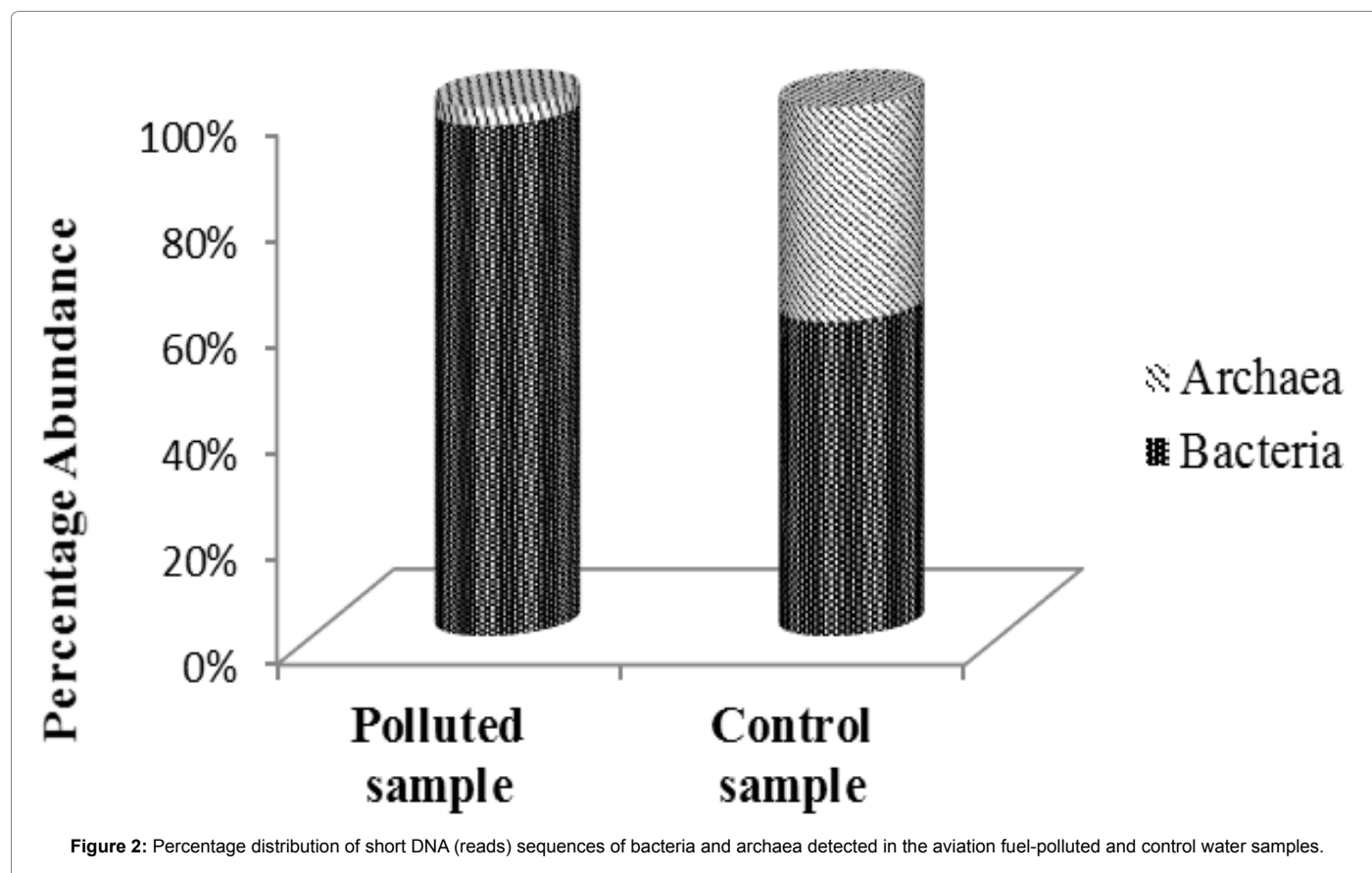
Bacteria showed a high occurrence in the two sites with the percentage composition of 93.83% in the polluted sample and 58.05% in the control sample.

Phylum level affiliations of the sequences recovered from the two samples revealed distinct difference in phylum-level community composition. Sequences from 26 and 20 phyla were retrieved from the polluted and control site, respectively. "Other" represents the sum total of all phyla with percentage read of less than one (1). As presented on Table 1, the top / dominant phyla in the bacterial community detected in the polluted water were Unknown, Proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria, and Chloroflexi and represented 37.52%, 33.86%, 7.31%, 6.19%, 3.65% and 2.84% respectively. The top / dominant phyla in the control water sample were Unknown, Proteobacteria, Firmicutes, and Actinobacteria and represented 21.50%, 39.69%, 27.87% and 2.61% of all classified sequences from the sample, respectively. Dominant phyla in the archaeal community retrieved for both waters were Euryarchaeota and Crenarchaeota representing 1.33% and 0.19% in the polluted sample and 0.24% and 0.23% in the control sample.

Sequence affiliated to other phyla like Planctomycetes (0.11%), Fibrobacteres (0.08%), Deinococcus-thermus (0.04%), Fusobacteria



**Figure 1:** Standard Metagenomics protocol. The dominance of bacteria in the different ecosystems as revealed in this study agrees to our previous findings (Udotong et al.) and is an indication of the water conditions being habitable to the bacterial group.



S No	Phylum	Percentage Reads (%)	
		Polluted sample	Control sample
1	Unknown*	37.52	21.50
2	Proteobacteria	33.86	39.69
3	Firmicutes	7.31	27.87
4	Actinobacteria	6.19	2.61
5	Cyanobacteria	3.65	0.48
6	Chloroflexi	2.84	0.47
7	Bacteroidetes	1.88	1.37
8	Ciliophora	1.82	0.57
9	Euryarchaeota	1.33	0.24
10	Other**	3.97	6.96

\*Unknown represents all phyla with % reads of >1 but not identified in the database while \*\*Other is the sum total of all phyla with % read count of <1.

**Table 1:** Top 8 Phyla and parentage distribution of bacterial and archaeal diversity retrieved from the aviation fuel-polluted and control (unpolluted) water bodies.

(0.01%), etc. and Chlorobi (0.12%), Spirochaetes (0.11%), Aquificae (0.06%), Gemmatimonadetes (0.05%), Fibrobacteres (0.03%), Fusobacteria (0.02%) in the contaminated and control samples, respectively, were found in extremely low abundance, as indicated in parentheses.

Sequences belonging to 40 and 34 classes of prokaryotes were retrieved from the polluted and control water samples, respectively. The top 12 Classes are represented on Table 2. Over thirty eight (38.08) percent of sequences retrieved from the polluted water sample and 21.56% of sequences from the control water sample were affiliated to the class “Unknown”. Also, 39.55% of sequences from the control site had affiliation to the class ‘Not assigned’ and were the highest in among the classes. While sequences similar to Betaproteobacteria

(20.13%), Alphaproteobacteria (8.10%) and Actinobacteria (6.17%) dominated in the contaminated water, Betaproteobacteria (20.75%), Gammaproteobacteria (4.29%) and Actinobacteria (2.57%) showed highest occurrence in the control sample.

Presented on Table 3 are the sequences retrieved from the polluted water sample that matched those of bacterial and archaeal diversity that belonged to the Order Unknown, Burkholderiales, Actinomycetales, and Rhizobiales while those in the control sample matched the ‘Not assigned’, Unknown and Burkholderiales, etc.

Table 4 presents the different families of bacteria and archaea identified in the polluted and control water samples. Majority of the detected sequences were affiliated to the family Unknown, with the percentage composition of 44.50% followed by *Alcaligenaceae*

S No	Class	Percentage Read Count (%)	
		Polluted sample	Control sample
1	Not assigned	0.05	39.55
2	Unknown*	38.08	21.56
3	Betaproteobacteria	20.13	20.75
4	Alphaproteobacteria	8.10	1.83
5	Actinobacteria	6.17	2.57
6	Bacillaceae	3.96	1.49
7	Cyanophyceae	3.38	0.44
8	Clostridia	3.36	1.40
9	Gammaproteobacteria	3.07	4.29
10	Chloroflexi	2.84	0.47
11	Deltaproteobacteria	1.93	0.64
12	Gymnostomatea	1.65	0.24
13	Bacteroidetes	1.54	0.37
14	Methanomicrobia	1.14	0.09
15	Other**	4.67	6.11

\*Unknown are Classes with percentage read count of >1 but not identified in the database while \*\*Other is the sum total of all Classes with percentage read count of <1

**Table 2:** Percentage distribution of top bacterial and arhaeal Classes detected in the aviation fuel-polluted and unpolluted water samples.

S No	Order	Percentage Read Count (%)	
		Polluted sample	Control sample
1	Not assigned	0.03	39.51
2	Unknown*	42.20	25.43
3	Burkholderiales	18.82	18.22
4	Actinomycetales	5.39	1.93
5	Bacillales	3.78	1.40
6	Rhizobiales	4.93	0.95
7	Chroococcales	3.38	0.44
8	Clostridiales	3.34	1.25
9	Spathidiida	1.56	0.24
10	Bacteroidales	1.54	0.37
11	Methanosarcinales	1.14	0.09
12	Sphingomonadales	1.04	0.04
13	Pseudomonadales	0.77	1.67
14	Neisseriales	0.11	1.13
15	Other**	10.05	9.32

\*Unknown are Orders with percentage read count of >1 but not identified in the database while \*\*Other is the sum total of all Orders with percentage read count of <1

**Table 3:** Representative Order of prokaryotes detected in the aviation fuel-polluted and control (unpolluted) water sample.

S No	Family	Percentage Read Count (%)	
		Polluted sample	Control sample
1	Unknown*	44.50	25.66
2	Comamonadaceae	1.71	8.12
3	Alcaligenaceae	16.55	0.72
4	Bacillaceae	3.62	0.93
5	Cyanobacteriaceae	3.38	0.44
6	Beijerinckiaceae	3.06	-
7	Streptomycetaceae	2.78	0.77
8	Coniocybaceae	0.03	39.51
9	Burkholderiaceae	0.38	9.36
10	Neisseriaceae	0.11	1.13
11	Pseudomonadaceae	0.36	1.11
12	Clostridiaceae	1.33	1.04
13	Eubacteriaceae	1.64	0.15
14	Spathidiidae	1.56	0.24
15	Chloroflexaceae	1.16	0.27
16	Methanosarcinaceae	1.14	0.04
17	Sphingomonadaceae	1.04	0.04
15	Other**	23.07	14.07

\*Unknown are Families with percentage read count of >1 but not identified in the database while \*\*Other is the sum total of all Families with percentage read count of <1

**Table 4:** Family classification of prokaryotic sequences detected in the aviation fuel-polluted and unpolluted samples.

Bacterial and Archaeal Species	Polluted sample	Control sample	% Identity Match	Accession number
Uncultured bacterium	+	-	93	KF023595.1
Uncultured bacterium	+	-	81	GU632587.1
<i>Mycobacterium</i> sp.	-	+	95	LN876401.1
Uncultured bacterium	+	-	83	GQ402641.1
Uncultured archaeon	+	-	96	KJ645016.1
<i>Acidovorax</i> sp.	-	+	96	KR088454.1
Uncultured <i>Streptophyta</i>	-	+	89	JQ701246.1
<i>Staphylococcus</i> sp.	-	+	83	AJ316320.1
<i>Saprospira</i> sp.	+	+	98	AY929064.1
Uncultured <i>Methanosarcina</i> sp.	+	-	95	AY454773.1
<i>Achromobacter</i> sp.	+	+	95	AM232721.1
Uncultured <i>Gloeotheca</i> sp.	+	+	93	AY874086.1
Uncultured <i>Verrucomicrobia</i> bacterium	-	+	73	AY874030.1
Uncultured <i>Bacteroidetes</i> bacterium	-	+	95	AY874003.1
Uncultured <i>Beijerinckia</i> sp.	+	-	94	AY806011.1
<i>Streptomyces</i> sp.	+	-	86	AB124448.1
<i>Streptomyces</i> sp.	-	+	91	AB124529.1
<i>Simkania negevensis</i>	+	+	98	SSU68460.2
<i>Pseudomonas aeruginosa</i>	+	+	81	AB126582.1
Unidentified bacterium	+	-	86	AJ518513.1
<i>Bacillus anthracis</i>	-	+	83	AE016879.1
<i>Bacillus</i> sp.	+	-	85	AB126768.1
<i>Bacillus sphaericus</i>	+	+	78	AY161044.1
Uncultured <i>Chloroflexi</i> bacterium	+	-	95	AY921865.1
<i>Acidovorax delafieldii</i>	+	-	81	AJ518818.1
Uncultured <i>Chloroflexus</i> sp.	+	+	95	AY862018.1
Unidentified eubacterium	+	-	95	AJ229218.1
<i>Pseudomonas</i> sp.	-	+	75	AJ278108.1
Uncultured <i>Methanosphaera</i> sp.	-	+	77	AY454780.1
Uncultured <i>crenarchaeote</i>	-	+	87	AY454669.1
Uncultured archaeon	+	-	96	DQ146728.1
<i>Spirulina subsalsa</i>	+	+	95	AF329394.1
<i>Pyramimonas parkeae</i>	-	+	98	AF393608.1
<i>Pseudomonas saccharophila</i>	-	+	77	AF396932.1
<i>Bradyrhizobium</i> sp.	-	+	82	AF363148.1
Uncultured <i>Methanobacteriaceae</i>	+	-	91	AM050403.1
Uncultured candidate division OD1	+	-	86	AY921841.1
Uncultured soil bacterium	+	-	96	AY850299.1
<i>Chlorella sorokiniana</i>	-	+	88	X65689.1
<i>Leptolyngbya</i> sp.	+	-	91	X84809.1
<i>Gemmata obscuriglobus</i>	-	+	94	X85248.1
<i>Methylocapsa acidiphila</i>	+	-	93	NR_028923.1

Key: +: Detected; -: Not detected

**Table 5:** Representative diversity of Prokaryotes in Aviation fuel-polluted and unpolluted water samples.

(16.55%) in the contaminated water and *Coniocybaceae* (39.51%) and Burkholderiaceae (9.36%) in the control water.

This study revealed a higher bacterial diversity in the aviation fuel-polluted water than in the unpolluted water. This may be attributed to presence of petroleum hydrocarbons and their effects on the diversity and population of prokaryotes especially the bacterial group in the freshwater system, an observation earlier reported by Atlas and Bartha [12]. Bacterial and archaeal species with gene sequences affiliated to those present at the two study sites together with their accession numbers are represented on Table 5. Both sites share some species in composition as observed in other taxa and here are indicated with a “+” sign. Majority of the prokaryotic sequences are those of uncultured bacteria as well as uncultured archaea. Some of the species in common include *Bacillus sphaericus* with accession number AY161044.1, *Achromobacter*

sp.-AM232721.1, Uncultured *Gloeotheca* sp.-AY874086.1, *Pantoea* sp.-AJ534866.1, *Stigonema ocellatum*- AJ544082.1, *Pseudomonas aeruginosa*-AB126582.1, *Simkania negevensis*-SSU68460.2, Uncultured *Chloroflexus* sp.-AY862018.1. Observably, plenty of the species found in the polluted sample are not found in the control sample and vice versa. For example, *Methylobacterium* sp.-AJ549955.1, *Bacillus gibsonii*-AB112017.1, *Dechloromonas* sp.-AY084087.2, *Nitrobacter winogradskyi* - L11661.1, *Methylocapsa acidiphila*- NR\_028923.1, *Methylocystis parvus* - AJ458508.1, etc.

## Conclusion

The 16S rDNA analysis of the prokaryotic diversity of the remote aviation fuel-polluted and unpolluted lentic ecosystems revealed an enormous composition of bacteria and archaea in both water



bodies. The polluted water had a greater composition of prokaryotes. Bacterial community got a higher diversity of the composition in both waters. Archaeal population of the polluted water was remarkably low compared to the high composition observed in the unpolluted and control samples.

According to Ntushelo [13], Approaches to identifying and studying bacterial diversity often relied on the traditional methods of plating bacteria on agar. These approaches are still relevant for culturable bacteria but fall short of detecting fastidious and unculturable bacteria. Molecular-based techniques like targeted sequencing of the 16S rRNA gene from gross DNA samples have facilitated surveys of bacterial diversity. The sequencing and cloning of individual sequences is however tedious and cannot provide a comprehensive survey of a bacterial community. The 16S rRNA gene can be amplified from pure bacterial colonies or can be amplified directly from a crude sample. Amplified from a crude sample, the 16S rRNA gene can be massively sequenced using high-throughput sequencing instruments. Direct amplification of the 16S rRNA gene and its massive sequencing has corrected the underrepresentation of bacteria in many bacterial communities. Analysis of bacterial communities is now made easier by the ample data generated from various bacterial communities survey projects, like this hydrocarbon polluted and unpolluted lentic ecosystem [14,15].

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