



A Comparative Study on the Microbial and Physicochemical Characterization of Borehole Water from Bonny and Omuku, Rivers State, Nigeria

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ABSTRACT

This study investigated the microbial and physico-chemical quality of borehole water in Omoku and Bonny, Port Harcourt, Nigeria. A total of four borehole sites in duplicates were sampled from each sample community, where water samples were collected from different sample points and mixed to form a composite sample for a community and analyzed for physico-chemical parameters (pH, temperature, TDS, turbidity, hardness, alkalinity, chloride, nitrate, and sulfate) and microbial

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contaminants including total Coliforms and faecal Coliforms. The results showed varying levels of physico-chemical parameters and microbial analysis revealed the presence of pathogenic bacteria only in samples from Omoku while that of Bonny yielded no microbial growth. The total heterotrophic bacterial counts (THBC) from Omoku ranged from 3.2×10^5 CFU/ml - 9.4×10^5 CFU/ml while the fungal counts ranged from 1.5×10^3 - 2.0×10^4 . A total of 7 strains of bacteria and 4 fungal isolates were identified from the water samples from Omoku to generic level. Bacterial genera encountered included *Staphylococcus* sp., *Enterobacter* sp. and *Proteus* sp. Fungal isolates identified included *Fusarium* sp., *Penicillium* sp., *Trichoderma* sp. and *Candida* sp. The Most Probable Number (MPN) method for total coliform determination revealed counts for samples from Omoku community borehole (14 colonies/100ml each), highlighting the need for continued and sustained monitoring. Physicochemical parameters such as pH which revealed the water to be slightly acidic (6.79-6.86), nitrate ranged from 8.63 - 15.74mg/l, turbidity ranged from 0.53 - 1.22 ntu, TDS ranged from 1.67 - 1.83 mg/l and total hardness ranged from 93.87 - 158.85 mg/l. This study highlights the need for regular water testing, proper borehole construction and maintenance, and effective water treatment to ensure safe drinking water. The findings contribute to the development of sustainable water management strategies, promoting public health and environmental sustainability in urban Nigeria.

Keywords: Physicochemical; microbial; coliforms; antimicrobial; borehole; water.

1. INTRODUCTION

Water, the elixir of life (Mythrey, et al., 2012) is a fundamental human need and an abundant natural resource, covering 70% of the Earth's surface (WHO, 2017) and has become an indispensable resource for all living organisms (Del, et al., 2009). Its availability, accessibility and quality are fundamental to human health, ecosystem integrity, and sustainable development (Watson & Lawrence, 2003, Kruk, et al., 2018).

Access to safe and potable water is a basic human right, yet it remains a significant challenge for millions globally, particularly in developing countries (Onyebuchi, et al., 2024). Contaminated water sources pose a serious threat to public health, acting as a vehicle for a wide range of waterborne diseases, including cholera, typhoid fever, diarrhea, and dysentery (Shayo, et al., 2023). These diseases disproportionately affect vulnerable populations, contributing to morbidity, mortality, and hindering socio-economic progress. The urgency to address water quality issues is underscored by the United Nations Sustainable Development Goal 6, which aims to ensure availability and sustainable management of water and sanitation for all (Evaristo, et al., 2023).

In Nigeria, a nation rich in natural resources, access to safe drinking water remains a persistent challenge (Balogun & Redina, 2019, Isukuru, et al., 2024). Rapid urbanization,

population growth, inadequate sanitation infrastructure, and industrial activities contribute to the pollution of surface and groundwater resources. (Rashid, et al., 2018). In addition, many communities rely on alternative water sources, such as boreholes, for their daily needs. Borehole water, often perceived as a safer alternative to surface water, is groundwater accessed through drilled wells (Saah, et al., 2020). However, the quality of borehole water can be compromised by various factors, including geological formations, improper well construction and maintenance, and contamination from surface runoff or nearby waste disposal sites (Abanyie, et al., 2023).

Rivers State, located in the Niger Delta region of Nigeria, is a crucial hub for the country's oil and gas industry. This industrial activity, coupled with the challenges mentioned above, can exert significant pressure on the region's water resources. Bonny and Omoku, two distinct communities within Rivers State, face unique environmental and socio-economic conditions that may influence the quality of their borehole water. Bonny, a coastal island, is a major oil and gas hub with a high population density and significant maritime activities. Its proximity to the coast makes it susceptible to saltwater intrusion and potential contamination from oil spills and industrial discharges. Omoku, on the other hand, is an inland community with a predominantly agricultural economy. While less industrialized than Bonny, Omoku may face challenges related to agricultural runoff, including pesticides and fertilizers, which can leach into groundwater and

contaminate borehole sources (Sarker, et al., 2021).

Identifying the specific water quality concerns that these communities experience is critical for implementing effective water management policies that protect public health. This study, therefore, focuses on a comparative analysis of the microbial and physicochemical characteristics of borehole water from Bonny and Omuku, Rivers State, Nigeria. The research aims to Assess the microbial load and physicochemical parameters of borehole water samples from both communities, identifying and quantifying the presence of bacteria and fungi, including potential pathogens. This will provide insights into the potential health risks associated with consuming untreated borehole water.

2. METHODOLOGY

2.1 Sample Collection

A total of 4 borehole sites in duplicates were sampled from each sample community, where water samples were collected from different sample points (taps) and mixed to form a composite sample. To obtain the samples, the nozzle of each of the taps closest to the tank was sterilized with cotton wool soaked in ethanol, and the taps were allowed to run for two minutes to ensure that water standing in the pipe was not collected. Samples were collected in sterile containers in duplicates from the four sampling points and transported to the laboratory following standard methods for analysis. The samples were analyzed for total heterotrophic bacterial count, total coliform count, and fecal coliform count. The physicochemical parameters analyzed were pH, turbidity, alkalinity, total dissolved solids (TDS), electrical conductivity, total hardness, salinity, and atomic absorption spectrometry (AAS). The selected salt content was analyzed for chloride, nitrate, sulfate, and fluoride.

2.2 Microbiological Analysis

Enumeration of Total Heterotrophic Bacteria:

Ten-fold serial dilutions were performed on the samples. One milliliter of the water sample was suspended in 9 mL of sterile normal saline (0.85% w/v NaCl) to create a stock solution. One milliliter of the aliquot was then pipetted into a test tube containing 9 mL of sterile normal saline to create 10^{-2} , 10^{-3} , and 10^{-4} dilutions. Enumeration was

performed on each medium after 24–48 hours of incubation.

Isolation of Total Heterotrophic Bacteria:

Using a sterile 1 mL pipette (or syringe), 0.1 mL of each dilution was inoculated onto nutrient agar plates using the spread plate technique. The inoculum was spread evenly using a sterile glass rod. The Petri dishes were incubated in an inverted position at 37°C for 24 hours. Colonies were enumerated on plates yielding between 30 and 300 colonies. Inoculations were performed in duplicate to minimize error, and the average count was recorded. The number of colonies formed was calculated by multiplying the number of colonies by the dilution factor and the inverse of the volume plated. Colonies were subcultured to obtain pure cultures, which were stored for further tests.

Isolation of *Enterobacter* species:

MacConkey agar was used for the selective isolation and differentiation of Gram-negative bacteria, particularly members of the Enterobacteriaceae family. It contains bile salts and crystal violet, which inhibit the growth of Gram-positive bacteria while allowing the growth of Gram-negative bacteria. The agar also contains lactose as a fermentable carbohydrate and the pH indicator neutral red. Using a sterile 1 mL pipette (or syringe), 0.1 mL of each dilution was inoculated onto MacConkey agar plates using the spread plate technique. The inoculum was spread evenly using a sterile glass rod. The Petri dishes were incubated in an inverted position at 37°C for 24 hours. Colonies were enumerated on plates yielding between 30 and 300 colonies. Inoculations were performed in duplicate to minimize error, and the average count was recorded. The number of colonies formed was calculated by multiplying the number of colonies by the dilution factor and the inverse of the volume plated. Colonies were subcultured to obtain pure cultures, which were stored for further tests.

Isolation of Total Heterotrophic Fungi:

Potato Dextrose Agar (Hi-Media, India) containing chloramphenicol (50 mg/L) was used for the isolation of fungi. The medium was prepared according to the manufacturer's instructions. Inoculation was performed using the spread plate method, and the inoculated plates were incubated at 28°C for 5 days. Emerging colonies were counted, calculated, and expressed as colony-forming units per gram (cfu/g). Further identification was performed through microscopy

using the lactophenol cotton blue technique. A 100 μ L aliquot of lactophenol cotton blue was placed on a pre-cleaned glass slide. A wire loop was used to collect a colony and tease it into the lactophenol cotton blue drop. A coverslip was placed on the preparation and examined under a $\times 40$ objective lens to observe the fungal structures (Cheesbrough, 2006).

Enumeration of Total and Fecal Coliforms:

The multiple tube fermentation technique, also known as the Most Probable Number (MPN) method, was used to estimate total and fecal coliform counts. Three sets of test tubes containing lactose broth and appropriate sample volumes were used. Tubes exhibiting acid and gas production were considered positive for the presence of the target organisms. The number of organisms present was determined statistically using the MPN table. This technique consists of three major steps: the presumptive, confirmed, and completed tests. The presence of fecal coliforms was further characterized by streaking positive tubes from the presumptive test onto Eosin Methylene Blue Agar (EMBA) plates. All distinct colony types were transferred from EMBA to Tryptic Soy Agar (TSA) plates. Colonies from TSA plates were Gram-stained, and biochemical tests were performed for identification.

The Presumptive Test: Total and fecal coliforms were enumerated using multiple tube fermentation tests. Coliform counts were obtained using the three-tube MPN technique. The presumptive coliform test was performed using lactose broth. The first set of three tubes contained 10 mL of double-strength lactose broth (DSLb), while the second and third sets contained 10 mL of single-strength lactose broth (SSLb). All tubes contained Durham tubes before sterilization. The three sets of tubes received 10 mL, 1 mL, and 0.1 mL of water sample, respectively, using sterile pipettes. The tubes were incubated at 37°C for 24–48 hours for the estimation of total coliforms and at 44.5°C for 24–48 hours for fecal coliforms. The tubes were examined for acid and gas production. Acid production was indicated by a color change of the broth from red to yellow, and gas production was determined by the entrapment of gas in the Durham tube. The MPN was then determined from the MPN table for the three sets of tubes.

The Confirmed Test: The confirmed test was performed by transferring a loopful of culture

from a positive tube from the presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth containing a Durham tube. The tubes were incubated at 37°C for 24–48 hours for total coliforms and at 44.5°C for 24–48 hours for fecal coliforms and observed for gas production.

The Completed Test: The completed test was performed by streaking a loopful of broth from a positive tube onto Eosin Methylene Blue (EMB) agar plates for pure colonies. The plates were incubated at 37°C for 24–48 hours. Colonies developing on EMB agar were further identified as fecal coliforms (*Escherichia coli*). Colonies exhibiting a green metallic sheen were confirmed to be fecal coliform bacteria with a rod shape.

Colonial Characterization and Identification of Isolates:

Isolates were identified based on their morphological and cultural characteristics on growth media and their reactions to biochemical reagents. Identification materials, reagents, and protocols described by Cheesbrough (2000) were used to identify discrete colonies from the bacteriological media of subcultured isolates. Morphological characterization of bacterial isolates was based on cell morphology, including shape, size, opacity, color, edge, elevation, Gram stain, and biochemical tests. The biochemical identification included tests for citrate utilization, catalase activity, motility, etc.

2.3 Physico-chemical Analysis

Measurement of Water pH: Water acidity (pH) was measured using a pH probe (Lanalyzer, model 407A, Orion Research, USA) calibrated with buffers at pH 4 and 7. pH, representing the concentration of H^+ ions, is measured on a logarithmic scale ranging from pH 1 (very acidic) through pH 7 (neutral) to pH 14 (very alkaline). The expected pH range for the water samples was between 5.5 and 8.5.

Determination of Conductivity: An electronic conductivity meter was used to measure the conductivity of the water. The probe was pre-calibrated with a potassium chloride (KCl) solution for 15 minutes, as per the manufacturer's instructions. Ten milliliters of the sample were brought to a temperature of 25°C by immersion in a water bath and the bottle was stoppered. The electrode was immersed in the sample, the bridge was balanced, and the resistance was read from the LCD of the equipment. The measurement was repeated for all eight sample locations.

Determination of Total Dissolved Solids (TDS) by Gravimetric Method: A portion of the water sample was filtered, and 10 mL of the filtrate were measured into a pre-weighed evaporating dish. Following the procedure for the determination of total solids, the total dissolved solids content of the water was calculated as follows:

$$\text{Total dissolved solids (mg/L)} = (W_2 - W_1) \text{ mg} \times \frac{1000 \text{ mL of filtrate used}}{10 \text{ mL of filtrate used}}$$

Where:

- W_1 = initial weight of evaporating dish
- W_2 = final weight of the dish (evaporating dish + residue)

Determination of Alkalinity: Fifty milliliters of the sample were pipetted into a clean 250 mL conical flask. Two drops of methyl red indicator were added, and the solution was titrated against a standard 0.01 M HCl solution to a pink endpoint.

$$\text{Total alkalinity (mg/L)} = [V \times M \times 100,000] / \text{mL of sample used}$$

Where:

- V = volume of acid used
- M = molarity of acid used

Determination of Turbidity: Turbidity was determined using a standardized Hanna HI98703 Turbidimeter. Samples were poured into the measuring bottle, and the surface of the bottle was wiped with silicone oil. The bottle was then inserted into the turbidimeter, and the reading was recorded.

Chloride Determination: Chloride content was determined in mg/L by the argentometric titrimetric method following APHA-AWWA-WPCF (1980). Chloride was precipitated as silver chloride, and potassium chromate indicator was used to mark the endpoint of the titration by a color change from yellow to pinkish yellow.

Reagents:

- Potassium chromate indicator solution: 5 g of potassium chromate was dissolved in a small amount of distilled water, and silver nitrate solution was added until the formation of a red precipitate. The solution

was allowed to stand for 12 hours and then filtered. The volume was adjusted to 100 mL with distilled water.

- Standard silver nitrate titrant (0.0141 N): 2.395 g of AgNO_3 was dissolved in distilled water and diluted to 1 liter. The solution was standardized against a standard sodium chloride solution.
- Standard sodium chloride solution (0.0141 N): 824.0 mg of NaCl (dried at 140°C) was dissolved in chloride-free water and diluted to 1 liter (1 mL of standard solution = 500 $\mu\text{g Cl}^-$).

Procedure: 100 mL of the wastewater sample, or a sample diluted to 100 mL, was titrated against the silver nitrate solution in the presence of potassium chromate indicator. The endpoint of the titration was indicated by the appearance of a pinkish-yellow color of silver chromate. A blank was also titrated simultaneously.

$$\text{Chloride (mg/L)} = (A - B) \times N \times 35,450 / \text{mL of sample}$$

Where:

- A = mL of titrant used for the sample
- B = mL of titrant used for the blank
- N = normality of titrant

Nitrate Determination: Nitrate was determined by the phenol disulfonic acid method. Alkali nitrate-N reacts with 2,4-phenol disulfonic acid to form a yellow color. The percent transmission of the yellow color was measured spectrophotometrically at 410 nm.

Reagents:

- Phenol disulfonic acid: 25 g of crystalline white phenol was dissolved in 150 mL of concentrated H_2SO_4 and heated in a water bath for 2 hours.
- Ammonium hydroxide
- Stock nitrate solution: 721.8 mg of anhydrous KNO_3 was dissolved in distilled water and diluted to 1 liter (1 mL = 100 μg).
- Standard nitrate solution: 20 mL of the stock nitrate solution was diluted to 100 mL with distilled water (1 mL = 20 $\mu\text{g NO}_3^-$).

Procedure: 50 mL of the wastewater sample was evaporated on a hot plate until a

residue formed, which was then dissolved in 3 mL of phenol disulfonic acid. The reaction was allowed to proceed for 10 minutes, and then 15 mL of distilled water was added. Subsequently, 7 mL of ammonium hydroxide solution was added, and the final volume was adjusted to 50 mL. The intensity of the yellow color as percent transmission was measured at 410 nm. The nitrate concentration in mg/L was determined using a calibration curve.

Nitrate-N (mg/L) = $\mu\text{g Nitrate} / \text{mL of sample}$

Sulfate Determination: Sulfate was determined in the wastewater using the turbidimetric method. Sulfate ions are precipitated in an HCl acid medium with barium chloride to form barium sulfate crystals of uniform size.

Reagents and Apparatus:

- **Conditioning reagent:** 50 mL of glycerol was mixed with a solution containing 30 mL of concentrated HCl, 300 mL of distilled water, 100 mL of 95% ethyl alcohol, and 75 g of NaCl.
- **Barium chloride (BaCl_2):** Crystals of 20–30 mesh were used.
- **Stock sulfate solution:** 147.9 mg of anhydrous sodium sulfate (Na_2SO_4) was dissolved in distilled water and diluted to 1000 mL (1 mL = 100 $\mu\text{g SO}_4^{2-}$).
- **Standard sulfate solution:** 1 mL of the stock solution was diluted to 100 mL with distilled water (1 mL = 1 $\mu\text{g SO}_4^{2-}$).
- **Measuring spoon:** Capacity of 0.2 to 0.3 mL.
- Magnetic stirrer with stirring bars.
- Stopwatch.

Procedure: 5 mL of the wastewater sample, diluted to 100 mL with distilled water, was taken in an Erlenmeyer flask. 5 mL of conditioning reagent was added and mixed using a stirring apparatus. While stirring, a spoonful of BaCl_2 crystals (approximately 0.5 g) was added and stirred for exactly 1 minute at a constant speed. Immediately after stirring, the solution was poured into a spectrophotometer, and the turbidity was measured at 30-second intervals for four minutes. The maximum turbidity reading obtained within the four-minute interval was

recorded. The sulfate concentration was calculated in mg/L using a calibration curve.

Sulfate (mg/L) = $\text{mg SO}_4^{2-} \times 1000 / \text{mL of sample}$

Fluoride Determination

Reagents:

- Acid Zirconyl-SPADNS Reagent
- Stock Fluoride (1 mL = 1 mg F^-)
- Standard Fluoride (1 mL = 0.01 mg F^-)
- Control Stock (1 mL = 0.005 mg and 1 mg)

Procedure: A 25 mL portion of the filtered sample, diluted to 25 mL, was taken, and 5 mL of SPADNS reagent was added. The optical density (OD) of the sample was measured and recorded as OD_{sample}. A control standard was prepared by taking 5 mL of the control stock solution and diluting it to 25 mL, followed by the addition of 5 mL of SPADNS reagent. The optical density of the control standard was also measured and recorded as OD_{control std}.

Calculation:

Fluoride (mg/L) = $\text{OD}_{\text{sample}} \times \text{Correction factor} \times \text{Dilution factor}$

Where: Correction Factor = $1 / \text{OD}_{\text{control std}}$

3. RESULTS

Table 1 shows the counts of Total Coliform Bacteria Count (TCBC), Total Heterotrophic Bacteria (THB), and Total Heterotrophic Fungi (THF) in water samples from boreholes in Omoku and Bonny. The table compares the microbial load between the two locations.

KEY: TCBC: Total Coliform Bacteria Count.
THB: Total Heterotrophic Bacteria Count.
THF: Total Heterotrophic Fungal Count.

Table 2 shows the results of the MPN test for total coliforms in the Omoku borehole samples. It lists the number of positive tubes (showing acid and gas production) for each sample at different dilutions (10 mL, 1 mL, and 0.1 mL) and provides the calculated MPN index per 100 mL along with the 95% confidence limits.

Table 1. Microbial counts from sampling sites

S/N	Sample Code	Composite Water sample from Omoku Boreholes			Composite Water Samples from Bonny		
		TCBC(CFU/ml)	THB(CFU/ml)	THF(CFU/ml)	TCBC	THB	THF
1	B1	3.8×10^4	8.3×10^5	1.3×10^4	Nil	Nil	Nil
2	B2	Nil	9.4×10^5	2.0×10^4	Nil	Nil	Nil
3	B3	8.5×10^3	Nil	1.5×10^3	Nil	Nil	Nil
4	B4	Nil	3.2×10^5	2.0×10^3	Nil	Nil	Nil

Table 2. Most Probable Number (MPN) of Total Coliform Based On The Presumptive Test Tubes

Sample ID	3 of 10mls (DS) Each	3 of 1ml (SS) Each	3 of 0.1ml (SS) Each	MPN INDEXPER 100ml	95% Confidence Limits	
					Lower	Upper
B1	3	3	3	-	-	-
B 2	3	0	0	8	24	3
B 3	3	2	0	14	35	6
B 4	3	1	1	14	35	6

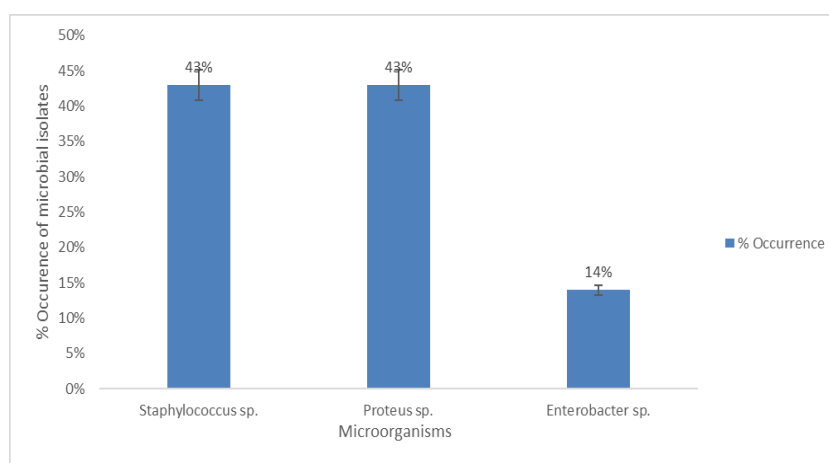


Fig. 1. Percentage occurrence of bacterial isolates from Omoku Boreholes

Table 3. Antimicrobial susceptibility features of the gram positive bacterial isolates

ISOLATE CODE	AZ	LEV	E	PEF	CN	APX	Z	AM	R	CPX
B1	S	S	I	S	S	R	R	R	R	S
B2	S	S	S	S	S	R	S	R	S	S
B4	I	S	I	S	I	R	S	I	S	S

Keys; S= Susceptible; I= Intermediate; R= Resistance

Table 4. Antimicrobial susceptibility features of the gram negative bacterial isolates

ISOLATE CODE	SXT	CH	SP	CPX	AM	AV	CN	PEF	OfX	X
B1	S	S	S	S	I	S	S	S	S	S
B2	S	R	S	S	R	R	I	S	S	S
B3	S	R	S	S	I	S	S	S	S	I
B4	S	I	S	S	I	I	S	S	S	S

Keys; S= Susceptible; I= Intermediate; R= Resistance

Table 5. Morphological characteristics of fungal Isolates

Isolate code	Macroscopy	Microscopy	Possible Genera
F1	White fluffy mycelia,fast growing, white reverse, smooth mycelia	Presence of branched thin wall long hyphae.	<i>Fusarium</i> sp.
F2	White cracked reverse, green folded dry mycelia, white margin,circular and entire.	Long hyaline hyphae with brushlike conidiospores	<i>Pennicillium</i> sp.
F3	Cream, raised, entire, circular, Smooth and dull, 9mm	Oval purple	<i>Candida</i> sp
F4	Cream, raised, entire, circular, Smooth and dull, 3mm	Oval purple	<i>Candida</i> sp
F5	White fluffy mycelia,fast growing, white reverse, smooth mycelia	Presence of branched thin wall long hyphae.	<i>Fusarium</i> sp.
F6	White fluffy mycelia,fast growing, white reverse, smooth mycelia	Presence of branched thin wall long hyphae.	<i>Fusarium</i> sp.
F7	Cream, raised, entire, circular, Smooth and dull, 7mm	Oval purple	<i>Candida</i> sp
F8	Greenish rough fast growing colony, presence of mycelia	Presence of septate branched hyphae	<i>Trichoderma</i> sp

Table 6. Physiochemical parameters of the water samples

Parameter	Bonny Borehole	Omoku Borehole	WHO Guideline	Suitability
pH	6.79	6.86	6.5-8.5	Acceptable
TDS (mg/L)	1.67	1.83	<500	Acceptable
Turbidity (NTU)	1.22	0.53	<1	Acceptable
Total Hardness (mg/L)	158.85	93.87	<300	Acceptable
E.C (us/cm)	427.12	273.95	-	Acceptable (based on TDS)
Nitrate (mg/L)	15.74	8.63	<50	Acceptable
Alkalinity (mg/L)	120.31	94.52	<200	Acceptable
Chloride (mg/L)	153.82	57.13	<250	Acceptable
Sulphate (mg/L)	86.41	41.68	<400	Acceptable
Fluoride (mg/L)	0.151	0.042	0.5-1.5	Slightly low
Salinity (mg/L)	10.77	4.76	<1000	Acceptable

Table 7. Heavy metal parameters of the water samples

Parameter	WHO Guideline	Bonny Borehole	Omoku Borehole	Comparison
Sodium (Na)	No strict guideline, but generally below 200 mg/L is recommended for those with sodium-sensitive conditions.	0.17482 mg/L	0.08328 mg/L	Both well below WHO guideline.
Potassium (K)	No strict guideline, but generally considered safe within normal dietary intake.	0.31126 mg/L	0.17309 mg/L	Both well below typical dietary intake.
Calcium (Ca)	20-100 mg/L	13.04752 mg/L	9.18927 mg/L	Both within the recommended range.
Magnesium (Mg)	20-30 mg/L	6.15693 mg/L	2.85101 mg/L	Both within the recommended range.
Iron (Fe)	0.3 mg/L	2.15186 mg/L	0.72152 mg/L	Both exceed the WHO guideline.
Manganese (Mn)	0.04 mg/L	0.16219 mg/L	0.42184 mg/L	Both exceed the WHO guideline.
Copper (Cu)	1-2 mg/L	5.23158 mg/L	7.92073 mg/L	Both exceed the WHO guideline.
Zinc (Zn)	3-5 mg/L	3.94857 mg/L	2.43108 mg/L	Both within the recommended range.

List 1. Table indicating the biochemical characteristics of microbial isolates from Omoku Borehole

Biochemical Characteristics Of Microbial Isolates From Omoku Borehole																			
Isolate Code	Color	Elevation	Surface	Form	Edge	Opacity	Size (mm)	Catalase	Citrate	Glucose	Lactose	Indole	MR	VP	Motility	TSIA	H ₂ S	Gram Stain	Possible Genera
B1	Cream	S. Raised	Smooth & Shiny	Round	Entire	Opaque	4	+	+	+	-	-	+	-	+	AB-	-	+ve Cocci	<i>Staphylococcus</i> sp.
B2	Cream	Flat	Smooth & Shiny	Round	Entire	Translucent	4	+	+	+	+	-	-	+	+	BA-	-	+ve Cocci	<i>Staphylococcus</i> sp.
B3	Cream	Flat	Smooth & Shiny	Irregular	Entire	Translucent	5	+	-	+	-	+	+	+	+	AA+	-	-ve Rod	<i>Proteus</i> sp.
B4	Cream	Flat	Smooth & Shiny	Round	Entire	Translucent	4	+	+	+	-	+	+	-	+	AB-	-	-ve Rod	<i>Proteus</i> sp.

4. DISCUSSION

Table 1. reveals varying levels of microbial contamination in the Omoku borehole samples. Total Heterotrophic Bacterial counts were detected in all Omoku samples, ranging from 3.2×10^5 CFU/mL to 9.4×10^5 CFU/mL. This indicates a substantial presence of bacteria in the borehole water, suggesting potential contamination. The highest THB count was observed in sample B2 (9.4×10^5 CFU/mL). Total Coliform Bacterial Counts were detected in two out of the four samples from Omoku. Sample B1 had a TCBC of 3.8×10^4 CFU/mL and B3 had a TCBC of 8.5×10^3 CFU/mL. The presence of coliform bacteria is a strong indicator of fecal contamination (Holcomb & Stewart, 2020) and suggests a potential health risk associated with consuming this water without treatment (Holcomb & Stewart, 2020, Bai et al., 2022). The varying levels suggest different sources or degrees of contamination at the different boreholes.

Total Heterotrophic Fungal counts were also observed in all Omoku samples, ranging from 1.5×10^3 CFU/mL to 2.0×10^4 CFU/mL. While not necessarily indicative of fecal contamination, the presence of fungi could still pose health concerns, depending on the species present as supported by the studies of Mirshekar et al. (2019). The highest THF count was found in B2 (2.0×10^4 CFU/mL). Interestingly, a striking observation from Table 1 is that no TCBC, THB, or THF were detected in any of the water samples from the Bonny water sources which corresponds to the finding of Ezekiel-Hart et al. (2021) where they reported the effectiveness of water treatment protocol in Bonny. This stark contrast to the Omoku samples suggests a significantly better microbial quality of water in Bonny's water supply. This could be due to effective water treatment processes (Ezekiel-Hart, et al. 2021), a more protected source, or a combination of factors.

For Sample B1, all three tubes were positive at all dilutions (3/3 for 10mL, 1mL, and 0.1mL). This indicates a high concentration of coliform bacteria in this sample, Samples B2, B3, and B4 shows a varying numbers of positive tubes where B2 Three positive tubes at 10 mL, zero at 1 mL, and zero at 0.1 mL, resulting in an MPN index of 8 per 100 mL. B3 and B4: Three positive tubes at 10 mL, two positive tubes at 1 mL, and zero positive tubes at 0.1 mL for B3, and three positive tubes at 10 mL, one at 1mL, and one at 0.1mL for B4. Both B3 and B4 have an MPN

index of 14 per 100 mL. Sample B1 appears to be the most contaminated, followed by B3 and B4 (which have the same MPN), and then B2.

Fig. 1. clearly shows that *Staphylococcus* sp. and *Proteus* sp. are the dominant genera, each accounting for 43% of the identified isolates. This co-dominance suggests that these two genera are prevalent in the sampled boreholes. *Enterobacter* sp. represents only 14% of the isolates, indicating a significantly lower occurrence compared to *Staphylococcus* and *Proteus*. However, *Proteus* species are often associated with decaying organic matter and can be opportunistic pathogens, particularly in individuals with compromised immune systems. Their presence may indicate fecal contamination or poor sanitation practices (Drzewiecka, 2016).

Proteus can also cause urinary tract infections and other health issues (Jamil, et al., 2023).

Enterobacter species are also opportunistic pathogens and can be found in various environments, including water and soil. Some species can cause respiratory or urinary tract infections (Salimiyan, et al., 2019). Tables 3 and 4 shows the Antimicrobial Susceptibility of bacterial isolates from Omoku to a range of antibiotics. Table 3 reporting gram positives antibiotics reveals that the *Staphylococcus* isolates (B1, B2, B4) exhibit varying susceptibility patterns. Notably, all isolates are resistant (R) to Ampicillin (AM) and Cephalexin (CPX). This is a significant finding, indicating potential antibiotic resistance in these bacteria. B1 and B2 are susceptible (S) to Azithromycin (AZ), Levofloxacin (LEV), Erythromycin (E), and Ciprofloxacin (CN), while B4 shows intermediate (I) resistance to some of these. This suggests that these antibiotics *might* be effective, but further testing is needed, especially for B4. The resistance to AM and CPX is a serious concern. Table 4 (Gram-Negative): shows the susceptibility of the Gram-negative isolates (B1, B2, B3, B4). Again, resistance is observed. Isolates B2 and B4 show resistance (R) to Chloramphenicol (CH) and Ampicillin (AM) respectively. B4 also shows intermediate (I) resistance to Ciprofloxacin (CN). B1, B3, and B4 are susceptible to most of the other antibiotics tested. The resistance to commonly used antibiotics is a concern.

The observed resistance to multiple antibiotics, especially Ampicillin and Cephalexin, is a serious public health concern. It suggests that infections

caused by these bacteria might be difficult to treat with these common antibiotics (Koch, et al., 2021, Bharadwaj, et al., 2022).

Table 5 lists the morphological characteristics of the fungal isolates and their possible genera.

The table suggests the presence of *Fusarium* sp., *Penicillium* sp., *Candida* sp., and *Trichoderma* sp. *Candida* was the most frequently isolated.

Tables 6 and 7 presents the results of the physico-chemical and heavy metal analyses of the water samples, comparing them to WHO guidelines.

Most of the parameters are within WHO guideline limits, which is good. However, *turbidity* in the Bonny borehole is slightly above the guideline. *Fluoride* levels in both Bonny and Omoku are *slightly low* compared to the recommended range.

The heavy metal analysis reveals that *Iron (Fe)* and *Manganese (Mn)* and *Copper (Cu)* levels in both Bonny and Omoku boreholes *exceed* the WHO guidelines. This is a significant finding and indicates potential heavy metal contamination. Long-term exposure to these metals can have adverse health effects (Jaishankar, et al., 2014, Ugwu, et al., 2024). The other metals tested (Sodium, Potassium, Calcium, and Zinc) are within acceptable limits. These findings support the need for water treatment before consumption. The presence of antibiotic-resistant bacteria poses a serious health risk. The elevated heavy metal concentrations also raise concerns about long-term health effects.

5. CONCLUSION

The comprehensive analysis of microbial quality and physicochemical parameters of borehole water in Omoku and Bonny, Port Harcourt, has brought into perspective the environmental safety of these water sources. It has shown that the water supply is not suitable for human consumption because of the presence of heavy metals and unacceptable range of total coliforms. The Most Probable Number (MPN) method revealed elevated total coliform counts in the samples from Omoku, emphasizing the importance of continued monitoring and management strategies to ensure water quality.

6. RECOMMENDATIONS

The borehole water in both locations, especially Omoku, requires treatment before consumption

to remove or inactivate pathogens and reduce heavy metal concentrations and further research is needed to identify the sources of contamination, determine the specific species of bacteria and fungi present, investigate the mechanisms of antibiotic resistance, and assess the potential health risks associated with consuming this water. Thus, community leaders and regulatory bodies should ensure regular monitoring of the water quality which has become essential in ensuring the continued safety of the water supply to the community.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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