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Comparative Evaluation of Microbial Diversity of Epipellic and Benthic Sediments using Cultural and Metagenomics Techniques

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Authors' contributions

This work was carried out in collaboration between all authors. All the authors designed the study and wrote the protocol. Author UOE performed the statistical analysis and wrote the first draft of the manuscript. All authors managed the analyses of the study. Author UOE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Studies have shown that molecular techniques are better in describing microbial diversity in various ecosystems than cultural techniques. The study was aimed at comparative evaluation of the microbial diversity of benthic and epipellic sediments using cultural and metagenomics techniques.
Methodology: Benthic and epipellic sediments were collected in triplicates from five locations from the Iko River estuary in Eastern Obolo. Total heterotrophic bacterial and fungal counts, and characterization of microbial isolates were done using the standard microbiological technique. Metagenomic DNA was extracted using ZYMO soil DNA extraction Kit (Model D601, Zymo Research, USA). Following extraction and amplification, the resulting DNA was sequenced using next-generation sequence on Miseq Illumina platform. Data from cultured based techniques were

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analysed using analysis of variance (ANOVA) and student t-test while resulting metagenomic data were analyzed using web-based bioinformatics tools.
Results: Bacteria and fungi counts ranged from 1.08 to 1.60 (x 10^6 CFU/g) and 0.10 to 2.2 (x 10^3 CFU/g) with benthic sediments having the highest abundance in both cases. Compared to cultural techniques which captured only bacterial and fungal kingdoms, metagenomics captured archaea, protozoa, viruses, plantae, and unknown kingdoms. Furthermore, 17 phyla were obtained using metagenomics compared to 3 phyla captured by cultural techniques. A total of 61 isolates were recovered spread across various genera (10 from benthic and 11 from epipellic). Most common isolates in both samples were *Bacillus, Micrococcus* and *Pseudomonas*. Although a total of 300 species were identified using metagenomics, about 78.92% and 71.19% of the species were uncultured bacterium for benthic and epipellic sediments, respectively. Furthermore, the species were dominated with species involved in nutrient recycling such as *Thiobacillus prosperus, Sulfurimonas* species and *Marinobacterium nitratireducens.* Surprisingly, 15(0.15%) of the reads showed sequence similar to Influenza A virus (H3N6) viral cRNA with accession number LC053487.1.
Conclusion: The results show that metagenomic assessment is better in capturing the bacterial diversity of sediment than cultural methods.

Keywords: Sediment; metagenomics; microbial diversity; Niger Delta region of Nigeria.

1. INTRODUCTION

Molecular based techniques have been shown by a number of studies to be able to capture the unculturable majority in samples obtained from extreme and non-extreme environments much better than culture based techniques [1-3]. Amongst these techniques, metagenomic based techniques remain the gold standard and is increasingly becoming popular around the world in the description of structural and functional composition or diversity of ecosystems [1-4]. The Niger Delta region of Nigeria is the biggest delta in Africa and third largest in the world [5]. The region boasts one of the richest crude oil deposits in Africa and its export remains the main stay of the Nigerian's economy [5-6]. Over six decades of oil exploration activities have resulted in incessant crude oil spillages in the region [5-6]. The impact of crude oil and its products on the various ecosystems including sediment is well documented in the region [7-12] and beyond [13]. Its ecological diversity is now threatened at an alarming pace from crude oil and associated pollutants.

Sediment is a particulate matter that can be transported by physical processes and eventually deposited. Sediment health is important for a number of reasons. First, sediments act as the most important reservoir for metals and other pollutants in the aquatic environment. Second, it helps explain the marine ecosystems and history of the ocean [8]. Sediments can be classified into a number of groups depending on a number of factors such as grain size, and origin of formation [14]. The benthic zone is the ecological zone that is at the lowest level of a body of water while the epipellic sediment is the part that is visible mostly at low tide. The Benthic zone starts at the shoreline and continues down until it reaches the floor, encompassing the sediment surface and subsurface layers. Although this zone may appear barren, it plays a vital role in the health of aquatic ecosystems. Tiny, microscopic benthic organisms live in this zone and act as a source of food for bottom feeding animals. Benthic organisms are very important as they are good indicators of water quality [8,14].

It has been estimated that the number of prokaryotic cells in the largely unexplored sediments is about 8–35 x 10^{29}, which represents 10–30 % of the total biomass on earth [15]. Such huge diversity cannot be accessed by the routinely used cultural methods such as plate counts and earlier molecular methods such as fatty acid analysis [1]. Metagenomics, although still in its infancy have been used in a number of studies to capture such huge diversities in various ecosystems much better than with culture-based techniques [1,9,16]. The aim of this study was therefore to compare the microbial diversity of benthic and epipellic sediments using cultural and metagenomics techniques.

2. SAMPLING LOCATION

Sampling location for this study was Emereoke II (Ward 5) community of Eastern Obolo Local Government Area of Akwa Ibom State, Niger Delta, Nigeria. Sediment sampling was done at coordinates 4º32′0″N & 7º42′0″E along the Okoro

River estuary. The sampling location is host to several multinational oil companies notably Shell Petroleum Development Company (SPDC). However, the majority of the inhabitants are occupied by peasant and subsistent farming activities.

2.1 Sediment Sample Collection

Benthic and epipellic sediment samples were collected in triplicates from five different locations for each sediment type. Briefly, a 22cm hand-held Dutch auger was used to aseptically collect the various epipellic sediment samples. For collection of the benthic sediment, a Shepek (Wiidco) mud grab was used to aseptically collect the samples at depths of 5-10m below sea level. All samples were collected at low tide and stored in amber coloured bottles and placed immediately in ice packs. The samples were then transported immediately to the laboratory for further analysis [4,9].

2.2 Enumeration of Total Aerobic Heterotrophic Bacteria and Fungi Counts (THBC and THFC)

Triplicate samples from each of the location were made into composite samples for a particular location and then used for THBC and THFC. This was carried out as previously described [17-19]. Briefly, from each of the composite samples, a ten-fold serial dilution was carried out (10^{-1} to 10^{-10}) using one gram of the benthic and epipellic sediments. Exactly one ml from the 10^{-5} and 10^{-3} dilutions were plated in duplicates onto freshly prepared nutrient agar and Sabouraud dextrose agar (SDA), respectively for the enumeration of total aerobic bacteria and fungi, respectively. The plates were incubated for 24 and 48 hours, respectively. Furthermore, the serial dilutions were also plated out on MacConkey agar and Salmonella Shigella agar. After incubation, the plates were then observed for growth and the colonies counted and recorded. Distinct colonies of bacteria and fungi were picked for further microbiological analysis.

2.3 Characterization and Identification of Microbial Isolates

Distinct colonies were maintained and purified as previously described [9]. Resulting pure bacteria isolates were characterized using gram staining, microscopy and biochemical tests. The biochemical tests included citrate, motility, indole, Voges-proskauer, methyl red, catalase test, triple sugar fermentation, iron sulphide, gas production and acid. These were carried out as previously described [20-21]. The fungal isolates were identified as previously described by Domsch et al. [22].

2.4 Genomics DNA Extraction and PCR Amplification

Exactly 0.25 grams of each sediment samples were weighed out and used for genomic DNA extraction. Extraction from sediment samples was performed using ZYMO soil DNA extraction Kit (Model D 6001, Zymo Research, USA) following the manufacturer's instructions. Following DNA extraction from the samples, the genomic DNA extracts were subjected to PCR amplification. The PCR was set up for 30 cycles for 2 hours at 96, 72 and 65^{o}C for denaturation, annealing and extension. The amplified genomic DNA (15 µl) were then subjected to 1.5% gel electrophoresis by mixing with 2 µl of loading dye. These were done using as previously described [1,23].

2.5 Next-generation DNA Sequencing and Analysis of Reads

DNA sequencing was performed at Inqaba Biotechnology Company in South Africa. This was done using Next Generation Sequencing (NGS) technology using sequencing primer -16S: 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'- ATTACCGCGGCTGCTGG-3'. The sequencing was performed using automated PCR cycle- Genome Sequencer™ MiSeq (Illumina). Analysis and alignment was performed using Vecton NTI suite 9 (InforMax, Inc.). Overall bioinformatics analysis was done using NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1. For every sample set, every read was BLASTED and the result file saved. Only reads of sufficient Q scores (>q20) and lengths were used in the analysis.

2.6 Statistical Analysis

All statistical analyses were done using Graphpad Prism 5.0 and Microsoft Excel 2010. Replicate counts of total heterotrophic bacteria and fungi are presented in bar charts. Mean counts were also analyzed further using student t-test and analysis of variance at 95% level of significance. Relative abundance plot was done on the resulting reads from the various phyla taxa represented as 100% stacked bar.

3. RESULTS

3.1 Microbiological Analysis

The results of the microbiological analysis are presented in Tables 1, 2 and figure 1. Total heterotrophic counts of bacteria in epipellic and benthic sediment are presented in Figure1. Tables 1 and 2 shows the bacterial and fungal isolates obtained in this study. The highest total heterotrophic bacterial count of 160 (x 10^5 CFU/g) was obtained from benthic sediment from locations BS5 and BS6. While the lowest count of 108 and 107 (x 10^5 CFU/g) were gotten from ES2 and BS2. Interestingly, epipellic sediment locations (2, 5 and 6) with high counts also had high count in their corresponding benthic locations. Total heterotrophic fungal counts were less than those of bacteria for both locations. The highest fungal count obtained from both sediments were 16 and 22 (x 10^3 CFU/g), respectively for ES2 and BS5. After 48 hours no growth was observed on locations 2 and 4 for epipellic and benthic sediment, respectively. Comparism with student t-test did not show any significant difference between the counts for both benthic and epipellic locations for bacteria but was for fungi.

A total of 61 isolates were obtained from both sediments and these were spread across 11 and 10 genera for epipellic and benthic sediments, respectively. Epipellic sediment isolates were *Bacillus cereus, Bacillus* species*, Micrococcus* species*, Proteus* species*, Pseudomonas* species*, Enterococcus* species*, Staphylococcus* species*, Aeromonas* species*, Citrobacter* species and *Corynebacterium sp.* Isolates from benthic sediment were *Enterobacter* species*, Citrobacter sp, Escherichia coli, Shigella* species*, Bacillus* species*, Micrococcus* species*, Serratia* species *and Pseudomonas* species*. Pseudomonas and Enterobacter* were species that were found in both types of sediments and most abundant as well. The isolates were distributed amongst three bacteria phyla namely firmicutes, actinobacteria and proteobacteria. The most abundant phyla were the firmicutes and proteobacteria in both samples. Fungi isolates from both sediments were more consistent and less diverse than bacterial isolates. They included *Penicillium* species*, Aspergillus niger, A. candidus, A. versicolour, P. expansum, Fusarium* species*, Aspergillus* species*, Alternaria* species*, Rhizopus* species*, A. oryzae, Mucor* species and *Saccharomyces cerevisiae*. Aspergillus isolates were the most frequent fungal isolates followed by the *Penicillium* species.

3.2 Metagenomic Analysis

Following whole community genome NGS, the sequences were sorted into kingdoms, phyla, classes, orders, families, orders, genera and species, and are presented in Tables 3 to 6. Table 3 shows the kingdom classification for both sediments and from the table it can be seen that bacteria was the most abundant with reads of 12,485 (98.52%) and 9,321(91.55%), respectively for epipellic and benthic sediments. In epipellic sediment, the top five kingdoms were bacteria, archaea, fungi, virus and unknown. However, in the benthic sediment, the top five kingdoms were bacteria, fungi, archaea, unknown and plantae. Compared to cultural techniques which captured only bacterial and fungal kingdoms, metagenomics captured 7 kingdoms.

Table 1. Probable isolates from the epipellic sediment and their corresponding phylum

Epipellic sediment	Phylum	Benthic sediment	Phylum
Bacillus cereus	Firmicutes	*Enterobacter* species	Firmicutes
Bacillus species	Firmicutes	*Shigella* species	Proteobacteria
Micrococcus species	Actinobacteria	*Citrobacter* species	Proteobacteria
Proteus species	Proteobacteria	*Bacillus* species	Firmicutes
Pseudomonas species	Firmicutes	*Micrococcus* species	Actinobacteria
Enterococcus species	Firmicutes	*Esherichia coli*	Proteobacteria
Pseudomonas species	Firmicutes	*Serratia* species	Proteobacteria
Staphylococcus species	Firmicutes	*Pseudomonas* species	Proteobacteria
Aeromonas species	Proteobacteria	*Citrobacter* species	Proteobacteria
Corynebacterium species	Proteobacteria		
Citrobacter species	Actinobacteria		

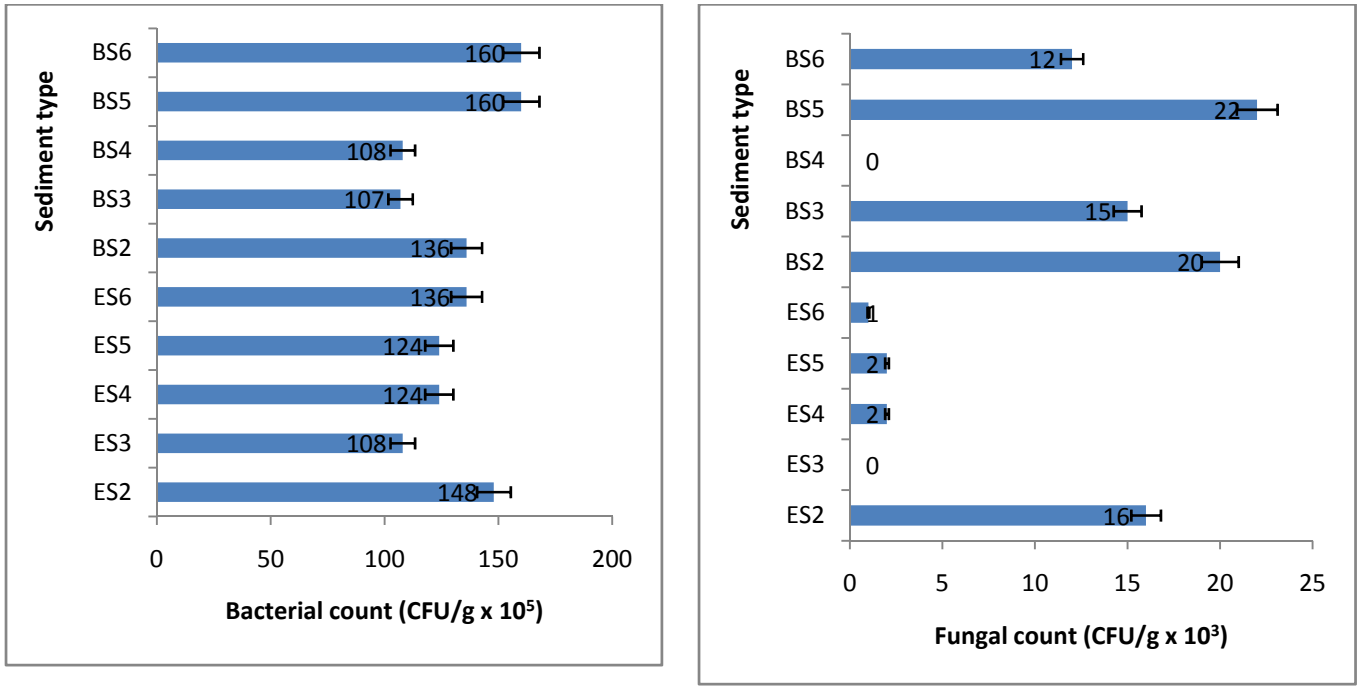

Fig. 1. Bar chart showing the total bacterial and fungal counts from the various samples. Analysis of variance of bacterial and fungal counts showed significance ($p < 0.05$). Student t-test showed no significance ($p > 0.05$) between the Benthic and Epipellic bacterial counts while that of fungal counts showed significant difference ($p < 0.05$)

Keys: ES and BS represents Epipellic and Benthic Sediments while 2,3,4,5 and 6 = various locations

Table 4 and Fig. 2 show the phyla classification of the sediment samples. A total of 17 phyla were obtained for both samples compared to just 3 obtained from cultural techniques. However, epipellic sample had more unknown phyla than the benthic sediment. Furthermore, the top eight (8) phyla were almost similar to each other in different orders of abundance of their reads. These top nine phyla were Unknown, Proteobacteria, Actinobacteria, Ascomycota, Firmicutes, Chloroflexi, Bacteroidetes and Planctomycetes. From the relative abundance bar chart plot, it can be seen that Ciliophora, Nitrospira and Fusobacteria were not detected in the epipellic sediment but only in the benthic sediment. However, Chlamydiae and Thermomicrobia were unique to the epipellic sediment.

Table 5 shows the various classes obtained from both sediments. Unlike the phyla classification, the top 10 classes were fairly similar in both samples. In all, a total of 18 out of 23 were similar in both samples. Halobacteria, Acidobacteria, Chlamydiae, Caldilineae and Thermomicrobia were in the epipellic but not in the benthic sediment. On the other hand, Thermoprotei, Polypodiopsida, Nitrospira, Epsiloproteobacteria and Fusobacteria were present in the benthic and not in the epipellic sediment sample.

Table 2. Fungal species isolated from both epipellic and benthic sediments

Fungi species	Epipellic sediment	Benthic sediment
Penicillium spp	Y	Y
Aspergillus niger	Y	Y
Aspergillus candidus	Y	Y
A. Versicolour	Y	N
Penicillum expansum	N	Y
Fusarium species	Y	N
Aspergillus spp	Y	Y
Rhizopus spp	Y	Y
Aspergillus oryzae	Y	N
Mucor species	Y	Y
Saccharomyces cerevisiae	Y	Y

Keys: Y= Isolated and N= Not isolated

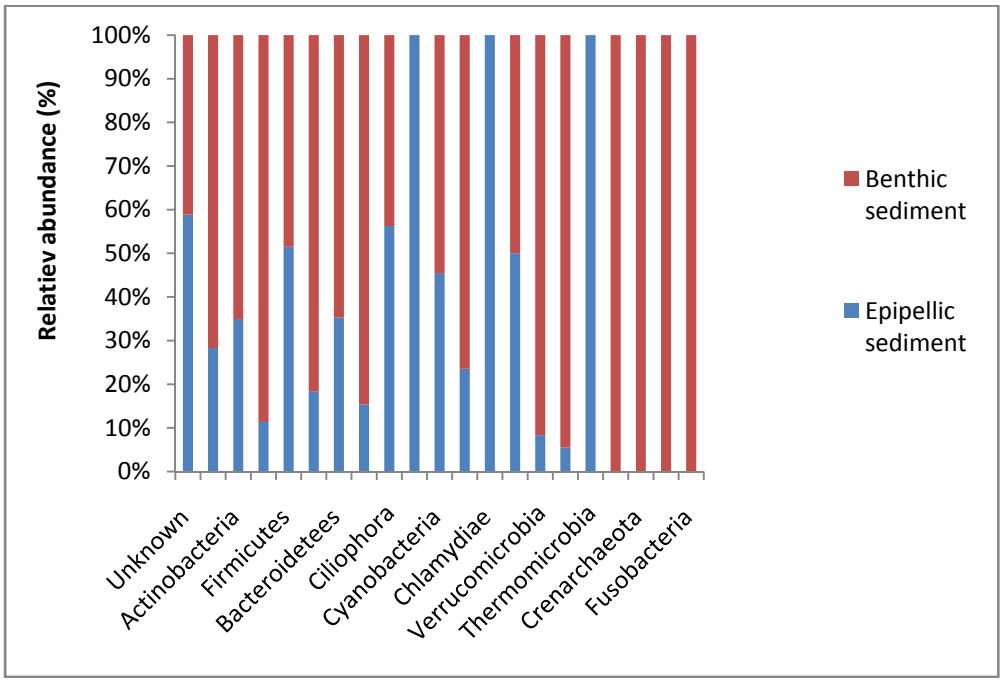

Fig. 2. Relative abundance plot of the microbial phyla taxa represented as 100% stacked bar

Table 3. Kingdom classification of sampled sediment samples

Kingdoms	Epipellic		Kingdoms	Benthic	
	Read counts	Percentage (%)		Reads counts	Percentage (%)
Bacteria	12485	98.52	Bacteria	9321	91.55
Archaea	63	0,50	Fungi	413	4.06
Fungi	53	0.42	Archaea	396	3.89
Virus	49	0.39	Unknown	23	0.23
Unknown	13	0.10	Plantae	17	0.17
Protozoa	9	0.07	Protozoa	7	0.07
Plantae	1	0.01	Virus	2	0.02

Table 4. Phyla classification of epipellic and benthic sediments

Phyla classification	Epipellic		Phyla classification	Benthic	
	Read counts	Percentage (%)		Read counts	Percentage (%)
Unknown	12025	94.89	Unknown	8403	82.54
Proteobacteria	275	2.17	*Proteobacteria*	697	6.85
Actinobacteria	233	1.84	*Actinobacteria*	434	4.26
Ascomycota	53	0.42	*Ascomycota*	413	4.06
Firmicutes	17	0.13	*Chloroflexi*	71	0.70
Chloroflexi	16	0.13	*Planctomycetes*	66	0.65
Bacteroidetes	12	0.09	*Bacteroidetes*	22	0.22
Planctomycetes	12	0.09	*Tracheophyta*	17	0.17
Ciliophora	9	0.07	*Firmicutes*	16	0.16
Euyarchaeota	6	0.05	*Acidobacteria*	13	0.13
Cyanobacteria	5	0.04	*Verrucomicrobia*	11	0.11
Acidobacteria	4	0.03	*Ciliophora*	7	0.07
Chlamydiae	2	0.02	*Cyanobacteria*	6	0.06
Gemmatimonadetes	1	0.01	*Crenarchaeota*	2	0.02
Veruucomicrobia	1	0.01	*Gemmatimonadetes*	1	0.01
Tracheophyta	1	0.01	*Nitrospira*	1	0.01
Thermomicrobia	1	0.01	*Fusobacteria*	1	0.01

Table 6 shows the various orders obtained with their reads. In addition to unknown and not assigned reads, benthic sediment had 36 orders compared to 35 orders obtained from the benthic sediment. The orders unique to epipellic sediment were *Thiotrichales*, *Halobacteriales*, *Caudovirales*, *Rhodobacterales, Pleurostomatida, Bifidobacteriales, Oceanospirillales, Flavobacteriales, Chlamydiales, Syntrophobacterales, Caldilineales, Myxococcales* and *Thermomicrobiales*. Those of benthic sediment were *Alteromonadales, Vibrionales, Desulfuromonadales, Kordiimonadales, Caudovirales, Pseudomonadales, Halanaerobiales, Bdellovibrionales, Nitrospirales, Desulfobacterales ,Fusobacteriales, Polypodiales* and *Enterobacteriales*.

Table 7 shows the top 25 BLAST and their read counts. Common hits corresponded to Uncultured bacterium, Uncultured candidates, uncultured gamma, Uncultured zeta, Uncultured archeaon, Uncultured actinobacterium and Uncultured delta. The most common genus in both samples was Corynebacterium. Surprisingly 15 (0.15%) of the reads showed sequence similar to Influenza A virus viral cRNA with accession number LC053487.1 (Influenza A virus: A/duck/Vietnam/LBM798/2014 (H3N6)).

4. DISCUSSION

Bacteria, fungi, algae, viruses and protozoa are the main kingdoms studied in microbiology. However, the first two have received much more attention than the rest because of the relative ease with which they can be cultured in the laboratory. The results of the cultural techniques showed the presence of bacteria and fungi with the latter being the most dominant group in this study. However, metagenomics analysis was

able to capture bacteria, fungi, algae, virus, protozoa and plantae in addition to unknown kingdoms. From the results of the study, the top three (3) dominant kingdoms in both studied location were bacteria, fungi and archaea in both ecosystems. More fungi and archaea were seen in the benthic than in the epipellic sediment with reads counts of 413 and 396, respectively. In a similar study by Udotong et al. [9], metagenomic assessment of lentic sediment ecosystem contaminated with aviation fuel revealed much more than cultural methods. Their cultural methods could not capture the important kingdom archaea but the 16s rRNA technique captured them. This was also similar to our findings in our study which showed that the archaea were more abundant than other kingdoms except for bacteria in epipellic sediment and the third most abundant kingdom in the benthic ecosystem. The archaea were not captured by cultural techniques were employed in this study.

Bacterial counts in our study were higher in our findings than those of an earlier report by Udotong et al. [9]. They reported counts of bacteria and fungi that ranged from 1.1 to 5.1 x 10^7 and 1.0 to 2.7x10^6 cfu/g, respectively for the benthic, epipellic and mangrove roots ecosystems sampled in Iko river estuary located in Iko town in Eastern Obolo community of Akwa Ibom state which were within range of our total heterotrophic bacteria counts that ranged from 1.08 to 1.60 (x 10^7 cfu/g). However, our fungal counts that ranged from 0.2 to 2.2 (x 10^4 cfu/g/) were much lower.

The epipellic sediment bacterial isolates were *Bacillus cereus, Bacillus* species*, Micrococcus* species*, Proteus* species*, Pseudomonas* species*, Enterobacter* species*, Staphylococcus* species*, Aeromonas* species*, Corynebacterium* species *and Citrobacter* species. Isolates from benthic sediment were similar and include *Enterobacter* species*, Shigella* species*, Citrobacter* species*, Bacillus* species*, Micrococcus* species*, Escherichia coli, Enterobacter* species*, Serratia* species *and Pseudomonas* species. *Pseudomonas, Enterobacter* and *Bacillus* species were species that were found in both types of sediments and most abundant as well. Similar isolates from microbiological analysis of Iko river sediments and mangrove roots ecosystems were *S. aureus,*

Table 5. Class classification of epipellic and of benthic sediment

Class	Read counts	Percentage (%)	Class	Read counts	Percentage (%)
Unknown	12035	94.97	Unknown	8423	82.73
Actinobacteria	229	1.81	*Actinobacteria*	433	4.25
Gammaproteobacteria	101	0.80	*Dothideomycetes*	413	4.06
Epsiloproteobacteria	78	0.62	*Gammaproteobacteria*	352	3.46
Betaproteobacteria	55	0.43	*Betaproteobacteria*	238	2.34
Dothideomycetes	53	0.42	*Chloroflexi*	71	0.70
Deltaproteobacteria	22	0.17	*Planctomycetacia*	66	0.65
Chloroflexi	15	0.12	*Deltaproteobacteria*	50	0.49
Alphaproteobacteria	14	0.11	*Alphaproteobacteria*	42	0.41
Planctomycetacia	12	0.09	*Bacteroidetes*	19	0.19
Clostridia	11	0.09	*Liliopsida*	16	0.16
Gymnostomatea	9	0.07	*Bacilli*	14	0.14
Bacteroidetes	7	0.06	*Acidobacteria*	13	0.13
Halobacteria	6	0.05	*Verrucomicrobiae*	11	0.11
Bacilli	6	0.05	*Gymnostomatea*	7	0.07
Acidobacteria	4	0.03	*Sphingobacteria*	2	0.002
Not assigned	4	0.03	*Thermoprotei*	2	0.002
Flavobacteria	3	0.02	*Clostridia*	2	0.002
Chlamydiae	2	0.02	*Flavobacteria*	1	0.01
Sphingobacteria	2	0.02	*Gemmatimonadetes*	1	0.01
Caldilineae	1	0.01	*Polypodiopsida*	1	0.01
Liliopsida	1	0.01	*Nitrospira*	1	0.01
Gemmatimonadetes	1	0.01	*Epsilonproteobacteria*	1	0.01
Verrucomicrobiae	1	0.01	*Fusobacteria*	1	0.01
Thermomicrobia	1	0.01	Not assigned	1	0.01

Bacillus cereus, Serratia marcescens, Enterobacter species, *Micrococcus and Escherichia coli* [9].

Jiang et al. [24] in an earlier study reported microbial abundance in the sediments that ranged from 10^8 cells/g at the water-sediment interface to 10^7 cells/g at a sediment depth of 42 cm. The abundance of more bacteria in both sediments can be seen in the abundance of more bacteria reads than other kingdoms and this was further confirmed by the more heterotrophic bacterial counts in both epipellic and benthic sediments. In line with our cultural results, more bacteria reads were obtained in the epipellic sediment than in the benthic sediment using metagenomics.

Table 6. Order classification of epipellic and benthic sediment

Order	Epipellic		Order	Benthic	
	Read counts	Percentage (%)		Read counts	Percentage (%)
Unknown	12186	96.16	Unknown	8966	88.07
Actinomycetales	185	1.46	Not assigned	413	4.06
Campylobacterlaes	78	0.62	*Hydrogenophilales*	233	2.29
Not assigned	53	0.42	*Alteromonadales*	135	1.33
Thioctrihales	32	0.25	*Actinomycetales*	114	1.12
Hydrogenophilales	19	0.12	*Chloroflexales*	71	0.70
Chloroflexales	15	0.09	*Chromatiales*	67	0.66
Planctomycetales	12	0.09	*Planctomycetales*	66	0.65
Clostridales	11	0.06	*Bacteroidales*	19	0.19
Bacteroidales	7	0.05	*Asparagales*	16	0.16
Spathidiida	6	0.05	*Acidobacteriales*	13	0.13
Halobacteriales	6	0.04	*Verrucomicrobiales*	11	0.11
Caudovirales	5	0.03	*Bacillales*	10	0.10
Rhodobacterales	5	0.03	*Spathidiida*	7	0.07
Chromatiales	4	0.03	*Lactobacillales*	4	0.04
Lactobacillales	4	0.03	*Vibrionales*	4	0.04
Bukholderiales	4	0.03	*Desulfuromonadales*	3	0.03
Acidomicrobiales	4	0.03	*Kordiimonadales*	2	0.02
Acidobacteriales	4	0.03	*Caudovirales*	2	0.02
Pleurostomatida	3	0.02	*Sphingobacteriales*	2	0.02
Bifidobacteriales	3	0.02	*Burkholderiales*	2	0.02
Oceanospirillales	3	0.02	*Rhodospirillales*	2	0.02
Rhodospirillales	3	0.02	*Rhizobiales*	2	0.02
Alteromonadales	3	0.02	*Xanthomonadales*	2	0.02
Flavobacteriales	3	0.02	*Pseudomonadales*	2	0.02
Sphingobacteriales	2	0.02	*Halanaerobiales*	1	0.01
Chlamydiales	2	0.02	*Syntrophobacterales*	1	0.01
Bacillales	2	0.02	*Gemmatimonadales*	1	0.01
Verrucomicrobiales	1	0.01	*Bdellovibrionales*	1	0.01
Syntrophobacterales	1	0.01	*Clostridiales*	1	0.01
Caldilineales	1	0.01	*Nitrospirales*	1	0.01
Xanthomonadales	1	0.01	*Desulfobacterales*	1	0.01
Myxococcales	1	0.01	*Fusobacteriales*	1	0.01
Asparagales	1	0.01	*Acidomicrobiales*	1	0.01
Rhizobiales	1	0.01	*Polypodiales*	1	0.01
Gemmatimonadales	1	0.01	*Flavobacteriales*	1	0.01
Thermomicrobiales	1	0.01	*Campylobacterales*	1	0.01
-			*Enterobacteriales*	1	0.01

Table 7. Top 25 BLAST and their read counts

Epipellic	Read counts	%	Benthic	Read counts	%
Uncultured bacterium	10013	78.92	Uncultured bacterium	7260	71.19
Uncultured candidate	1428	11.25	Uncultured zeta	413	4.05
Sulfurimonas species	*73*	*0.58*	Uncultured archaeon	391	3.83
Corynebacterium deserti	*59*	*0.47*	Uncultured actinobacterium	313	3.07
Uncultured gamma	58	0.46	*Thiobacillus prosperus*	233	2.82
Uncultured zeta	53	0.42	Uncultured gamma	141	1.38
Uncultured archeaon	45	0.35	Marinobacterium nitratireducens	126	1.24
Uncultured actinobacterium	41	0.32	*Uncultured thioprofundum*	119	1.71
Uncultured beta	32	0.25	Uncultured chloroflexi	71	0.70
Thiomicrospira sp	*31*	*0.24*	Halothiobacillus kellyi	*60*	*0.59*
Pseudoclavibacter sp	*25*	*0.20*	*Uncultured delta*	*42*	*0.41*
Uncultured delta	20	0.16	Uncultured alpha	34	0.33
Thiobacillus prosperus	16	0.13	*Uncultured Proteobacterium*	14	0.14
Rhodococcus ruber	16	0.13	*Uncultured verrumicrobia*	10	0.10
Uncultured episilon	15	0.12	*Alicycbacillus ferroxydans*	7	0.07
Actinomycetes sp	14	0.11	*Uncultured aciditerrimonas*	7	0.07
Sanguibacter antarcticus	7	0.06	*Uncultured cyanobacterium*	6	0.06
Uncultured ilumatobacter	7	0.06	Corynebacterium pseudotuberculosis	5	0.05
Clostridium sp	7	0.06	*Thioalkalispira microaerophila*	3	0.03
Corynbacterium glutamicum	7	0.06	*Phycicoccus sp*	3	0.03
Uncultured euryarchaeote	12	0.09	*Bacterium mebic09124*	32	0.31
Uncultured planctomycete	10	0.08	*Uncultured dehalococcoides*	22	0.22
Uncultured firmicutes	10	0.08	*Uncultured bacteroidetes*	19	0.19
Uncultured chloroflexaceae	8	0.06	*Uncultured candidate*	19	0.19
Uncultured nocardioidaceae	8	0.06	*Influenza A virus*	15	0.15

Elsewhere, Fernandes et al. [25] carried out a study in order to appreciate differences in benthic bacterial community composition at the relatively pristine Tuvem and the anthropogenic influenced Divar mangrove ecosystems in Goa, India. They employed parallel tag sequencing of the V6 region (a highly conserved region) of 16S rDNA and their results revealed that the phylum Proteobacteria was dominant at both locations comprising 43-46% of total tags. The Tuvem ecosystem was characterized by an abundance of members belonging to the class Deltaproteobacteria (21%), ~ 2100 phylotypes and 1561 operational taxonomic units (OTUs) that shared > 97% similarity. At Divar, the Gammaproteobacteria were ~ 2x higher (17%) than at Tuvem. These findings agreed completely with our findings as proteobacteria were the second most abundant phyla after the unknown phyla. In our findings, proteobacteria were the most abundant phyla in both sediment types. Although deltaproteobacteria were not the most abundant class, they were, however, amongst the top 8 classes classified in our sediment samples.

In a recent study by Bucci et al. [26] to examine seasonal changes in microbial community structure in freshwater stream sediment in a North Carolina River Basin using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) and molecular fingerprint analysis of 16S rRNA genes. They found out that gamma, alpha and beta proteobacteria were prevalent species of microbial taxa represented among all sites and this agrees completely with our findings even though we sampled only during dry season alone (March). Furthermore, they found out that actinobacteria were the next most prevalent species observed, with greater occurrence in dry compared to the wet season. This finding was in

line with our findings as actinobacteria were also the most abundant class of bacteria with more prevalence in the benthic environment than in the epipellic sediments.

Korlevic et al. [27] while examining the bacterial diversity of polluted surface sediments in the northern Adriatic Sea using 16S rRNA, they reported ranging from low (200 detected genera) to high (1000+ genera) biodiversity (using just 1 g of the sample), with lowest biodiversity observed in polluted samples. This indicated that there was considerable biodiversity in all sediment samples but it was severely restricted after exposure to crude oil selection pressure. In our study, 0.25 g of sediment from each of the sediment sample was used for assessment of the whole community in our study. This revealed a combined total of 300 genera of the bacterium with over 70% of unknown species. This was still higher than the number of genera obtained using cultural methods (a total of 21 genera) for both sediments. This implies that over 93% of the diversity could not be captured by the cultural methods. The overall low number of genera captured in this study by both techniques could be explained by the selection pressure put on the ecosystems from the oil spillages that is frequent in the study location. Furthermore, *Thiobacillus prosperus, Sulfurimonas* species and *Marinobacterium nitratireducens* were dominant with species involved in nutrient recycling. Similar findings were reported by Mason et al. [28] who annotated several genes involved in nitrogen cycle to bacterial phylotypes in the Mina Stream sediment following functional gene analysis.

5. CONCLUSION

Sediments have outstanding ability to accumulate anthropogenic sources of pollutants which can alter its microbial diversity. Based on the results in this study, it can be concluded that the metagenomics assessment of both types of sediments was better than culture-based technique in capturing microbial diversity. The finding of a similar sequence to Influenza a virus H3N6 obtained from a duck in Vietnam poses a potential health risk. This calls for routine surveillance of sediment microbial community.

ETHICAL APPROVAL

During sampling, permission was sought and obtained from the inhabitants even though the location is not privately owned or protected in any way. They were assured that the sampling would not in the way involve endangered or protected species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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