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Microbiological Examination and Physicochemical Analysis of Estuary Water Used as a Point of Source Drinking Water

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Authors' contributions

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

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ABSTRACT

Introduction: Access to potable water is a fundamental human right. However, this is hardly the case in the rural areas of oil producing communities of the Niger Delta.
Aim of Study: The primary aim of this study was therefore to evaluate the physicochemical and microbiological qualities of estuary water used as potable water.
Method: Estuary water samples were collected from Eastern Obolo community in Akwa Ibom State, Niger Delta, Nigeria and analysed for physiochemical using standard techniques, and microbiological parameters using standard cultural and metagenomics techniques. Replicate data from microbiological and physicochemical analyses were analyzed using analysis of variance (ANOVA) while bioinformatic analyses were done using Vecton NTI suite 9 (InforMax, Inc.), NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1.

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Results: Physicochemical analysis showed that the levels of metals such as chromium, nickel, zinc and iron and as well as turbidity were significantly higher than acceptable standards. Bacterial counts ranged from 0.64 to 1.00 (x 10^5) cfu/ml. Fungal counts ranged from 2.00 to 4.00 (x 10^2) cfu/ml. *Esherichia coli, Citrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Flavobacterium*, *Shigella, Salmonella* and *Vibrio* species were isolated using cultural means. Total coliforms counts were higher in location E6 which was closest to the community. Metagenomics analysis gave a total of six kingdoms namely bacteria, unknown, protozoa, archae, plantae and fungi in decreasing order of read counts. Furthermore, it revealed a total of 16 phyla, 24 classes, 38 orders, 39 families and 209 species of bacteria. Some pathogens not captured by cultural means such as *Clostridium species, Vibrio alginolyticus, Vibrio neresis, Staphylococcus kloosii, Corynebacterium diptheriae,* and uncultured species such as u*ncultured Helicobacter* were all captured by metagenomics.
Conclusion: Although, cultural methods used in this study were able to capture water borne disease pathogens, metagenomics captured much more kingdoms and species. Where possible, both techniques should be used in the microbiological examination of water samples.

Keywords: Estuary; potable water; metagenomics; cultural methods; water borne diseases; Niger Delta.

1. INTRODUCTION

Historically, the Niger Delta was defined to comprise the present day Bayelsa, Delta and Rivers States of South-South Nigeria [1]. Starting from the year 2000, it was expanded administratively to cover nine states namely Akwa Ibom, Abia, Bayelsa, Cross River, Delta, Edo, Ondo, Imo and Rivers States. The region is very important because of its abundance of crude oil deposits. Geographically, the region has a land mass of about 25,640 km^2 (comprised of a low land area of 7,400 km^2, fresh water swamp of 11,700 km^2, salt water swamp of 5,400 km^2 and Sand Barrier Islands of 1,140 km^2) [2-3].The Niger Delta mangrove ecosystem is the largest in Africa and third in the world [4]. It is one of the world's most fragile ecosystems and holds the highest diversity of fresh water fish species in West Africa [5]. Its estuary waters play very important roles in the Niger Delta ecosystem. It serves as a source of fish, food, transportation and "portable" water in some areas and the cultural heritage of the people. Exploration and production activities since the late 1950's have brought enormous foreign exchange to the country and the region. However, this has come at the expense of the Niger Delta environment [4].

Access to safe drinking water eludes millions of people around the world especially those in sub-Saharan Africa. Nigeria is a member of the United Nation Declaration of the Right to water. This declaration states that everyone living in Nigeria has the right to sufficient, affordable, safe and acceptable water for personal and domestic uses [6] The report estimated that 63 and 100 million Nigerians still do not have access to improved sources of drinking water and basic sanitation facilities, respectively. Furthermore, the report stated that urban and rural populations as at 2012 stood at 84.84 and 83.99 million with 12% and 33.33% still in the practice of open defecation, respectively. This is even made worst in the Niger Delta because inhabitants of its riverine communities are in the habit of disposing sewage directly into surrounding and nearby water bodies. The situation is further worsen by incessant crude oil spillage which usually have far reaching health and economic implications beyond the aquatic habitats. A classical case is the Ogoni Land situation as revealed by the United Nations Environmental Programme report [4]. Access to unclean water is very significant from a public health point of view. It remains the main transmission route of water washed, water based and water borne diseases [7]. Waterborne diseases results in considerable morbidity and mortality amongst children under five years of age, elderly and immune-compromised persons [7].

Cultural based methods remain the most used methods for biological examination of water samples. These conventional techniques include total coliform test, multiple fermentation tubes or the most probable number and recently, the defined substrate and hydrogen sulphide techniques [7-8]. Cultural methods as reviewed recently are plagued with problems. These challenges include being time consuming, non-sensitive to viral and protozoan communities that might be present, and also limited to small

culturable minority [7-10]. More worrisome is the formation of biofilms in natural water sources and even in drinking water distribution systems [8]. This has prompted the increasing use of more sensitive molecular based techniques [7-9].

In the Niger Delta, a number of studies exist that have used cultural techniques to examine water quality. However, to the best of our knowledge, no studies exist that have employed metagenomics in examination of estuary water in a rural setting it is used domestically often without prior treatment. Thus, the prime aim of this study was therefore to examine estuary water quality using cultural and metagenomic techniques in addition to physicochemical analysis.

2. METHODS

2.1 Study Location

The study location chosen for this study was Emereoke II (Ward 5) community of Eastern Obolo Local Government Area, Akwa Ibom State, Nigeria. Eastern Obolo is located on co-ordinates 4°32′0″N 7°42′0″E. The study location is host to several multinational oil companies notably Shell Petroleum Development Company (SPDC). (See Fig. 1)

2.2 Sample Collection

Estuary water samples were collected from a total of 5 different locations (2, 3, 4, 5 and 6). From each location, the samples were collected in triplicates using sterile sample bottles. The samples were transported immediately to the laboratory for microbiological, metagenomics and physicochemical analyses. These were done as previously described [10-11]. Location 2 and 3 were located closest to the pipelines, 3 and 4 were further away from human settlement and the pipelines while 5 and 6 were located closest to human settlement. Sample 6 was taken closest to the community.

2.3 Physiochemical Analysis

Triplicate samples from all the location were subjected to physicochemical analysis. The various physicochemical parameters analyzed were pH, temperature, electrical conductivity, turbidity, total dissolved solids, total hardness, dissolved oxygen, biochemical oxygen demand, calcium, potassium, magnesium, N-nitrate, N-nitrite, copper, zinc, sulphide, N-ammonia, chromium, nickel and cobalt. These were all carried out using standard methodologies described previously by APHA 1992 & 1995, and WHO 1992 [12-14].

Fig. 1. A plate showing an abandoned oil well. From this location, sample number E6 was obtained

2.4 Microbiological Analysis

The water samples collected in triplicates were made into five composite samples of 1 litre each and used for the microbiological analyses. From each of the water samples, a ten-fold serial dilution were carried out (10^{-1} to 10^{-10}) as described previously by APHA (1989), Antai, & Antai et al. [15-17]. For each of the water samples, dilutions from 10^{-2} and 10^{-3} were plated in duplicates on freshly prepared Nutrient agar and Sabouraud Dextrose agar (SDA) for the enumeration of total aerobic bacteria and fungi, respectively. In addition, they were also plated on Salmonella Shigella agar, Mac Conkey agar and Thiosulphate Citrate Bile Salt agar (TCBS). The plates were then incubated for 24 and 48 hours, respectively. After incubation, the plates were then observed for growth and the colonies counted. Distinct colonies were purified, maintained and identified as previously described [18-21]. Total coliform was carried out using a *x2-1B* machine, freshly prepared Eosin Methylene Blue (EMB) agar, and advanced membrane filter (of pore size 0.45 mm and diameter 47 mm). Briefly, the filtration chamber was uncoupled and sterilized using an autoclave at 121°C under15 psi. After autoclaving, the chamber was aseptically coupled back and the membrane filter inserted. Exactly 100 ml of water sample was filtered through and the membrane filter aspectically removed, and placed on the EMB. This was repeated for all samples and the plates incubated at 28±2°C for total coliform enumeration.

2.5 DNA Extraction from Sample and Polymerase Chain Reaction (PCR)

The five composite water samples were further made into one composite sample for metagenomic analysis. DNA Extraction was performed using ZYMO soil DNA extraction Kit (Model D 6001, Zymo Research, USA) by following strictly the manufacturer's instructions. Briefly, one ml of the water sample was measured out using a graduated Eppendorf tube. The measured water sample was then placed in a ZR Bashing Bead ™ Lysis Tube followed by the addition of 750 µl Lysis Solution to the tube. The content of the 2 ml tube was centrifuged at 1,000 rpm for 5 minutes. The ZR Bashing Bead ™ Lysis Tube was centrifuged in a micro centrifuge at ≤10,000 x *g* for 1 minutes. After this, 400 µl of the filtrate was added to a Zymo-Spin ™ IV Spin Filter in a Collection Tube and centrifuge at 7,000 rmp (7,000 x *g*) for 1 minute. This was followed by the addition of 1,200 µl of DNA Binding Buffer to the filtrate in the Collection Tube. Exactly, 800 µl of the mixture from above was added to a Zymo-Spin ™ IIC Column in a Collection Tube and centrifuge at 10,000 x *g* for 1 minute. Flow through from the Collection Tube was discarded and this particular step was repeated with the remaining filtrate. Exactly 200 µl of DNA Pre-Wash Buffer was thereafter added to the Zymo-Spin™ IIC Column in a new Collection tube and centrifuged at 10,000 x *g* for 1 minute after which 500 µl Soil DNA Wash Buffer was added to the Zymo-Spin ™ IIC Column and centrifuged at 10,000 x *g* for 1 minute. The Zymo-Spin ™ IIC Column was transferred into a clean 1.5 ml micro centrifuge tube and 100 µ DNA Elution Buffer added directly to the column matrix. This was centrifuged 10,000 x *g* for 30 seconds to elute the DNA. The eluted DNA was transferred into a filter unit of Zymo-Spin ™ IV-HRC Spin Filter in a clean 1.5 ml micro centrifuge tube and centrifuged at exactly 8,000 x *g* for 1 minute.

Following DNA extraction from the samples, the genomic DNA extracts were subjected to PCR amplification. The PCR was set at 30 cycles for 2 hours at 96, 72 and 65°C for denaturation, annealing and extension. The amplified genomic DNA (15 µl) were then subjected to 1.5% gel electrophoresis after mixing with 2 µl of loading dye. These were done using as previously described by Salaam et al. and Edet et al. [22-23]

2.6 Next Generation DNA Sequencing and Bioinformatics Analysis

DNA sequencing was performed using Next Generation Sequencing with sequencing primer - 16S: 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'- ATTACCGCGGCTGCTGG-3'. The sequencing was done using automated PCR cycler Genome Sequencer™ on MiSeq (Illumina) platform. 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.

2.7 Statistical Analysis

The resulting counts and readings for physicochemical analysis were subjected to analysis of variance (ANOVA) to test for significance at 95 % confidence limit.

3. RESULTS

3.1 Physicochemical Analysis

Table 1 shows the results of the physicochemical and heavy metals analyses of the estuary water from the various sampled locations. From the results, it can be seen that the pH ranged from 6.62 to 7.14 with locations 5 and 6 recording the lowest and highest values, respectively. From the samples, location 2 recorded the lowest temperature of 28.5°C while locations 5 and 6 recorded the highest temperature of 29.50°C. The conductivity result indicates that E6 recorded the highest conductivity of 40.10 (μs/cm) and E5 recorded the least of 36.90 (μs/cm). The turbidity result shows that locations E3 and E6 had values of 25.40 and 26.60 NTU and these values were about two times higher than those of the other locations. The hardest water sample was that from location 6 (61.00 mg/L) followed by location 5 (34.20 mg/L). The biological oxygen demand for the various sampled locations was the most consistent parameter as it ranged from 6.31 to 6.72 mg/L, with location 6 having the highest values. Furthermore, calcium was below detection levels in all the sampled locations while potassium levels ranged from 0.20 to 1.50 mg/L. Magnesium was not detected in locations 2 to 4 but was detected in locations 5 and 6 with values of 0.17 mg/L and 0.73 mg/L, respectively.

N-nitrite levels were lower than those of N-nitrate and locations 4 and 6 recorded the highest N-nitrate values of 2.60 and 2.50 mg/L, respectively. Similarly both locations recorded the highest levels of N-nitrite. Ammonia was detected in all the sampled locations with locations 5 and 6 having the highest values of 0.27 and 0.44 mg/L, respectively. Heavy metals analyses indicate that copper, zinc, iron, nickel, chromium and cobalt were detected in the sampled locations. Iron levels were higher than those of copper and zinc in locations 2, 3 and 6. Sulphide levels were the highest of all the sampled parameters even though was not recorded in locations 4 and 5. However, it was highest in location 2 with a value of 480.00 mg/L followed by location 3 with a value of 274.00 mg/L. Copper zinc, and iron were below detection levels in locations 4 and 5.

3.2 Microbiological Analysis

Table 2 shows the total bacterial and fungal heterotrophic counts (THBC and THFC) results. The results show that THBC counts were more than two fold higher than those of THFC. The highest THBC and THFC values of 100 (x 10^3) and 8 (x 10^2) counts were seen in location 4. The least THBC of 64 (x 10^3) was recorded in location 2.

Table 3 shows the total coliform counts of the various water samples. However, location 6 recorded total coliform that was too numerous to count (TNTC).

Table 4 shows the bacterial and fungal isolates obtained in this study. *Esherichia coli* was the most abundant species from the estuary water samples. In addition, other isolates obtained were *Citrobacter*, *Bacillus*, *Micrococcus*, *Pseudomonas* and *Flavobacterium*, *Salmonella, Shigella, Flavobacterium* and *Vibrio* species.

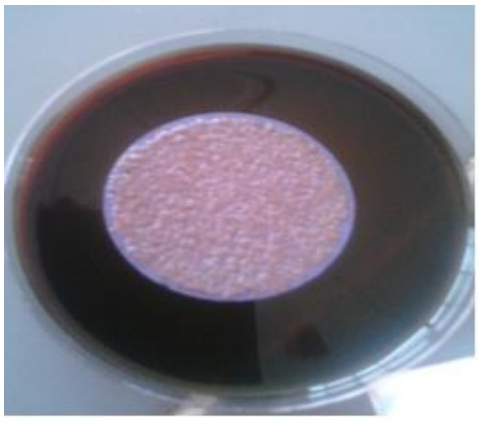

Fig. 2. A plate showing total coliform from location 6 which was too numerous to count (TNTC) after 24h

Furthermore, Table 4 also shows the fungi species isolated from the estuary water. The fungal isolates were six in number and these were *Aspergillus, Candida, Rhizopus, Fusarium, Mucor* and *Penicillium* species. Aspergillus species were the most abundant species.

3.3 Metagenomics Analysis

The results of the 16S rRNA metagenomic assessment are shown in Tables 5 through 10. Table 5 shows the various kingdoms, their reads counts and their percentages. From the results, it can be seen that bacteria was the most abundant with a read count and percentage of 12,225 and 98.64%, respectively. The second most abundant kingdom was the unknown kingdom with a read count of 70 (0.56%) and this was followed closely by protozoa with a read count of 58 (0.47%). Interestingly, the kingdom fungi was the least most abundant kingdom.

Table 1. Physicochemical characteristic and heavy metal profile of estuary water samples from the various locations

Parameters	E2	E3	E4	E5	E6	WHO	NIS
pH	6.72±1.14*	6.93±0.01*	7.06±0.10*	6.62±0.20*	7.14±0.02*	6.5-8.0	6.5-8.5
Temperature (^{o}C)	28.50±1.71	29.00±0.01	29.5±0.01	29.60±0.10	29.60±0.00	ND	Ambient
Conductivity (us/cm)	37.50±0.10	38.70±0.01	38.30±0.01	36.90±1.71	40.10±1.41	ND	1000
Turbidity (NTU)	18.60±0.20	25.40±0.10	13.5±1.11	13.5±1.21	26.60±1.71	<0.10	5
TDS (mg/L)	23.30±0.02	23.22±0.02	22.98±0.04	22.14±1.71	24.06±0.10	1000	500
TH (mg/L)	19.20±2.41	7.1±0.01	18.60±1.44	34.20±1.14	61.00±1.71	ND	150
BOD (mg/L)	6.31±0.01	6.56±0.01	6.61±0.02	6.43±0.00	6.72±0.01	ND	ND
DO (mg/L)	1.90±0.10	2.56±0.01	2.41±0.07	1.71±0.04	2.40±0.10	ND	ND
Calcium (mg/L)	BDL	BDL	BDL	BDL	BDL	ND	ND
Potassium (mg/L)	0.30±0.01	0.50±0.10	0.40±0.10	0.20±0.01	1.50±.0.07	ND	ND
Magnesium (mg/L)	BDL	BDL	BDL	0.17±0.10	0.73±0.10	0.01-0.20	0.2
N-nitrate (mg/L)	1.80±0.01	1.10±0.01	2.60±0.10	1.50±0.01	2.50±0.10	50.00	50
N-nitrite (mg/L)	0.03±0.01	0.02±0.00	0.03±0.00	0.02±0.00	0.06±0.01	3.00	0.2
Copper (mg/L)	2.25±0.10	1.93+0.01	BDL	BDL	1.58±0.10	2.0	1.0
Zinc (mg/L)	0.85±0.01	0.82±0.10	BDL	BDL	0.68±0.10	0.01-0.05	3.0
Iron (mg/L)	5.00±1.14	1.45±0.10	BDL	BDL	6.32±0.01	>1.00	3.0
Sulphide (mg/L)	480.00±14.40	274.00±7.14	BDL	BDL	141.00±11.42	ND	ND
N-ammonia (mg/L)	0.06±0.10	0.28±0.10	0.24±0.01	0.27±0.20	0.44±0.02	>0.20	ND
Chromium (mg/L)	0.07±0.01	0.06±0.01	0.09±0.01	0.07±0.01	0.11±0.01	0.05	0.05
Nickel (mg/L)	0.09±0.01	0.15±0.10	0.09±0.01	0.09±0.01	0.08±0.01	0.07	0.02
Cobalt (mg/L)	0.31±0.01	0.75±0.10	0.31±0.01	0.32±0.10	0.25±0.10	ND	ND

*Keys: TDS=Total dissolved solutes, TH= total hardness, BOD= biological oxygen demand, ND= No data available, DO= Dissolved oxygen, BDL= below detection limit, E2, E3, E4, E5 and E6 = estuary water from locations 2,3,4,5 and 6. *Represent Mean±SD from three readings that were significant (P< 0.01). WHO = World Organization Standard and NIS = National Industrial Standard.*

Table 2. Mean total heterotrophic bacterial and fungal counts

Locations	THBC (x 10^3)	THFC (x 10^2)
ES2	64	4
ES3	68	4
ES4	100	8
ES5	68	2
ES6	69	2

ES, = Estuary water and 2,3,4,5 and 6 = locations 2, 3, 4, 5, and 6

Table 3. Total coliform counts of the various estuary water samples

Locations	Counts per 100ml	USEPA standard per 100ml
E2	8	1
E3	3	1
E4	26	1
E5	TNTC*	1
E6	20	1

*ES, = Estuary water and 2,3,4,5 and 6 = locations 2, 3, 4, 5, and 6. *Represents total coliform counts obtained from location 6. TNTC = Too Numerous To Count. USEPA = United State Environmental Protection Agency*

Table 4. Bacterial and fungal isolates from the estuary water samples

Bacterial isolates	*Fungal isolates*
Esherichia coli	*Aspergillus species*
Klebsiella species	*Candida species*
Citrobacter species	*Rhizopus species*
Bacillus species	*Fusarium species*
Micrococcus species	*Candida species*
Citrobacter species	*Mucor species*
Pseudomonas species	*Penicillium species*
Salmonella species	*Rhizopus species*
Shigella species	
Proteus species	
Lactobacillus species	
Flavobacterium species	
Vibrio species	

Table 5. Kingdom classification of the 16S rRNA metagenome from the composite estuary water sample

Kingdom	Read counts	Percentage (%)
Bacteria	12255	98.64
Unknown	70	0.56
Protozoa	58	0.47
Archaea	38	0.31
Plantae	2	0.02
Fungi	1	0.01

Table 6 shows the phyla classification of the composite estuary water sample and from the result, it can be seen that unknown phyla had the highest read counts of 6,929 (55.77%) which was followed by Proteobacteria with read counts of 2,610 (21.01%), *Actinobacteria* with read counts of 2,180 (17.55%), Bacteroidetes with counts of 240 (1.93%) and then Firmicutes in fifth position with read counts of 227 (1.83%). Other less abundant phyla identified were Ciliophora, Chloroflexi, Crenarchaeota, Acidobacteria, Verrucomicrobia, Tracheophyta, Cyanobacteria, Gemmatimonadetes, Euryarchaeota and Ascomycota with reads counts of 58, 37, 37, 13, 8, 2, 2, 1, 1 and 1, respectively.

Table 6. Phyla classification of the 16S rRNA metagenome from composite estuary water sample

Phyla classification	Read counts	Percentage (%)
Unknown	6929	55.77
Proteobacteria	2610	21.01
Actinobacteria	2180	17.55
Bacteroidetes	240	1.93
Firmicutes	227	1.83
Planctomycetes	78	0.63
Ciliophora	58	0.47
Chloroflexi	37	0.30
Crenarchaeota	37	0.30
Acidobacteria	13	0.10
Verrucomicrobia	8	0.06
Tracheophyta	2	0.02
Cyanobacteria	2	0.02
Gemmatimonadetes	1	0.01
Euryarchaeota	1	0.01
Ascomycota	1	0.01

Table 7 shows the various classes in the composite estuary water sample. Unknown class had a read count of 6,953 (55.96%). The next top nine classes were Actinobacteria, Gammaproteobacteria, Deltaproteobacteria, Bacilli, Flavobacteria, Alphaproteobacteria, Planctomycetacia, Sphingobacteria and Gymnostomatea. The least abundant classes were Liliopsida, Thermococci, Dothideomycetes, Gemmatimonadetes and Cycadopsida that had read counts of 1 representing 0.01% each.

Table 8 shows the order classification and this shows that the unknown had a read count of 7,783 representing 62.64%. The next top nine orders were Actinomycetales, Alteromonadales, Oceanospirillales, Flavobacterium, Bacillales, Planctomycetales, Sphingobacteriales,

Spathidiida and *Enterobacteriales* The last seven orders were *Thermococcales*, *Cycadales*, *Sphinogomonadales, Gemmatimonadales*, Not assigned, *Asparagales* and *Desulfobacterales* with reads counts of 1 each representing 0.01%.

Table 7. Class classification of the 16S rRNA metagenome from composite estuary water sample

Class	Read counts	Percentage (%)
Unknown	6953	55.96
Actinobacteria	2180	17.55
Gammaproteobacteria	2111	16.99
Deltaproteobacteria	324	2.61
Bacilli	169	1.36
Flavobacteria	159	1.28
Alphaproteobacteria	131	1.05
Planctomycetacia	78	0.63
Sphingobacteria	68	0.55
Gymnostomatea	58	0.47
Thermoprotei	37	0.30
Chloroflexi	37	0.30
Mollicutes	29	0.23
Clostridia	29	0.23
Betaproteobacteria	14	0.11
Acidobacteria	13	0.10
Bacteroidetes	13	0.10
Epsilonproteobacteria	8	0.06
Verrucomicrobiae	8	0.06
Liliopsida	1	0.01
Thermococci 1	1	0.01
Dothideomycetes	1	0.01
Gemmatimonadetes	1	0.01
Cycadopsida	1	0.01

Table 9 shows the family classification and the results indicate that unknown family accounted for 7,812 reads (62.88%). The next ten abundant families were Micrococcaceae, Halomonadaceae, Alteromondadeae, Idiomarinaceae, Alcanivoracaceae, Flavobacteriaceae, Planctomycetaceae, Bacillaceae, Spathidiidae and Staphylococcaceae. The last four families were had reads counts of 11 representing 0.09% each.

Table 10 shows the read counts of the top 20 orgnaisms at genus and species levels. *Uncultured bacterium* gave a read count of 5,434 representing 43.72%. this was followed by *Nesterenkonia sp* with counts of 1,832 (14.74%), followed by an *Uncultured marine sp* with counts of 1,118 (9.00%). Others were *Uncultured halomonas*, *Uncultured alteromonas, Uncultured alteromonas, Uncultured gamma, Ponticaulis koreensis, Idiomarina tainanensis, Halomonas species, Uncultured actinobacterium, Idiomarina seosinensis, Alcanivorax species, Kangiella species, Uncultured bacillus, Salinicoccus species, Coccinistipes vermicola, Bacterium i1-2, Spongiibacter marinus and Leeuwenhoekiella species with read counts of* 577, 463, 388, 195, 168, 96, 82, 78, 56, 49, 48, 47, 43, 43, 37 and 26 respectively.

Table 8. Order classification of the 16S rRNA metagenome from composite estuary water sample

Order	Read counts	Percentage (%)
Unknown	7783	62.64
Actinomycetales	2095	16.86
Alteromonadales	842	6.78
Oceanospirillales	783	6.30
Flavobacteriales	159	1.28
Bacillales	121	0.97
Planctomycetales	78	0.63
Sphingobacteriales	68	0.55
Spathidiida	58	0.47
Enterobacteriales	49	0.39
Lactobacillales	48	0.39
Desulfurococcales	37	0.30
Chloroflexales	37	0.30
Pseudomonadales	33	0.27
Rhodobacterales	31	0.25
Clostridiales	29	0.23
Acholeplasmatales	24	0.19
Rhodospirillales	19	0.15
Rhizobiales	17	0.14
Kordiimonadales	14	0.11
Acidobacteriales	13	0.10
Bacteroidales	13	0.10
Burkholderiales	12	0.10
Vibrionales	11	0.09
Verrucomicrobiales	8	0.06
Caulobacterales	8	0.06
Campylobacterales	8	0.06
Bdellovibrionales	6	0.05
Incertae sedis 8	5	0.04
Xanthomonadales	5	0.04
Bifidobacteriales	3	0.02
Thermococcales	1	0.01
Cycadales	1	0.01
Sphingomonadales	1	0.01
Gemmatimonadales	1	0.01
Not assigned	1	0.01
Asparagales	1	0.01
Desulfobacterales	1	0.01

Table 9. Family classification of the 16S rRNA metagenome from composite estuary water sample

Family	Read counts	Percentage (%)
Unknown	7812	62.88
Micrococcaceae	2027	16.32
Halomonadaceae	676	5.44
Alteromonadaceae	469	3.77
Idiomarinaceae	330	2.66
Alcanivoracaceae	106	0.85
Flavobacteriaceae	105	0.85
Planctomycetaceae	78	0.63
Bacillaceae	66	0.53
Spathidiidae	58	0.47
Staphylococcaceae	55	0.44
Cryomorphaceae	54	0.43
Enterobacteriaceae	49	0.39
Carnobacteriaceae	43	0.35
Not assigned	42	0.34
Desulfurococcaceae	37	0.30
Chloroflexaceae	35	0.28
Rhodobacteraceae	31	0.25
Pseudomonadaceae	31	0.25
Flexibacteraceae	29	0.23
Clostridiaceae	28	0.23
Acholeplasmataceae	24	0.19
Intrasporangiaceae	23	0.19
Corynebacteriaceae	21	0.17
Rhodospirillaceae	17	0.14
Kordiimonadaceae	14	0.11
Brevibacteriaceae	13	0.10
Acidobacteriaceae	13	0.10
Sphingobacteriaceae	12	0.10
Hyphomicrobiaceae	11	0.09
Prevotellaceae	11	0.09
Comamonadaceae	11	0.09
Vibrionaceae	11	0.09

4. DISCUSSION

The quality of water bodies be it oceans, seas, estuaries, fresh waters, lakes and streams is very complex [24] and important as the survival of flora and fauna, microorganisms and humans depends on it. As it is common with most riverine communities, these natural water bodies are most often the only source of drinking water. Often, this is always threatened by anthropogenic activities such as oil and gas exploration, industrial activities and domestic sewage disposal [25]. The quality is often assessed using microbiological parameters such as total coliform and physicochemical parameters such as pH, temperature, phosphate, nitrates, heavy metals, and pollutants [26,27]. Estuary water body health or quality becomes even more important when the Niger Delta ecosystem is involved because it sustains the economic activities of the inhabitants such as fishing and farming. It is also at the receiving end of legal and illegal refinering of crude oil activities, and their attendant oil spillages leading to environmental pollution and degradation.

Table 10. Read counts of the top twenty (20) organisms at genus and species levels

Organisms	Read counts	Percentage %
Uncultured bacterium	5434	43.72
Nesterenkonia species	1832	14.74
Uncultured marine	*1118*	*9.00*
Uncultured halomonas	*577*	*4.64*
Uncultured alteromonas	463	3.73
Uncultured gamma	388	3.12
Ponticaulis koreensis	195	1.57
Idiomarina tainanensis	168	1.35
Halomonas species	96	0.77
Uncultured actinobacterium	*82*	*0.66*
Idiomarina seosinensis	*78*	*0.63*
Alcanivorax species	56	0.45
Kangiella species	49	0.39
Uncultured bacillus	49	0.39
Salinicoccus species	48	0.39
Coccinistipes vermicola	47	0.38
Alkalibacterium species	43	0.35
Bacterium i1-2	43	0.35
Spongiibacter marinus	37	0.30
Leeuwenhoekiella species	26	0.21

From the results of the physicochemical analysis, it can be seen that some of the parameters failed to met the Nigerian Industrial Standard [27] and the World Health Organization [27]. For the sampled locations, pH, temperature and conductivity met the standards as they were all within acceptable range. This was the same for total dissolved solids and total hardness which were also below standards. However, there were

no guidelines for BOD, DO, calcium, and potassium. Turbidity was far above the maximum limits recommended WHO and NIS of < 0.10 and 5 NTU in all the sampled locations. Magnesium was not below detection limits in location 2, 3 and 4. However in location 6, it was above the limits of 0.01-0.20 and 0.2 for WHO and NIS, respectively. Nitrate and nitrite levels were all below safety limits of WHO and NIS. Copper limits in the locations detected were above NIS maximum levels of 1.0 mg/l. Zinc levels were more than those of WHO but within limits of NIS. Chromium and nickel were above the maximum levels set by both standards.

In a study carried out by Emuedo et al. [25] to assess the water quality of Nembe, Okrika and Okpare in Bayelsa over 24 months period, heavy metals were detected in levels that were agreeable to levels of copper and iron but higher than those of chromium, nickel and cobalt in our study. In another study by Seiyaboh et al. [28] that was carried out to access the physicochemical characteristics of sediment from Epie Creek, Bayelsa State, Nigeria, they reported nitrate and nitrite levels that were just slightly higher than our findings over their five sampled locations. However, their pH readings of 6.67-6.77 were within our pH range. Physicochemical parameters of estuary waters from Lagos lagoon in an earlier study showed agreeable temperature (27.00-33.00°C), conductivity (20.00-280.00 μs/cm), ammonia nitrogen (0.73-2.53 mg/L), turbidity (9.00-121 NTU) and dissolved oxygen level (2.0 -7.40) to our findings [29]. In a recent study by Onojake et al. [30], surface water physicochemical parameters of pH, temperature, DO, BOD, turbidity and heavy metals of Bonny/New Calabar River Estuary were very agreeable to our findings apart from conductivity that was higher than our findings.

Variations of physicochemical parameters examined in this study are known to influence microbial diversity. Total coliform bacteria include a wide range of aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehydes within 24 h at 35–37°C. Typical examples include bacteria in the genus *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter.* The presence of coliform in the various estuary water samples in our study is a call for concern as it indicates recent sewage contamination [27].

A total of ten bacteria were isolated, and they were *E. coli, Citrobacter sp, Bacillus sp, Micrococcus sp, Pseudomonas sp* and *Flavobacterium sp*. *E. coli* remained the most frequent isolate. The fungal isolates were *Aspergillus, Candida sp, Rhizopus, Fusarium sp, Mucor and Penicillium* sp. In a study to assess the bacteria diversity of a water body affected by refinery effluent by Obikwu and Otokunefor [31], they isolated similar isolates seen in our study. The similar isolates were *E. coli, Micrococcus, Bacillus sp, Pseudomonas sp, Flavobacterium sp and Citrobacter sp*. Interestingly, the seasonal variation of bacteria and fungi counts they reported were similar to our findings (2.00-4.00 $x10^2$ cfu/ml) especially for the months of November – March ($4.11x10^2$-$1.13x10^4$ cfu/ml). For the month of March, their bacteria count ranged from 2.50×10^4 – 9.40×10^8 and this was also within range of our findings (6.4×10^4 – 1.08×10^6 cfu/ml). However, Unimke et al. [32], reported slightly higher total heterotrophic bacteria and fungi counts from surface and sub surface waters during wet and dry season of Imo river estuary of 2.23-2.39 $x10^6$ and 1.17-1.38 $x10^5$, respectively for bacteria and fungi, respectively.

Anthropogenic activities especially crude oil spillages have been shown affect bacteria diversity [33]. Furthermore, the classical cultural techniques of describing bacteria diversity are usually not able to capture more than 1% of the bacteria diversity [24,34-35]. Sadly, the use of molecular techniques in assessment of microbial diversity studies in the Niger Delta is completely lacking or non-existent. Molecular assessment of the composite estuary water sample showed that bacteria were the most predominant kingdom, followed by unknown kingdom and protozoa as second and third most abundant kingdoms. Fungi diversity was very low and this explains in part the low fungi count observed in our study. In our study, detected phyla were Proteobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Plantocmycetes, Ciliophora, Chloroflexi, Crenarchaeota, Acidobacteria, Veruucomicrobia, Tracheophyta, Cyanobacteria, Gemmatimonadetes, Euryarchaeota and Ascomycota in descending order of abundance. In an earlier study, Bobrova et al. [34] used metagenomics (16S rRNA) to access the microbial diversity in the Black Sea estuaries in South-West of Ukraine, They found that the top most abundant phyla were Cyanobacteria, Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia and Planctomycetes across all

three location they analysed. These phyla were also found in our study.

In another study by Lamendella et al. [35] to assess the impact of the deepwater oil spill in the Gulf of Mexico coast microbial communities. The 16S data revealed that highly contaminated samples had higher abundance of Alpha and Gammaproteobacteria sequences. Our 16s rRNA results also showed that these two classes made the top seven classes with abundance of 1.05% and 16.99%, respectively. Their presence in the top seven classes also indicates that these classes may be thriving here because of the abundance of hydrocarbons contamination. Beale et al. 36] using a community multi-omics approach towards the assessment of surface water quality in an Urban River system reported similar top 10 orders to include *Actinomycetes, Rhodospirillales, Bukholderiales, Sphingobacteriales, Alteromonadales, Cyanobacteria family II, Flavobacteriales Planctomycetales, Acidimicrobiales* and *Rhizobiales*. These orders were well represented in our findings apart from *Cyanobacteria family II*.

In an earlier study by Gomez-Alvarez et al. [37] using pyrosequencing to examine the ecology of free chlorine and monochloramine treated samples observed proteins of bacterial descent in addition to eukaryotic, archaeal and viral proteins. They also reported genes associated with virulence and antibiotics resistance. Similar and dominant classes in their study to that of our findings were Actinobacteria, Betaproteobacteria, Gammaproteobacteria and Alphaproteobacteria. In an earlier study, beta lactamase genes were reported from the same water samples [23].

A survey of the 209 species revealed by metagenomic approach showed that species that are unculturable and culturable. Among these were *Nesterenkonia sp. UT 4-03, Ponticaulis koreensis strain GSW-23, Idiomarina tainanensis culture-collection MCCC: 1A02633, Idiomarina seosinensis culture-collection MCCC 1A0721, Kangiella sp, Uncultured marine bacterium clone SHFB611, Uncultured Halomonas sp. clone HA_102, Uncultured Alteromonas sp. clone ASTS_NEM_500m_340* and *Uncultured gamma proteobacterium clone JL-ETNP-F5.* Although pathogenic bacteria were not amongst the twenty dominant species, they were however present. They included *Clostridium sp, Vibrio alginolyticus, Vibrio neresis* and *Staphylococcus kloosii*.

5. CONCLUSION

The composition of estuary water is a very complex one that varies depending on the degree of anthropogenic interference. Given the findings in this study, it can be seen that physicochemical parameters especially the heavy metals copper, zinc and iron were higher than safe standards. Furthermore, 16s rRNA was more robust than the culture dependent methodology that was employed in this study. Although total coliform test were positive for estuary water samples, the use of metagenomics was able to capture more pathogenic microorganisms. There is a need for provision of potable water for the inhabitants of the sampled community.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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