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Original Article

Effects of Physicochemical Properties on the South-South Estuaries' Microbial Community Structure

Ewaoche Sunday Itodo[1], Alade Tolulope[2], Pius Osagioduwa Igbinomwanhia[3], Lusa Haruna Yadock[1], and Tatfeng Youtchou Mirabeau[2]*

[1] Baze University, Abuja, Nigeria.
[2] University of Benin, Benin City Nigeria.
[3] Niger Delta University, Wilberforce Island, Bayelsa State Nigeria.
*Author for correspondence ORCID ID: https://orcid.org/0000-0002-7947-5628; Email: ewieise@gmail.com

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ABSTRACT

The conceivably chemical gold of the marine environment is affected by a myriad of natural and artificial challenges. The physical and chemical challenges faced by microbes in the ocean are one among the many myriads of activities that affect marine microbial life. As the depth of the ocean increases, temperature declines, salinity increases, and the availability of nutrients dwindle, pollution from a variety of sources such as recreation, fish culture, and the assimilation and transport of pollution effluents through river can greatly affect the physiochemical and biodiversity of the ocean life. The total community DNA was extracted using the ZR soil microbe DNA Miniprep kit. The amplicon Library was prepared using the reversible terminator sequencing on Miseq, illumina's integrated next-generation sequencer by Inqaba Biotechnological as per 16S metagenomic sequencing Library preparation. For the determination of pH, the pH meter was used; the conductivity meter was used for conductivity; the salinometer was used for salinity, the heating method was used for total dissolved solids (TDS); Nephelometer was used for turbidity; the filter method was used for total suspended solids (TSS); palintest Nitratest method was used for Nitrate determination; Total alkalinity was determined by the standard titrimetric method; for Total hardness, the EDTA titrimetric method was used; the wet ashing method was used for metals; Hydrogen carbonate was determined by titrimetric method; sulfate and Chloride were determined by turbidimetric and argentometric method respectively. The total read counts from the kingdom across the three zones are 4981 (82.70%), 991 (94.11%), and 45835 (46.64%) for Okrika, Ibaka, and Brass, respectively. The total read counts from the phyla across the three zones are 1222 (21.73%) for Proteobacteria, 221 (21.33%) for Firmicutes, and 14712 (14.97%) for Firmicutes, respectively. The salinity and sodium values were 2.96 and 3.23 and 567.31 and 422.91 for the top and bottom, respectively, in Ibaka. The salinity and sodium values were 13.55 and 12.94 and 1877.68 and 1874.02 for top and bottom, respectively, for Okrika. The salinity and sodium values were 11.65 and 8.51 and 1238.69 and 1338.82 for the top and bottom, respectively,

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for Brass. The number of bacteria phyla obtained from the sediment was found to depend on the physicochemical parameters (sodium, salinity, pH, etc.).

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INTRODUCTION

Billions of living microbial cells underpin all of the world's oceans, which is 200 times the total biomass of humans on the planet. These microbes' relationship with time and energy is fundamentally different from that of humans. What appears to be a day to them could be a thousand years to us. They are not interested in the sun, they are not interested in growing quickly, and they are probably not interested in the petri dish. We need time and sunlight to replicate the exact conditions of the deep sea. The sun provides us with the motivation to move quickly as well as the energy to do so. There is currently no theoretical limit to the life span of a single cell if microbes can access energy; they can live for a long time by replacing broken parts over time (Orphan et al., 2001).

Microorganisms from the sediments tend to have more tolerance for the harsh condition of the ocean, unlike other types of microbes that will be damaged and sometimes killed if exposed to the same conditions (Cohen, 2003).

The quality of marine water resources is primarily determined by physicochemical characteristics. Monitoring and determining physicochemical parameters of seawater (vertical and spatial sea surface water profile) such as pressure, temperature, pH, conductivity, salinity, turbidity, dissolved oxygen, and nutrient composition (such as nitrite, nitrate, ammonium, and phosphate, among others) becomes critical for assessing the status of the near coastal environment.

Long-term irrigation with this sewage effluent contaminates soil and crops to such an extent that it becomes poisonous to plants and promotes soil deterioration (Quinn & Syers, 1978; Hemkes et al., 1980). This contains a significant number of potentially dangerous compounds, such as soluble salts and heavy metals such as Fe2+, Cu2+, Zn2+, Mn2+, Ni2+, and Pb2+. These heavy metal additions are unwanted. Plants can accumulate heavy metals in their tissues at levels above the permitted levels, posing a threat to the lives of humans and animals feeding on these crops and potentially contaminating the food chain, as observed in soil and plants that received irrigation water mixed with industrial effluent (Amin et al., 2010).

The biogeochemical parameters exhibit considerable seasonal variations depending on the local conditions, such as rainfall, tidal incursions,

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various abiotic and biotic processes, and the quantum of freshwater inflow that affects the nutrient cycle of the coastal environment (Chavan et al., 2005).

This work, therefore, seeks to evaluate the impact of the physicochemical parameters on the distribution of microbial life across the three estuaries in the South-South region of Nigeria.

MATERIALS AND METHODS

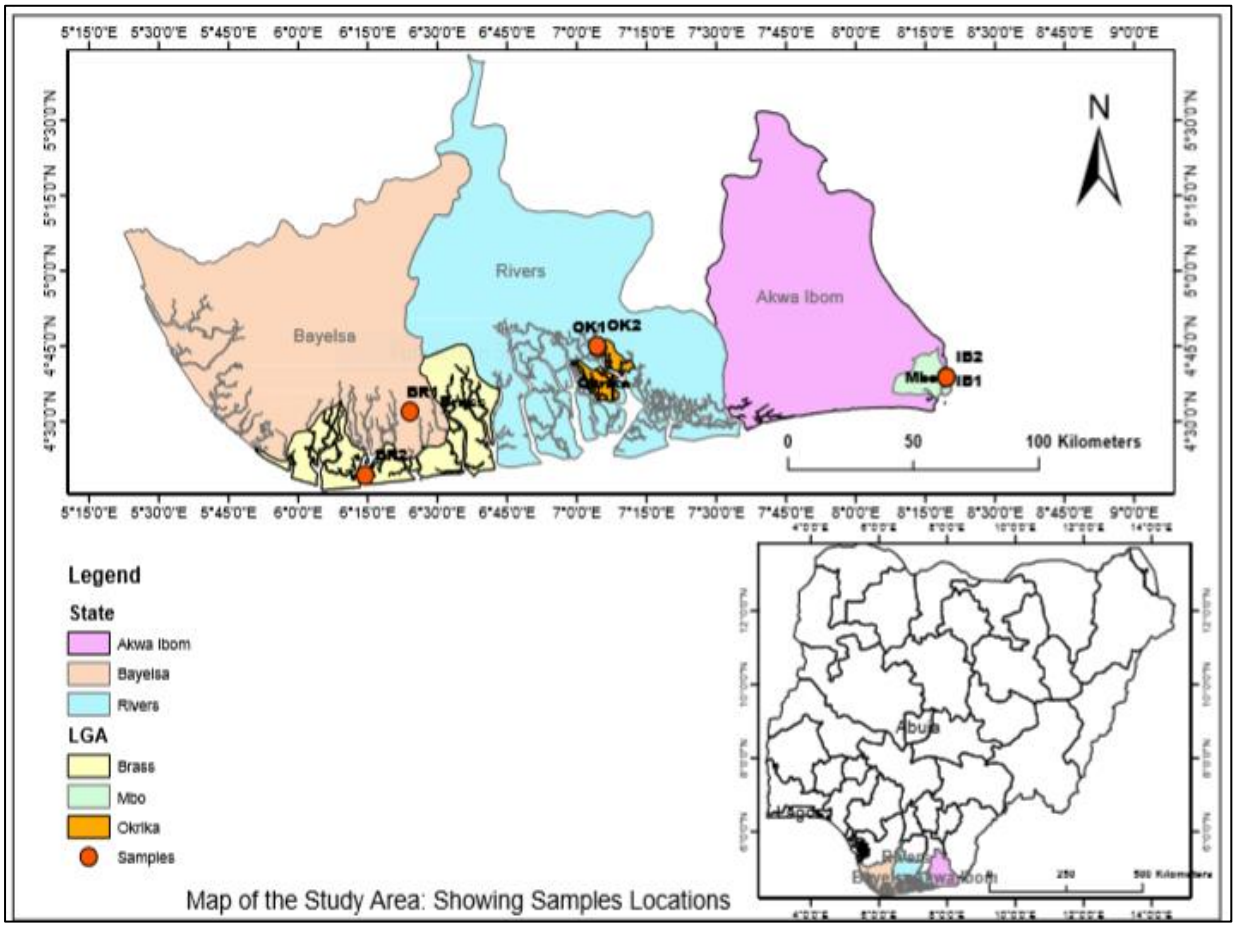
Study Area

The research was conducted in Akwa Ibom state's Ibaka estuary (040 38'43.0" N, 0080 19'36.6" E), Rivers state's Okrika estuary (040 44'34.9" N, 0070.04'36.0E), and Bayelsa state's Brass estuary (04.18.11.7" N, 0060 14'30.5 "E). The samples were examined at Niger Delta University's Medical Laboratory Science Department's Molecular and Diagnostic Laboratory Unit in Amassoma, Bayelsa State. Bayelsa is in the tropics and can be found at latitude 040 150N, longitude 05 0220W, and latitude 06 045E. Delta State borders the state to the north, the Atlantic Ocean to the southeast, and Rivers State to the west.

Sample Collection

Water and sediment samples were collected from Ibaka estuary in Akwa Ibom state (040 38'43.0" N, 0080 19'36.6" E), Okrika estuary in Rivers state (040 44'34.9" N, 0070.04'36.0E), and Brass estuary in Bayelsa state (04.18.11.7" N, 0060 14'30.5" E) at depths ranging from 10 to 15 meters. Water and sediment samples from the epipelagic and bathypelagic zones were collected in sterile polypropylene bottles and transported to the laboratory for further analysis.

Figure 1: Map of the study area showing samples locations



Source: (Researchers, 2023)

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DNA Extraction (Miniprep)

The ZR Soil Microbe DNA MiniPrep kit was used to extract the total community DNA from the sediment samples. In brief, one gram of deep-sea sediments was aliquoted into the ZR Bashing bead lysis tube, which was then filled with 750 ml of lysis solution. The bashing beater was placed in a 2 ml tube holder assembly and proceeded for 5 minutes at maximum speed. Following that, the bashing bead lysis tube was centrifuged at 10,000 xg for 1 minute, the supernatant was transferred to a collection tube with a Zymo-spin IV Spin Filter (orange top) and centrifuged at 7,000 x g for 1 minute, followed by the addition of 1200 ml of soil DNA binding buffer to the filtrate from the previous tube. In a collection tube, 800 ml of the previous step's mixture was transferred to a Zymo-Spin IIC Column and centrifuged at 10000 x g for 1 minute. The flow from the collection tube was discarded, and the preceding step was repeated. In a new collection tube, 200 ml of DNA pre-wash buffer was added to the zymo-spin IIC Column and centrifuged at 10,000 x g for 1 minute. The zymo-spin IIC was transferred to a clean 1.5 ml microcentrifuge tube, and 100 ml DNA elution buffer was directly added to the column matrix before centrifuging at 10,000 x g for 30 seconds to elute the DNA. The previously eluted DNA was transferred to a prepared zymo-spin IV-HRC spin filter (green top) in a clean 1.5 ml microcentrifuge tube and centrifuged at 8000 x g for 1 minute.

The method of Malmstrom *et al. (*2007) was adopted for the Cultivation-independent techniques. In order to accurately determine the abundance of microbes in the sediment samples, the DNA sample was processed and sequenced using the MiSeq2500 Illumina system.

Physicochemical Analysis

Determination of pH

The pH of the water samples was determined using the pH meter. The meter was calibrated (standardized) before use with two buffer solutions, pH 4 and pH 9 to ensure accurate pH readings. After thoroughly rinsing the electrode in distilled water, it was dipped in the water sample. The pH of the water sample was determined using a consistent pH readout. A pH meter is an instrument that we use when quick and accurate pH measurements are required. The sensory electrode is in contact with the to-be-tested solution. The pH meter was calibrated so that a specific difference in voltage between silver chloride and the test solution read a specific pH value.

Determination of Conductivity (µscm-1)

The electrical conductivity of the water sample was measured using a conductivity meter that also included a salinity meter. The meter's probe was dipped into the water sample, and the control switch was set to conductivity mode, yielding a steady readout as the electrical conductivity of the water sample.

Determination of Salinity

The control switch on the same meter as in the previous example was set to salinity, and a steady readout of the salinity of the water sample was recorded. The container was filled with water, and the straw was carefully inserted (clay-covered end down), with clay added until the straw floated at the desired depth. The depth at which the salinometer floats in the water was marked with a permanent marker (0 per cent salt solution). Dissolved salts were measured in parts per thousand (ppt). The density of the water is measured by the hydrometer:

Determination of Total Dissolved Solids

The total dissolved solid was carried out by weighing an empty evaporating dish in analytical balance. The water sample was well mixed and poured into a funnel with filter paper. One hundred (100) ml of water sample was placed in the evaporating dish and evaporated to dryness in a six-hole water bath. The dish and its contents were dried to a constant weight in an oven at 105^0C. The weight of the residue obtained was proportional to the volume of the sample used. The weight difference is expressed as the total dissolved solid in ppm. (EPA, 2012).

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Total solid (TS) = Total dissolved solid (TDS) + Total suspended solid (TSS)

Total dissolved solids (mg / L) = [(TDSA – TDSB)] × 1000 / sample (ml)

Where TDSA = weight of dried residue + dish in milligrams and TDSB = weight of dish in milligrams.

Determination of Turbidity

The sample was brought to room temperature and thoroughly mixed to disperse the solids. It was allowed to wait until air bubbles disappeared. The water sample was then poured into the turbidimeter tube. The turbidimeter was zero and calibrated with a standard calibration solution. The water sample was then inserted into the meter, and the turbidity was directly measured in NTU units.

Measurement of Suspended Solids

Eleven (11) cm diameter filter paper was oven dried at 105 ^{0}C to a constant weight and the weight was recorded. Filter papers were used to filter 100 ml water samples. The filter papers and residues were oven-dried to a constant weight at 105 ^{0}C. The increase in weight was used to calculate the total suspended solid (Gao et al., 2006).

Total suspended solids (mg / L) = [(TSSA – TSSB)] 1000 / sample (ml), where TSSA is the weight of the dish and filter paper plus the dried residue and TSSB is the weight of the dish and filter paper in milligrams.

Determination of Nitrate (NO_3)

The Nitratest tube was filled to 20 ml mark with water sample, one level spoonful of Nitratest powder, and one Nitratest tablet were added, and the tube was shaken for one minute. It was left to stand for one minute before being gently flipped three times to aid in flocculation. The clear solution was decanted into a test tube with a 10 ml mark. One Nitricol tablet was crushed and combined to dissolve, then allowed to rest for 10 minutes for full-colour development before recording the photometer reading at 570 nm concentration in mg/L.

Determination of Total Alkalinity (TA)

One hundred (100) ml of filtered water samples were placed in a 250 ml conical flask, along with two drops of methyl orange indicator to give the orange colour.

This was titrated to a light pink endpoint with 0.02M HCl.

$$TA = \frac{VT\ x\ M\ x\ 100000}{Vol.of\ sample}.$$

Where VT = volume of HCl. M = Molarity of HCl, 100,000 = Molar mass of $CaCO_3$ in (mg)

Determination of Total Hardness (TH)

A titration of 50 ml Burette was filled to the '0' mark with a 0.01M EDTA solution. A 250 ml conical flask was filled with 100 ml of filtered water samples. The flask was then filled with 1 ml of ammonia/ammonium chloride buffer. To achieve the red-wine colour, 3 drops of Eriochrome Black T indicator were added. This was titrated to a marine-blue finish (colour).

$$Total\ Hardness\ (TH) = \frac{VT\ x\ A\ x\ 1000}{Vol.of\ sample}$$

Where: VT = volume of EDTA; A = mg equivalent of 1 ml EDTA which is (1 ml = 10 units).

Analysis of Minerals (Metal Ions)

Preparation of samples: (Wet ashing method) – Haynes (1980) One hundred (100) ml of water samples were dispensed into 250 ml conical, and 25 ml of 2M HNO_3 was added and thoroughly mixed. This was evaporated to dryness over a six-hole water bath, cooled to room temperature, and then 25 ml of concentrated HNO_3 was added to the residue in the flask and heated on a hot plate to near boiling, which was repeated until the solution was dry. This process was repeated until the residue became white. After that, distilled water was added, and the residue was washed and filtered in 100 ml volumetric flasks before being made up to the mark with distilled water.

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Determination of Metals

Flame photometry was used to determine sodium (Na) and potassium (K). AAS (Absorption Spectrophotometer) was used to determine calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) at various wavelengths using an Acetylene/Air-gas mixture. Four calibration standards for Na, K, Fe, Zn, Mn, Ca, and Mg were created according to Greenberg, Clesceri and Eaton (1992).

The pre-treatment procedure was carried out prior to the analysis. Clean polyethene bottles were used to collect the sample. Polyethene bottles were used in these analyses because glass bottles absorb metals and thus cause analysis inaccuracy. Immediately after collection, the water was filtered through a 0.45 m membrane filter. The first 50-100 ml of sample was used to rinse the equipment. Thereafter, the required sample volume was collected. To stabilize the metal content, acidification with (1:1) nitric acid to a pH of 2 was used. For acidification, approximately 3 ml of (1:1) nitric acid per litre of the sample was sufficient. The same initial procedure was used if the suspended solids content was required. The filter containing the suspended solids, on the other hand, was kept and stored in a suitable container. The beaker was covered with a watch glass, which serves as a surface to evaporate a liquid or as a cover for a beaker while solids are being weighed. The sample was heated until all of the water had evaporated and the sample was dry. The solution was then cooled, and 3 ml of HNO_3 was added. The solution was then heated once more until digestion was complete. This could be indicated by the residue of a light colour. The residue was then dissolved by adding 2 ml of HCl (1:1) and gently heating it again. The watch glass and beaker were washed and filtered with H_2O. The filter was washed before being discarded. The filtrate was diluted with H_2O to concentrate within the instrument's range (Greenberg, Clesceri & Eaton, 1992).

Total metal analysis was performed using the sum of dissolved and suspended metal ions (Greenberg et al., 1992). In each case, each sample was run in triplicate to ensure high accuracy in the quantitative results. Metal analysis results were reported as X 2 in ppm units. The characteristic concentration check value is the element concentration (in mg/L) that produces a signal of approximately 0.2 absorbance units under ideal conditions at the listed wavelength. The characteristic concentration check allows the operator to determine whether the instrumental parameters have been optimized and the instrument is performing to specifications. An external calibration curve was used to calibrate the AAS. The external calibration curve was created using a solution containing known concentrations of the sample element, also known as stock solution. The stock solution was created using high-purity metal salts dissolved in high-purity acids. The stock standard was diluted to create working standards.

Hydrogen Carbonate HCO_3^-

Two (2 drops) of the mixed indicators were added to the hydroxide acid titration solution. The solution was titrated to the pink endpoint with 0.02M standard HCl.

Determination of Sulphate (SO_4)

One hundred (100) ml of sample was placed in a conical flask with a capacity of 250 ml. 5 ml of conditioning reagent was added and thoroughly mixed. The content was placed on a magnetic stirrer for 1 minute after adding 0.3 g of Barrium Chloride. After that, the solution was poured into a measuring cell (culvette). The spectrophotometer was calibrated to 425 nm. Absorbance values were taken and read off the calibration curve, as well as the transmittance using a standard sulphate solution.

Determination of Chloride

The "Argentometric method" was used to determine chlorides. Briefly, one hundred (100) ml of water samples were dispensed into separate 250 ml conical flasks, and 5 ml of potassium chromate was added to each and properly mixed. The mixture was then titrated with a standard silver nitrate solution to a brick-brown endpoint.

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1 ml silver Nitrate ($AgNO_3$) = 1mg Chloride Cl- All values were recorded in triplicate before calculating the means and standard deviation.

RESULTS

Okrika sediment samples contained 4981 bacteria read counts from 2699 OTUs representing bacteria species from 19 phyla, 25 classes, and 15 genera. *Proteobacteria, Actinobacteria, Actinomycetales, Rhodospirillaceae, Nanoarchaneum,* and *Natranaerobium thermophilus* were the most abundant phylum, class, order, family, genus, and species (*Table 1*).

Table 1: Organisms identified in Okrika through metagenomic characterization by Miseq 2500 illumina platform NGS

Total Reads (Kingdom)	4981(82.70%)
Abundant phylum	Proteobacteria (1222/21.73%)
Abundant class	Actinobacteria
Abundant order	Actinomycetales
Abundant family	Rhodospirillaceae
Abundant genus	Nanoarchaeum
Abundant species	Natranaerobius

Ibaka had 991 bacteria read counts from 117 OTUs representing bacteria species from nine phyla, fourteen classes, and fourteen genera. *Firmicutes*, *Gammaproteobacteria*, *Natranaerobiales*, *Natranaerobiaceae*, *Natranaerobius*, and *Natranaerobius thermophilus* were the most abundant phylum, class, order, family, genus, and species (*Table 2*).

Table 2: Organisms identified in Ibaka through metagenomic characterization by Miseq 2500 illumina platform NGS

Total Reads (Kingdom)	991(94.11%)
Abundant phylum	*Firmicutes* (221/21.33%)
Abundant class	Gammaproteobacteria
Abundant order	Natranaerobiales
Abundant family	Natranaerobiaceae
Abundant genus	Natanaerobius
Abundant species	Natranaerobius thermophilus

The brass sediment sample contained 45835 bacteria read counts from 2426 OTUs representing bacteria species from 7 phyla, 10 classes, and 27 genera. *Firmicutes*, *Clostridia*, *Clostridiales*, *Clostriaceae*, *Clostridium,* and *Bacillus flexus* were the most abundant taxonomic groups of bacteria (*Table 3*).

Table 3: Organisms identified in Brass through metagenomic characterization by Miseq 2500 illumina platform NGS

Total Reads (Kingdom)	45835 (46.64%)
Abundant phylum	*Firmicutes* (14712/14.97%)
Abundant class	Clostridia
Abundant order	Clostridiales
Abundant family	Clostridiacea
Abundant genus	Clostridium
Abundant species	Bacillus flexus

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According to our findings, the pH gradients of Ibaka's estuary deep and surface waters were 6.41 and 6.77, respectively. The salinity of the surface water, however, was 2.96 ppm. The TSS had a wider range of 96.63 and 172.41 for surface and deep waters, respectively. When values from deep and surface waters were compared, other parameters such as conductivity, turbidity, total hardness, total alkalinity, calcium, and magnesium were shown not to depend on the distribution of microorganisms in the estuaries (*Table 4*).

Table 4: Physicochemical properties of the deep and surface sea waters of Ibaka

Parameter	Ibaka Top	Ibaka Bottom
pH	6.41	6.77
Sal. (ppm)	2.96	3.23
Cond.(µS/cm)	5909	6617.67
Turb. (NTU)	63.74	80.36
TDS	2955	3308.67
*TSS	96.63	172.41
$NO_{3(ppm)}$	12.74	10.38
Cl(ppm)	2203	1681
$SO_{4(ppm)}$	24.25	36.41
HCO_3	2.37	3.13
TA	551	493.33
TH (ppm)	1180	1421
*Ca(ppm)	1121.28	848.81
*Mg(ppm)	280.33	214.84
Na(ppm)	567.31	422.91
K(ppm)	140.15	111.57
Fe(µg/l)	0.1403	0.1243
Mn(ppm)	0.0208	0.0202

Keys: pH: Potential of hydrogen; Sal: Salinity; Cond: Conductivity; Turb: Turbidity; TDS: Total dissolved solid; TSS: Total suspended solid; NO_3: Nitrate; Cl: Chloride; SO_4: Sulphate; HCO_3: Bicarbonate; TA: Total alkalinity; TH: Total hardness; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Fe: Iron; Mn: Manganese.

The study found that the sulphate levels in Okrika's estuary surface and deep waters were 107.32 ppm and 75.36 ppm, respectively. The TA and conductivity values obtained between the surface and deep waters had wider ranges (5.45–771 and 44998 S/cm–48797.67 S/cm, respectively). When the surface and deep-water values were matched, the remaining parameters, such as pH, salinity, Chloride, TSS, Fe, Mn, Na, NO_3 and turbidity, were shown not to depend on the microbial distribution (*Table 5*).

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Table 5: Physicochemical properties of the deep and surface sea waters of Okrika

Parameter	Okrika Top	Okrika Bottom
pH	6.77	6.56
Sal. (ppm)	13.55	12.94
Cond.(µS/cm)	44998	48797.67
Turb. (NTU)	5.51	6.95
TDS	22499	24398.67
*TSS	0.55	0.65
$NO_{3(ppm)}$	15.45	16.61
Cl (ppm)	7349	7404.33
$SO_{4(ppm)}$		75.36
HCO_3	4.233	3.10
TA	545	771
TH (ppm)	2625	2563
*Ca(ppm)	3755.33	3748.03
*Mg(ppm)	938.70	937.04
Na(ppm)	1877.68	1874.02
K(ppm)	469.40	468.23
Fe(µg/l)	0.246	0.1712
Mn(ppm)	0.012	0.0142

Keys: pH: Potential of hydrogen; Sal: Salinity; Cond: Conductivity; Turb: Turbidity; TDS: Total dissolved solid; TSS: Total suspended solid; NO_3: Nitrate; Cl: Chloride; SO_4: Sulphate; HCO_3: Bicarbonate; TA: Total alkalinity; TH: Total hardness; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Fe: Iron; Mn: Manganese.

For surface and deep waters, the total hardness and total alkalinity values of Brass ranged from 171.33–981 and 1955–1855, respectively. In the TH measurement, the deep-water value was higher than the surface-water value. Almost all parameters, with the exception of Fe, Mn, HCO_3, TSS, and pH, were found to be dependent on the microbial distribution (*Table 6*).

Table 6: Physicochemical properties of the deep and surface sea waters of Brass

Parameter	Brass Top	Brass Bottom
Ph	6.91	6.36
Sal. (ppm)	11.65	8.51
Cond.(µS/cm)	28905	16270
Turb. (NTU)	10.61	12.56
TDS	14452.67	8135
*TSS	1.63	2.65
$NO_{3(ppm)}$	14.84	13.75
Cl (ppm)	4881	5300
$SO_{4(ppm)}$	54.52	38.42
HCO_3	4.40	3.0
TA	171.33	981
TH (ppm)	1955	1855
*Ca(ppm)	2477.39	2677.63
*Mg(ppm)	619.34	669.41
Na(ppm)	1238.69	1338.82
K(ppm)	309.67	334.70
Fe(µg/l)	0.0243	0.125
Mn(ppm)	0.0153	0.026

Keys: pH: Potential of hydrogen; Sal: Salinity; Cond: Conductivity; Turb: Turbidity; TDS: Total dissolved solid; TSS: Total suspended solid; NO_3: Nitrate; Cl: Chloride; SO_4: Sulphate; HCO_3: Bicarbonate; TA: Total alkalinity; TH: Total hardness; Ca: Calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Fe: Iron; Mn: Manganese.

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In summary, the deep-water salinity values for the three regions of Ibaka, Okrika, and Brass were 3.23 ppm, 12.94 ppm, and 8.51 ppm, respectively. The conductivity values of the same depth in Ibaka and Okrika were higher (6617.67 ppm and 48797.67 ppm, respectively) than in Brass (16270 ppm). This was found to depend on the microbial distribution. The turbidity level in Ibaka was higher than in Okrika and Brass. Okrika had the highest TDS value when compared to Brass and Ibaka. TSS was higher (172.41 ppm) in Okrika than in Brass (0.6 ppm and 2.65 ppm, respectively). Apart from HCO_3, Na, K, and Fe, the other parameters varied more when compared across regions.

The surface waters across the three regions show that the salinity, conductivity, and turbidity values for the surface water of Ibaka, Okrika, and Brass were 2.96 ppm, 13.55 ppm, and 11.65 ppm; 5909 S/cm, 44998 S/cm, and 28905 S/cm; and 63.74 NTU, 5.51 NTU, and 10.61 NTU, respectively. This may depend on the microbial distribution. When compared to pH, Fe, and Mn, the TDS, TSS, and NO_3 values were higher. Across all regions, the difference in the parameters (TDS, TSS, and NO_3) might have encouraged microbial distribution. The parameters (pH, Fe, and Mn) differed significantly between the three regions. When compared to Ibaka (2203 ppm) and Brass, Okrika had a higher chlorine value (7349 ppm) than Ibaka (2681). Except for pH and TH, all parameters across all regions might have encouraged microbial distribution.

DISCUSSIONS

The majority of organisms identified through metagenomic characterization using the Miseq 2500 illumina platform NGS belong to two phyla: *Proteobacteria* and *Firmicutes*. Bacterial species from the phylum *Proteobacteria* alone had a total read count of 1222, accounting for 21.73% in Okrika, and *Firmicutes* had a total read count of 221, accounting for 21.33% in Ibaka. Brass had the fewest phylum-*Firmicutes*, accounting for 14.97% of the total read count of 14712 (*Tables 1, 2* & *3*). This is consistent with the findings of Krishna *et al.* (2020), Yadav & Sharma (2019), Flandroy *et al.* (2018) and Gibbons *et al.* (2014), who identified similar bacteria from the Himalayan urban lake. In terms of pH, it was slightly acidic in all three regions, both surface and deep waters (*Tables 4, 5* & *6*), and it could be deduced that *Proteobacteria* and *Fermicutes* in Okrika, Ibaka, and Brass must have benefited from the condition. The number of bacteria phyla obtained from the sediment was found to be dependent on physicochemical properties such as salinity, pH, conductivity, total dissolved solids, total alkalinity, and sodium, as expected. Okrika had high sodium and salinity values, which must have aided in the spread of the phylum *Proteobacteria* and class *Actinobacteria*.

In general, the abundance of microorganisms in marine sediment decreases with increasing depth and sediment age (Parkes *et al.,* 2014; Kallmeyer *et al.,* 2012). Major elements that may limit the deep sedimentary biosphere include temperature, pressure, pH, salinity, water availability, sediment porosity, and permeability, in addition to a lack of nutrients and an inadequacy of energy-yielding substrates. The salinity of the estuaries obtained varied greatly between Ibaka, Okrika, and Brass. Salinity decreased with depth from top to bottom in all of the zones except Ibaka. According to this analysis, Ibaka had the lowest salinity and thus the lowest bacterial load when compared to Okrika and Brass (see *Tables 4, 5* & *6*).

Although there is a large relative difference in the occurrence of *Bacteriodetes* and *Firmicutes* (0.05%: 14.96% in Brass and 6.03%: 11.52% in Okrika, according to our findings, it is worth discussing because of the role they play in diabetes and inflammatory bowel disease (IBD). *Firmicutes* and *Bacterioidetes* are the two major bacterial phyla in the gastrointestinal tract.

The *Firmicutes*/*Bacteroidetes* (F/B) ratio has been linked to homeostasis maintenance, and changes in this ratio can result in a variety of pathologies. Increases in the abundance of specific *Firmicutes* or *Bacteroidetes* species, for example, cause obesity and bowel inflammation, respectively (Abenavoli *et al.,* 2019; Shen *et al.,* 2018).

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Firmicutes are gram-positive bacteria with rigid or semi-rigid cell walls, primarily from the genera *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus*, and *Ruminicoccus* (Rinninella *et al.*, 2019; Seong *et al.*, 2018). While there are approximately 7,000 different species of gram-negative bacteria in the phylum *Bacteroidetes*, the majority of them are from the genera *Bacteroides, Alistipes*, *Parabacteroides*, and *Prevotella* (Gibiino *et al.*, 2018).

Although there is a lot of emphases on the F/B ratio, it is important to remember that this ratio can be affected by an increase in other phyla and that dysbiotic increases in other phyla do not always change the F/B ratio. *Proteobacteria* was found to be the most variable phylum, which contributes to dysbiosis (Shin *et al.*, 2015) and is associated with a decrease in *Firmicutes* and general microbial diversity in inflammatory bowel disease (IBD) (Morgan *et al.*, 2012).

More than 1000 species of bacteria in the gut belong to six dominant phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*. *Firmicutes* and *Bacteroidetes* bacteria are the most common, accounting for 90% of the gut microbiota (Rinninella *et al.*, 2019).

A healthy person's gut microbiota differs in different parts of the gastrointestinal tract and changes over time as a result of ageing (including infant development) and environmental factors such as dietary habits, lifestyle, and antibiotic use. Individuals' microbiota composition varies greatly, with differences attributed to age, ethnicity, lifestyle, and diet (Rinninella *et al.*, 2019; Lynch & Pedersen, 2016). Arumugam *et al.* (2011) distinguished three distinct enterotypes of microbiota. Such variations are thought to be physiological and are in line with healthy microbiota. Nonetheless, changes in microbiota composition are frequently linked to diseases, also known as dysbiosis. However, the relationship between altered microbiota and various diseases is frequently ambiguous.

While the source of our phyla, *Firmicutes* and *Proteobacteria, was* not directly from the human gut, it could be succinctly said that due to the proximity of our sample collection, especially in Okrika and Ibaka, where the sediment samples were taken just a few meters from the shores, the increased human activity must have contributed to their abundance. The slightly acidic content of the sampled area may have contributed to the belief that they originated in the human gut. The results might have presented a picture of the gut microbiome of the inhabitants of the sample collection sites.

The high salinity, sodium, and conductivity values of Brass must have favoured *Clostridium* accumulation. Again, the high salinity and sodium values of Brass must have contributed to the low level of *Firmicutes*; this is consistent with the work of Yang *et al.* (2018).

The low levels of sodium (Na) and salinity found in Ibaka compared to Okrika and Brass must have contributed to the phylum *Firmicutes*' high abundance. In comparison to Ibaka, Okrika and Brass have higher salinity and sodium (Na) values, which must have favoured the expansion of the phylum *Proteobacteria* and a lower level of *Fermicutes*. Brass's high salinity, sodium (Na) and conductivity values must have favoured *Clostridium* accumulation. In comparison to Okrika, Ibaka's low sodium (Na) conductivity and turbidity values must have favoured *Natranaerobius* abundance, in line with the findings of Yang *et al.* (2018).

At the genus level, three genera- *Nanoarchaeum, Natranaerobius* and *Clostridium*- were found in each of the three zones (*Tables 1, 2* & *3*). The common denominator that unites all of the genera is their proclivity to survive at combined pH and salt concentration extremes. This is well documented in the literature (Mesba *et al.*, 2009; Bowers *et al.*, 2009; Mesbah *et al.*, 2009). However, recent research has revealed their proclivity to grow in three extreme conditions: temperature, pH, and salt. This complexity has placed them in an unusual group of 'polyextremophiles known as halophilic alkali

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thermophiles (Mesbah & Wiegel, 2009; Mesbah *et al.*, 2009).

According to our findings, the anaerobic alkalimophile *Clostridium* was obtained from Brass with a slightly acidic pH range, as well as *Natranaerobius* with a slightly acidic pH range for Ibaka and Okrika (*Tables 4* & *5*). This is in contrast to Feely *et al.* (2012), Havas & Hutchinson (2011) and Lacoul *et al.* (2011) who reported a relatively alkaline pH of 10.5.

The study found that there was a greater difference in sulphate values between Okrika's surface and deep waters, but the difference was not dependent on the microbial distribution. The TA and conductivity values obtained between the surface and deep waters had wider ranges but did not affect the microbial distribution. When the surface and deep-water values were matched, the remaining parameters such as pH, salinity, Chloride, TSS, Fe, Mn, Na, NO_3 and turbidity, showed no difference in the microbial distribution (*Table 5*); this is consistent with the work of Oluwaseun *et al.* (2007).

The total hardness and total alkalinity values within the Brass range were significantly higher than the surface water in the TH measurement yet, had no effect on the distribution of microorganisms in the sampled estuaries. Almost all parameters, with the exception of Fe, Mn, HCO_3, TSS, and pH, showed a significant difference in the surface and deep-water values were compared. Again, this is in line with the invitro work of Oluwaseun *et al.* (2007).

CONCLUSION

The number of bacteria phyla obtained from the sediment was found to depend on the physicochemical parameters (sodium, salinity, pH, etc.). The availability of nutrients which reshapes the microbial composition and abundance is greatly influenced by the physicochemical properties of the ocean. There were marked effects of the physicochemical parameters on the abundance and distribution of microbial life across the three regions of Ibaka, Okrika, and Brass, much more than within the region.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

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