

Microbiological examination and physicochemical analysis of estuary water used as a point of source drinking water

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 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 physicochemical 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.

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 ($\times 10^5$) cfu/ml with fresh water having the highest. Fungal counts ranged from 2.00 to 4.00 ($\times 10^2$) cfu/ml. *Escherichia 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, archaea, 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* and *Staphylococcus kloosii*, *Corynebacterium diptheriae*, and uncultured species such as uncultured *Helicobacter*.

Conclusion: Although, cultural methods used in this study were able to capture water born 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

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. Geographically, the region has a land mass of

about 25,640 km² (comprised of a low land area of 7,400km², fresh water swamp of 11,700 km², salt water swamp of 5,400 km² and Sand Barrier Islands of 1,140 km²) [2-3]. The Niger Delta mangrove ecosystem is the largest in Africa and third largest 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 and in the livelihoods of the people where 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 but it has come at the expense of the Niger Delta environment [4].

Access to safe drinking water eludes millions of people around the world and especially 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]. Furthermore, the report estimates that 63 and 100 million Nigerians still do not have access to and improved sources of drinking water basic sanitation facilities, respectively. According to the report, urban and rural population as at 2012 stood at 84.84 and 83.99 million with 33.33% and 12% still in the practice of open defecation. This is even made worst in the Niger Delta because of the habit of sewage disposal directly into surrounding and nearby water bodies by inhabitants of river-line communities. The situation is further worsen by crude oil spillage which usually far reaching health and economic implications far beyond the aquatic habitats. A classical case in point here is the Ogoni Land 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 route of transmission for water washed, water based and water borne diseases [7]. Waterborne diseases results in considerable morbidity and mortality especially in children under five years of age, elderly and immunocompromised person [7].

Cultural based methods remains the most used methods for biological examination of water samples. These conventional techniques include total coliform test, multiple fermentation tube or the most probable number techniques and recently the defined substrate and hydrogen sulphide methods [7-8]. Cultural methods as reviewed recently are plagued with problems. These include time consumption, non-sensitive to viral and protozoan communities that might be present and also limited to small culturable majority [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 were done that have employed metagenomics in examination of estuary water in a rural setting where such water is used domestically often without prior treatment. Thus, the aim of this study was therefore to examine estuary water quality using cultural and metagenomic techniques in addition to physicochemical analysis.

Methods

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 figure one below)



Figure 1: A plate showing an abandoned oil well belonging to SPDC. From this location, sample number E6 was obtained.

Sample collection

Estuary water samples were collected from a total of 5 different locations (Emereoke 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 by Etim et al [11]. Location 2 and 3 were located close to the SPDC pipelines, 3 and 4 were further away from human settlement and the pipelines while 5 and 6 were located close to human settlement with 6 taken closest to the community.

Physicochemical 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].

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, (1990) & Antai et al (1989) [15-17]. For each of the water samples, dilution 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 and Mac Conkey agar and Thiosulphate Citrate Bile Salt (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 by Cruickshank et al (1975), Domsch et al (1980) and Udotong et al (2015) [19-21]. Total coliform was carried out using a x2-1B machine, freshly prepared Eosin Methylene Blue (EMB) agar, and advanced membrane filter (of pore size 0.45mm and diameter 47mm). Briefly, the filtration chamber was uncoupled and sterilized using an autoclave at 121°C under 15psi. After autoclaving, the chamber was aseptically coupled back with the membrane filter inserted, after which 100ml of water sample was filtered through and the membrane filter aseptically removed and placed on the EMB. This was repeated for all samples and the plates incubated at $28\pm 2^{\circ}\text{C}$ for total coliform enumeration.

DNA extraction from sample and polymerase chain reaction (PCR)

Resulting 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 manufactures 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 µ 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 $\leq 10,000 \times g$ 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 rpm ($7,000 \times g$) 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 $10,000 \times g$ for 1 minute. Flow through from the Collection Tube was discarded and this particular step was repeated with the remaining filtrate. 200 µl of DNA Pre-Wash Buffer was thereafter added to the Zymo-Spin™ IIC Column in a new Collection tube and centrifuged at $10,000 \times g$ for 1 minute after which 500 µl Soil DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuge at $10,000 \times g$ for 1 minute. The Zymo-Spin™ IIC Column was transferred into a clean 1.5 ml micro centrifuge tube and add 100 µ DNA Elution Buffer directly to the column matrix. This was centrifuge $10,000 \times g$ for 30 seconds to elute the DNA. The elute DNA from was transferred into a filter unit of Zymo-Spin™ IV-HRC Spin Filter in a clean 1.5 ml micro centrifuge tube and centrifuge at exactly $8,000 \times g$ for 1 minutes. The filtered DNA was the used for PCR and DNA sequencing. 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°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 by Salaam et al (2017) and Edet et al (2017) [22-23]

Next generation DNA sequencing and bioinformatics analysis

DNA sequencing was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of all microorganism present in the **water** sample using sequencing primer -16S: 27F: 5'-GAGTTTGATCCTGGCTCAG-3' and 518R: 5'- ATTACCGCGGCTGCTGG-3'. The sequencing was performed by Next Generation Sequencing Technique to determine the nucleotide sequence of all microorganisms present in the **water** sample using automated PCR cycle- Genome Sequencer™ MiSeq (Illumina). Analysis and alignment was performed using Vectors NTI suite 9 (InforMax, Inc.). Overall bioinformatics analysis was done using NCBI-BLAST-2.2.24 and CLC bio Genomics workbench v7.5.1.

Statistical analysis

The resulting counts from the duplicate plates were then recorded and subjected to **analysis of variance to test for significance at 95 % (confidence limit).**

Results

Physicochemical analysis

Table 1 shows the results of the physicochemical and heavy metals analyses of the estuary water from the various sampled location. From the results, it can be seen that from the various sampled estuary water locations, it can be seen that the pH of the sampled ranged from 6.62 to 7.14 with location 5 and 6 recording the lowest and highest ranges **respectively**. From the samples, location 2 recorded the lowest temperature of 28.5 ° c. 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 (25.40) and E6 (26.60 NTU), recorded turbidities values that were about two time higher than the rest of the other locations. The hardest water sample was that from location 6 (61.00mg/L) followed by location 5 (34.20mg/L). The biological oxygen demand for the various samples locations was the most consistent parameter as it ranged from 6.31 to 6.72 mg/L, with location 6 and 4 recording the highest and second to highest values. Furthermore, calcium was below detection levels in all the samples 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 0.17mg/l and 0.73mg/l respectively.**

Table 1: Physicochemical characteristic and heavy metal profile of fresh and marine water samples

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 (°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±0.0 1	18.60±1.4 4	34.20±1.1 4	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

211 Keys: TDS=Total dissolved solutes, TH= total hardness, BOD= biological oxygen
212 demand, ND= No data available, DO= Dissolved oxygen, BDL= below detection limit,
213 E2, E3, E4, E5 and E6 = estuary water from locations 2,3,4,5 and 6. *Represent
214 Mean±SD from three readings that were significant (P< 0.01). WHO = World
215 Organization Standard and NIS = National Industrial Standard.
216

217 N-nitrite levels were lower than those of N-nitrate and locations 4 and 6 recorded the
218 highest N-nitrate values of 2.60 and 2.50, respectively. Similarly both locations
219 recorded the highest levels of N-nitrite. Ammonia was detected in all the sampled
220 locations with locations 5 and 6 having the highest values of 0.27 and 0.44 mg/L.
221 Iron levels were higher than those of copper and zinc in locations 2, 3 and 6. Heavy
222 metals analyses indicate that copper, zinc, iron, nickel, chromium and cobalt were
223 detected in the sampled location. Sulphide levels were the highest of all the sampled
224 parameters even it was not recorded in location 4 and 5. However, it was highest in

location 2 with a value of 480.00mg/l followed by location 3 with a value of 274.00mg/L. copper zinc, and iron were below detection levels in locations 4 and 5.

Microbiological analysis

Table 2 shows the total bacterial and fungal heterotrophic counts (THBC and THFC). The results shows that THBC counts were more than two fold higher than those of THFC. The second highest THBC $100 (x 10^3)$ and THFC $8 (x 10^2)$ counts were seen in location 4 for estuary water samples collected. The highest THBC was seen in fresh water (FW). The least THBC of $64 (x 10^3)$ was seen in location 2.

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 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, MW and FW = Estuary, marine and fresh waters and 2,3,4,5 and 6 = locations 2, 3, 4, 5, and 6. *Represent total coliform counts obtained from location 6. TNTC = Too Numerous To Count. USEPA = United State Environmental Protection Agency.

Table 3 shows the total coliform counts of the various water samples. As can be seen in the table 4 and in figure 1, marine water showed no growth but just deposit of salts. However, location 5 recorded total coliform that was too numerous to count (TNTC).



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

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

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

Bacterial isolates	Fungal isolates
<i>Escherichia coli</i>	<i>Aspergillus species</i>
<i>Klebsiella species</i>	<i>Candida species</i>
<i>Citrobacter species</i>	<i>Rhizopus species</i>
<i>Bacillus species</i>	<i>Fusarium species</i>
<i>Micrococcus species</i>	<i>Candida species</i>
<i>Citrobacter species</i>	<i>Mucor species</i>
<i>Pseudomonas species</i>	<i>Penicillium species</i>
<i>Salmonella species</i>	<i>Rhizopus species</i>
<i>Shigella species</i>	
<i>Proteus species</i>	
<i>Lactobacillus species</i>	
<i>Flavobacterium species</i>	
<i>Vibrio species</i>	

Furthermore, Table 4 also shows the fungi species isolated from the estuary water. From the table, it can be seen that the isolates were seven in number and these include *Aspergillus*, *Candida*, *Rhizopus*, *Fusarium*, *Mucor* and *Penicillium* species. *Aspergillus* species were the most abundant species.

Metagenomics analysis

The results of the 16S rRNA metagenomic assessment are shown in the tables 6 through 14. Table 5 shows the various kingdoms, their reads counts and their percentage. 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, fungi were the sixth most abundant kingdom.

Table 5: Kingdom classification of the 16S rRNA amplicon from the composite estuary water sample

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

268

269 Table 6 shows the phyla classification of the composite **estuary water** sample and
270 from the result, it can be seen that unknown phyla had the highest read counts of
271 6929 (55.77%) which was followed by proteobacteria with read counts of 2610
272 (21.01%), actinobacteria with read counts of 2180 (17.55%), bacteroidetes with
273 counts of 240 (1.93%) and then firmicutes in fifth position with read counts of 227
274 (1.83%). Other phyla that were less abundant were identified as Ciliophora,
275 Chloroflexi, Crenarchaeota, Acidobacteria, Verrucomicrobia, Tracheophyta,
276 Cyanobacteria, Gemmatimonadetes, Euryarchaeota and Ascomycota with reads
277 counts of 58, 37, 37, 13, 8, 2, 2, 1, 1 and 1 respectively.

278 Table 6: Phyla classification of the 16S rRNA amplicon from composite estuary **water**
279 **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

280

281 Table 7 shows the various classes present in the composite estuary water samples.
282 **The unknown class of bacteria had a read count of 6.953 that comprised of 55.96%.**
283 The next nine top classes were Actinobacteria, Gammaproteobacteria,
284 Deltaproteobacteria, Bacilli, Flavobacteria, Alphaproteobacteria, Planctomycetacia,
285 Sphingobacteria and Gymnostomatea. The least abundant classes were Liliopsida,
286 Thermococci, Dothideomycetes, Gemmatimonadetes and Cycadopsida that had
287 read counts of 1 representing 0.01% each.

288
289

Table 7: Class classification of the 16S rRNA amplicon 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

290

291 Table 8 shows the order classification and this shows that the unknown had a read
292 counts of 7,783 representing 62.64% while the next nine top orders were
293 Actinomycetales, Alteromonadales, Oceanospirillales, Flavobacterium, Bacillales,
294 Planctomycetales, Sphingobacteriales, Spathidiida and Enterobacteriales The last
295 seven orders were Thermococcales, Cycadales, Sphingomonadales,
296 Gemmatimonadales, Not assigned, Asparagales and Desulfobacterales with reads
297 counts of 1 each representing 0.01%.

298
299

Table 8: Order classification of the 16S rRNA amplicon 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

300 Table 9 shows the family classification and the results indicate that unknown family
301 accounted for 7812 reads that represented 62.88%. The next ten abundant families
302 were Micrococcaceae, Halomonadaceae, Alteromonadaceae, Idiomarinaceae,
303 Alcanivoracaceae, Flavobacteriaceae, Planctomycetaceae, Bacillaceae,
304 Spathidiidae and Staphylococcaceae. The last four families were with reads counts
305 of 11 representing 0.09% each.

306 Table 9: Family classification of the 16S rRNA amplicon from composite estuary
307 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

308

309 Table 10 shows the read counts of the top 20 organisms at genus and species levels
310 and from the table it can be seen that Uncultured bacterium gave a read count of
311 5434 representing 43.72%. this was followed by *Nesterenkonia* sp with counts of
312 1832 (14.74%), followed by an uncultured marine sp with counts of 1118 (9.00%).

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

Organisms	Read counts	Percentage %
<i>Uncultured bacterium</i>	5434	43.72
<i>Nesterenkonia species</i>	1832	14.74
<i>Uncultured marine</i>	1118	9.00
<i>Uncultured halomonas</i>	577	4.64
<i>Uncultured alteromonas</i>	463	3.73
<i>Uncultured gamma</i>	388	3.12
<i>Ponticaulis koreensis</i>	195	1.57
<i>Idiomarina tainanensis</i>	168	1.35
<i>Halomonas species</i>	96	0.77
<i>Uncultured actinobacterium</i>	82	0.66
<i>Idiomarina seosinensis</i>	78	0.63
<i>Alcanivorax species</i>	56	0.45
<i>Kangiella species</i>	49	0.39
<i>Uncultured bacillus</i>	49	0.39

<i>Salinicoccus species</i>	48	0.39
<i>Coccinistipes vermicola</i>	47	0.38
<i>Alkalibacterium species</i>	43	0.35
<i>Bacterium i1-2</i>	43	0.35
<i>Spongiibacter marinus</i>	37	0.30
<i>Leeuwenhoekiella species</i>	26	0.21

Tables 10 shows the blast output of the top 20 uncultured species obtained from the composite estuary water samples. These were *uncultured bacterium*, *Nesterenkonia species*, *Uncultured marine bacterium*, *Uncultured halomonas*, *Uncultured alteromonas*, *Uncultured alteromonas*, *Uncultured gamma*, *Ponticaulis koreensis*, *Idiomarina tainanensis*, *Halomonas sp*, *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 5434, 1832, 1118, 577, 463, 388, 195, 168, 96, 82, 78, 56, 49, 48, 47, 43, 43, 37 and 26 respectively.

Discussion

The quality of water bodies be it oceans, seas, estuaries, fresh water, 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 [25]. The quality is often assessed using microbiological parameters such as total coliform and physicochemical parameters such as pH, temperature, phosphate, nitrates and 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 refining of crude oil, and their attendant oil spillages leading to environmental pollution and degradation.

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 meet the standards as they were all within acceptable range. This was the same for turbidity, 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, 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 1.0mg/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 detected heavy metals 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 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 $\mu\text{s}/\text{cm}$), ammonia nitrogen (0.73-2.53mg/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 pH, temperature, DO, BOD and turbidity, 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 types of drinking water sampled in the study site is a call for concern as it indicates recent sewage contamination ([27].

A total of ten bacteria were isolated, nine of which were Gram positive and two Gram negative. These species were *E. coli*, *Citrobacter* sp, *Bacillus* sp, *Micrococcus*, *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 (2014) [31] showed the presence of similar seen in our study. 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 $\times 10^2$ cfu/ml) especially for the months of November – March (4.11 $\times 10^2$ -1.13 $\times 10^4$ cfu/ml). For the month of March, their bacteria count ranged from 2.50 $\times 10^4$ – 9.40 $\times 10^8$ and this was also within range of our findings (6.4 $\times 10^4$ – 1.08 $\times 10^6$ $\times 10^6$ cfu/ml). However, Unimke et al (2014)[32], reported slightly higher total heterotrophic bacteria and fungi counts from surface and sub surface water during wet and dry season of Imo river estuary 2.23-2.39 $\times 10^6$ and 1.17-1.38 $\times 10^5$, 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][35]. Sadly, the use of molecular is assessment in microbial diversity studies in the Niger Delta is completely lacking or non-existent. Molecular assessment of the composite estuary water sample showed that bacteria was 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, Verucomicrobia, 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 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. Beale et al (2017)[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, Burkholderiales, 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 uncultured and cultured genus were dominant species in aquatic environment. Among these are 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. Metagenomic analysis revealed a total of 209 microorganisms. Although pathogenic bacteria were not amongst the twenty dominant species, they were however present. These were Clostridium sp, Vibrio alginolyticus, Vibrio neresis and Staphylococcus kloosii.

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 pathogenic microorganisms.

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