MICROBIAL DIVERSITY OF A REMOTE AVIATION FUEL CONTAMINATED SEDIMENT OF A LENTIC ECOSYSTEM IN IBENO, NIGERIA

BY

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PRESENTATION OUTLINE

- Introduction
- Methodology
  - Description of study Area
  - Culture-Dependent Approaches
  - 16S Metagenomic studies
- Results & Discussion
- Conclusion

INTRODUCTION

- **Nigerian economy relies heavily on oil revenue providing 70% of gov't revenue and about 95% of foreign exchange earnings**
- **Akwa Ibom State, one of the 9 States of Niger Delta region is the highest crude oil and condensate producer in Nigeria with Ibeno LGA hosting MPNU, an IOC and a subsidiary of ExxonMobil.**
- **Oil discharged into Ibeno environment were not cleaned up :**
  - **oil in process water,**
  - **oil discharges from tanker washing (ballast water),**
  - **oil spills from pipelines bursts**
  - **oil spilt during the Nigeria civil war of 1968 - 1970, when many oil installations were either bombed or sabotaged**
  - **Etc**

A former chairman, Ibeno Clan Council of chiefs, late Chief E. W. Ndarake; the current Paramount ruler of Ibeno LGA, HRM (Dr) Effiong Bassey Archianga and a former LG Council Chairperson, Hon. Mrs. Regina Ekpe, said that every inch of Ibeno land and water can be described as an oil-polluted site and needs to be cleaned up







Approximately 2,000 crude oil polluted sites needing remediation in the Niger Delta region, Nigeria







INTRODUCTION Contd

- **Between 1997 and 2001, about 2,097 oil spill incidents were recorded in Nigeria.**
- **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 bbls 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.**

14 YEARS AFTER

- **Microbial diversity of the spill site is monitored using the culture-dependent methods (Udotong *et al*, 2011)**
- **The findings only revealed <1% of the microbial population. These results were unrealistic and therefore did 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.**


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A: Study site as at date of sampling; B, C & D: Oil sheen on water while sampling

RESEARCH OBJECTIVE

This study is aimed at identifying the microbial communities that inhabit the sediment of a lentic ecosystem polluted by aviation fuel about 14 years ago using:

- **the conventional culture-dependent methods, and**
- **the 16S metagenomic (culture-independent) approaches.**

- **This is the first time, to the best of our knowledge, that 16S metagenomic tools is used to determine the ecological integrity of any environmental media in the Niger Delta region, Nigeria.**

METHODOLOGY: STUDY AREA

- **Ibeno LGA is located in the coastal area of Akwa Ibom State, Nigeria.**
- **It lies between Latitude 4º 30'N and 4º 36'N and Longitude 7º 48'E and 8º 18'E**
- **It is one of the 31 LGAs that make up Akwa Ibom State, NIGERIA**
- **It hosts the operational base of MPNU, an IOC and a subsidiary of ExxonMobil, and**
- **Qua Iboe Terminal (QIT)**

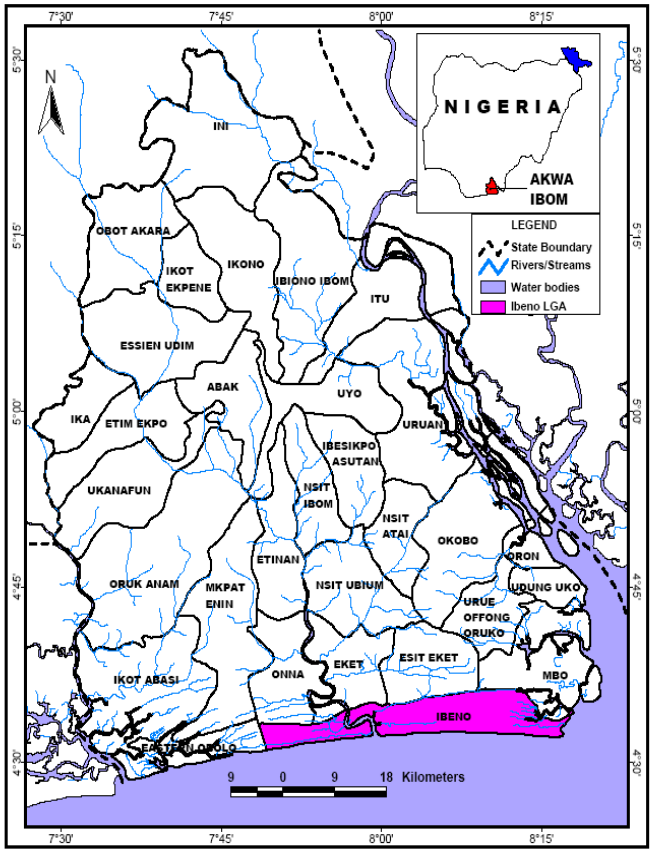
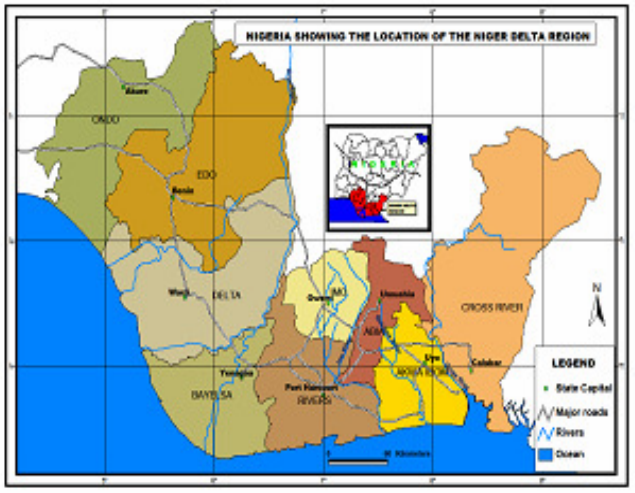
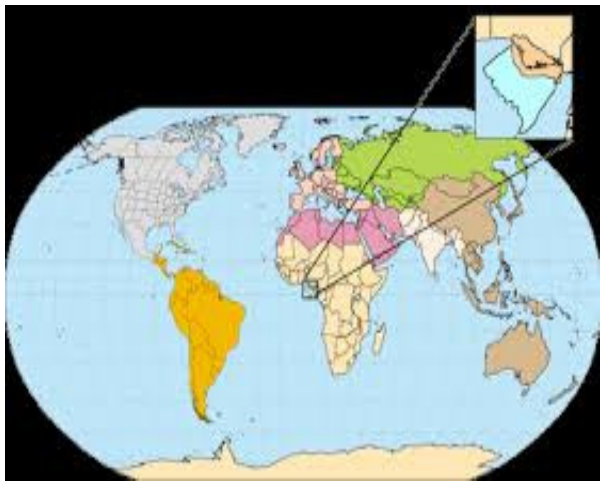


Fig.1.0 Akwa Ibom State: Location of Ibeno LGA

a.



b.



SEDIMENT SAMPLE COLLECTION







CULTURE-DEPENDENT METHODS

- **Serial Dilution**
- **Inoculation and Incubation**
- **Maintenance of Pure Culture**
- **Characterization and Identification of Microbial Isolates**

16S METAGENOMIC METHODS

DNA Extraction and Purification

- **Community / metagenomic DNA was extracted using the ZymoR soil DNA extraction Kit (Model D 6001, Zymo Research, USA) following the manufacturer's instructions.**
- **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 appropriate primers pairs.**
- **Successful amplification of DNA was verified by electrophoresis.**
- **The nucleotide sequence of the purified products was determined by 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.**

MICROBIAL GROWTH ON NUTRIENT MEDIA

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	-	-	-	-

MICROBIAL COUNTS

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

Culture medium	Morphology	Gram reaction	Cell type	Urease	Citrate	Methyl red	Voges Proskauer	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*

TAXONOMIC LEVELS

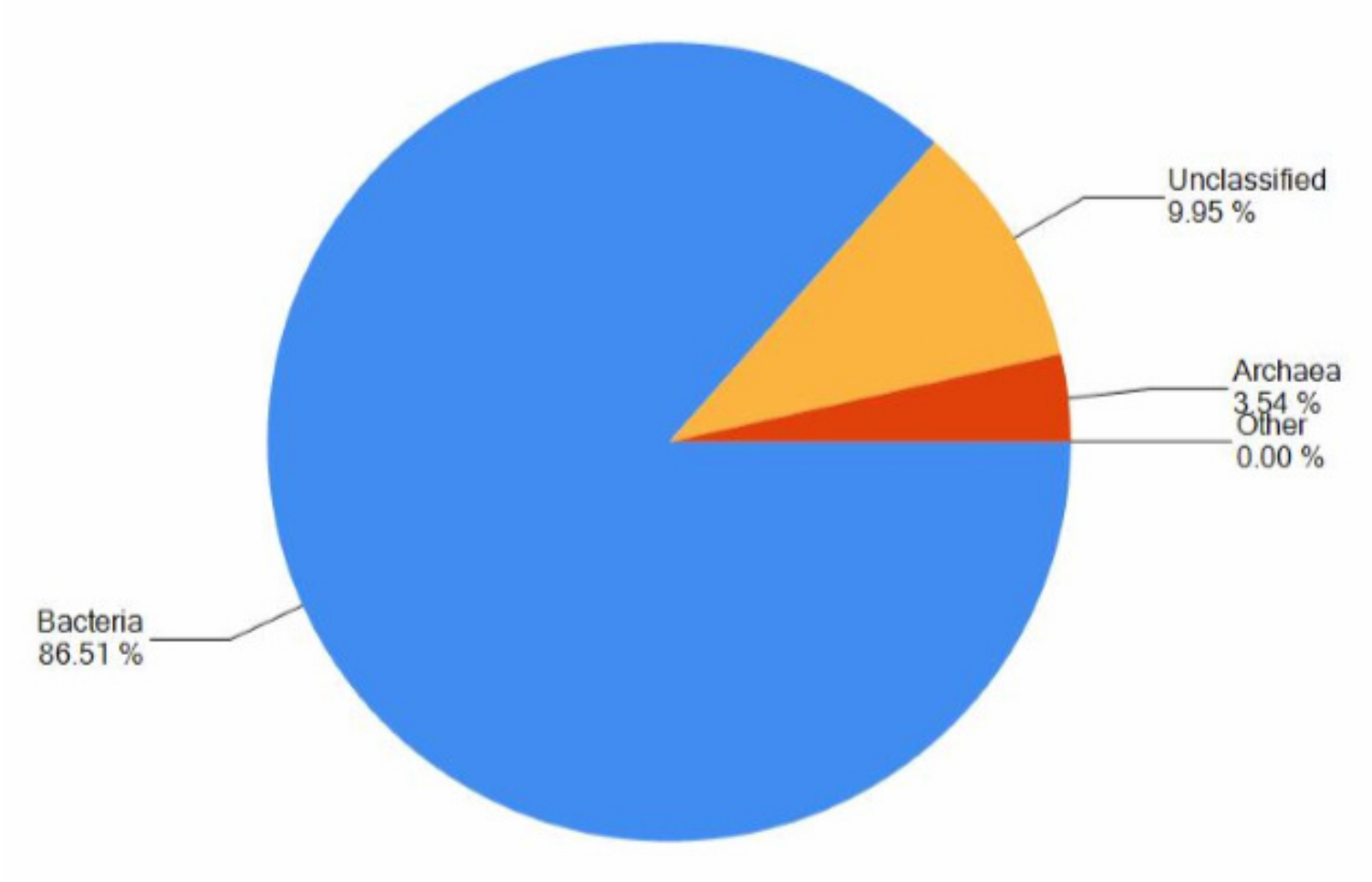
Taxonomic Level	Frequency
Phylum	1
Class	3
Order	4
Family	5
Genus	5
Species	6

16S METAGENOMICS STUDY

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

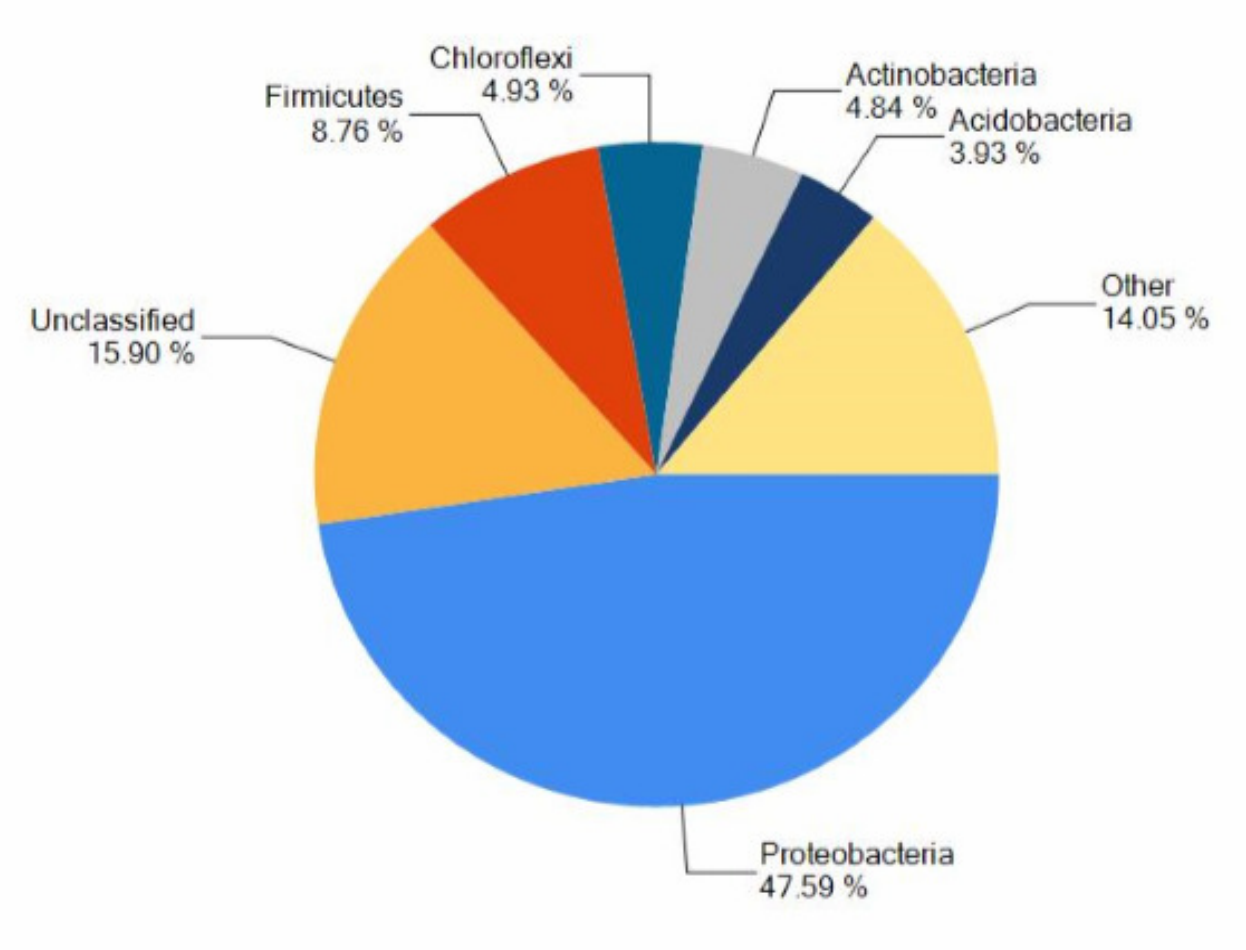
CLASSIFICATION RATE SUMMARY

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 %

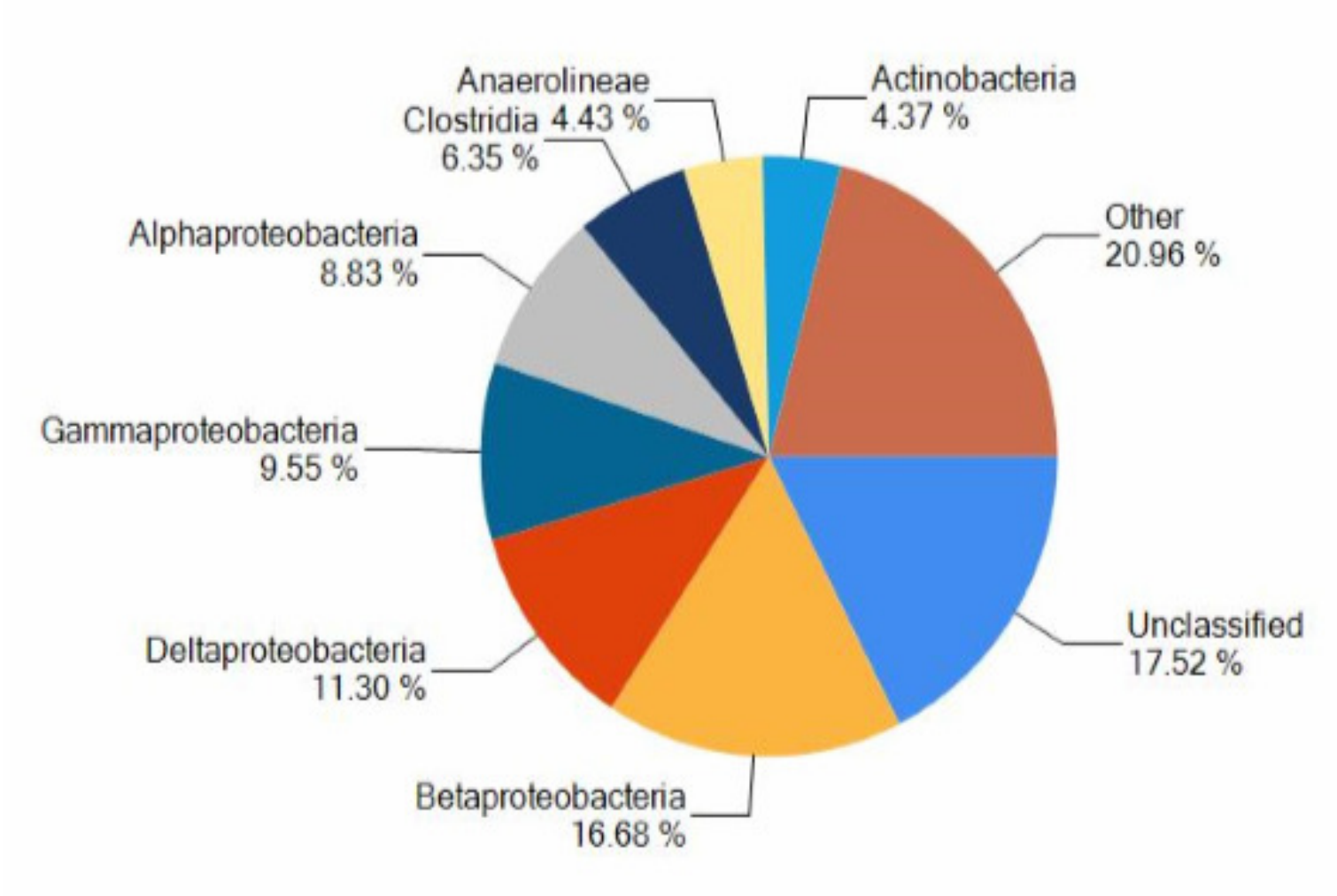


Kingdom (domain) classification of microorganisms in sediment from a remote aviation fuel-contaminated lentic ecosystem

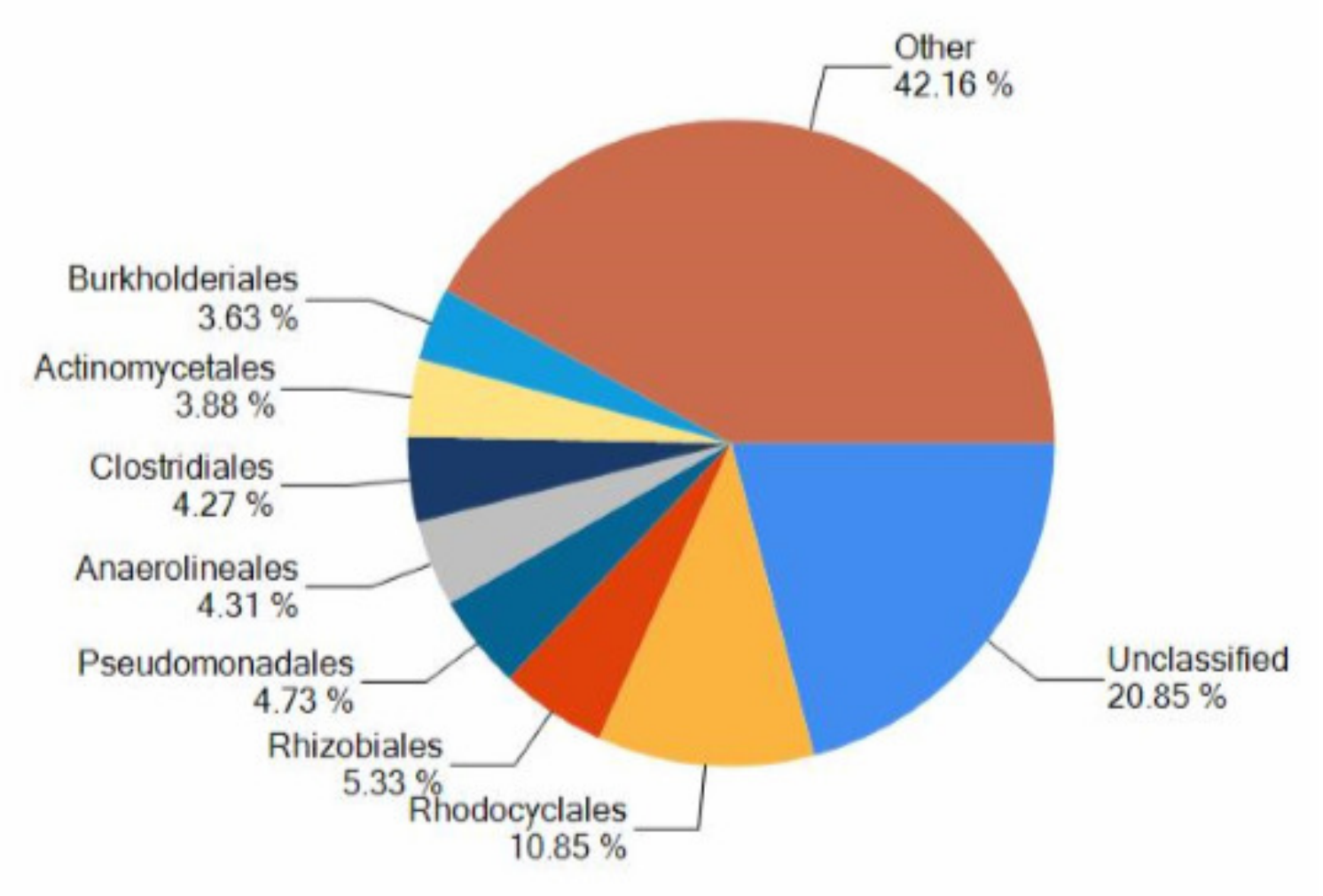
TOP 5 OF 27 PHYLA CLASSIFICATION



TOP 7 OF 57 CLASSES CLASSIFICATIONS



TOP 7 OF 109 ORDERS CLASSIFICATIONS



TOP 7 OF 224 FAMILIES CLASSIFICATION

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 %**

TOP 7 OF 498 GENERA 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 %**

TOP 7 OF 718 SPECIES 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 %**

ISOLATES FROM SAME SAMPLE

- Culture-dependent
  (The only isolates)
  - *Xanthomonas* sp
  - *Bacillus subtilis*
  - *Micrococcus* sp.
  - *Staphylococcus albus*
  - *Proteus* sp.
  - *Lactobacillus* sp
  - *B. cereus*
- 16S Metagenomics
  (Top 7 of 718 species)
  - *Zoogloea resiniphila*
  - *Longilinea arvoryzae*
  - *Edaphobacter modestus*
  - *Candidatus blochmannia rufipes*
  - *Peptoniphilus coxii*
  - *Uliginosibacterium gangwonense*
  - *Chondromyces pediculatus*

Comparison of Classification at Taxonomic level

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

CONCLUSION

- **Only six (6) species of bacteria, no archaeal representative were detected from the conventional culture-dependent techniques**
- **16S metagenomic analysis detected a total of 718 species comprising both bacterial and archaeal diversity.**
- **None of the 6 isolates were among the 7 most predominant of the 718 species community members of the aviation fuel-contaminated lentic sediment.**
- **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.**

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EBIPCO RESEARCH GROUP



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