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Physicochemical and Bacteriological Characteristics of Groundwater in Rumuigbo, Obio-Akpor Local Government Area of Rivers State, Nigeria

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Abstract
Water is one of the most important and abundant compounds on earth, and it is vital to the survival of all life forms. Groundwater is the most utilized in the southern part of Nigeria. Many groundwater extraction boreholes exist in Rumuigbo, Obio-Akpor Local Government Area of Rivers State, Nigeria. This study was carried out to assess the physicochemical and bacteriological characteristics of groundwater in the communities within Rumuigbo. Water samples were collected from groundwater extraction boreholes in the four communities within Rumuigbo: Omoi/Orosi, Nkpolu, Mgbuadu, and Mgbu-Esilaru. The samples were analyzed for Electrical conductivity (EC), salinity, pH, turbidity, nitrate, phosphate, iron, lead, zinc, total heterotrophic bacterial population (THBP), Vibrio, Shigella, and Salmonella populations using standard physicochemical and bacteriological methods. The results showed that EC of the groundwater in the four communities was 3.74±0.38 to 17.08±1.03 µS/cm; salinity was 1.02±0.01 to 1.66±0.54 mg/L; pH was 5.94±0.01 to 6.95±0.23; turbidity was 0.04±0.01 to 0.05±0.02 NTU; nitrate was 0.46±0.06 to 0.71±0.02 mg/L; phosphate was 2.85±0.03 to 3.34±0.15 mg/L; iron was 2.34±0.55 to 6.74±0.08 mg/L; lead was 0.18±0.04 to 0.40±0.14 mg/L; and zinc was 0.40±0.06 to 3.91±0.13 mg/L; THBP was 1.8±2.6 to 3.4±3.2 ×10^2 CFU/ml. There were no Vibrio and Shigella in the groundwater samples, except in one sampling point in the Mgbu-Esilaru and Mgbu-adu communities, respectively. The bacteria identified include Proteus, Bacillus, Micrococcus, Pseudomonas, Salmonella, Erythrobacter, and Klebsiella species. It is revealed that groundwater from the communities fulfills some of the requirements for portable drinking water but does not meet the standards for iron, lead, zinc, and Salmonella. Some form of groundwater treatment from the communities is required to meet all the requirements for potable drinking water.
Keywords: Groundwater, physicochemical characteristics, Coliforms, Salmonella, Total heterotrophic bacteria

INTRODUCTION

In southern Nigeria, groundwater is an easily accessed drinking water source for many people. It is assumed that groundwater is cleaner than surface water because purification is thought to occur as water flows down the soil profile (Egbueri, 2020). Also, clear and odorless water gives the impression that it is safe for consumption. This may not always be true as many bacteria and objectionable matter that may have been added naturally or, due to certain activities, can be invisible. Therefore, it is essential to know the suitability of water from a groundwater source for consumption and domestic activities.

Water quality is judged by standards established by regulatory bodies such as the World Health Organization (Adimalla, 2019). Drinking water that does not meet specified standards may lead to diseases. Chemically, assessing groundwater's suitability requires determining parameters such as pH, electrical conductivity, total dissolved solids, turbidity, nitrate, phosphate, iron, lead, and zinc. These parameters have value limits at or below which water can be adjudged safe for drinking and domestic use (Ayotte *et al.*, 2011).

Contamination of groundwater reduces the water quality for drinking purposes and can lead to health issues of public concern (Al-Ruwaih & Ben-Essa, 2004). Groundwater contamination can occur due to percolation of leachate from landfills, dumpsites, septic tanks, and treatment stations. It can also arise from flooding and sitting of boreholes close to soakaway pits or septic tanks (Adimalla *et al.*, 2018). Also, bacteria, soluble inorganic substances, and soluble organics can leach into underlying groundwater due to improper management of human, agricultural, industrial, and domestic waste before discharge into the environment.

Industrial effluents usually contain atmospheric deposits, inorganic substances, hydrocarbons, and microorganisms (Douterelo *et al.*, 2014). Infiltrating these constituents into the food chain through soil or water will be potentially hazardous to plants, animals, and humans. For the assessment of the pollution status of groundwater, the following water quality parameters are important: pH, conductivity, temperature, total dissolved solids, alkalinity, hardness, cations & anions, carbonates & bicarbonates, and sulfates (Ram *et al.*, 2021).

Various pathogens can contaminate groundwater. It is difficult to test for the different pathogens owing to the great diversity and low abundance of the different species. Therefore, regulatory bodies have established the use of indicator pathogens (Edokpayi *et al.*, 2018). Indicator pathogens used to evaluate microbial contamination of water include total and fecal coliforms, and *Salmonella*. Of the fecal coliforms, *Escherichia coli* have been suggested to be more appropriate for indication of fecal contamination of surface- or groundwater (Briancesco, 2005). The presence of indicator pathogens in water from groundwater extraction boreholes is an indication that public consumption of water from such boreholes will lead to the emergence of water-borne diseases. Li *et al.* (2022) stated that 80 % of diseases and 50 % of child deaths are suggested by the World Health Organization (WHO) to be related to poor drinking water quality. Treacy (2019) has suggested that the priority of water distribution agencies in developing countries should be to provide water from a source that requires little or no treatment. Therefore, it is of prime importance to have information on the quality of groundwater resources available.

Rumuigbo is a clan in Obio-Akpor Local Government Area of Rivers State in Nigeria. The primary source of water for drinking available to its inhabitants is water from groundwater extraction boreholes. Due to the level of anthropogenic activities and flooding in Rumuigbo, there is the danger of an increase in the amount of chemical pollutants and bacteria which can percolate into the underlying aquifers. Therefore, this study was carried out to evaluate the physicochemical and bacteriological qualities of groundwater in the communities within Rumuigbo.

MATERIALS AND METHODS

Study Area

The study area was the four communities (Omoi/Orosi, Nkpolu, Mgbuadu, and Mgbu-Esilaru) in Rumuigbo in Obio-Akpor Local Government Area of Rivers State, Nigeria. The locations of the sampling points within the communities as geo-referenced with the aid of a calibrated compass app on an Android phone are presented in Table 1.

Table 1: Geo-coordinates of the sampling points

SC	SPC and coordinates
Omoi/Orosi	O1 (4°50'35" N, 6°59'48" E); O2 (4°50'34" N, 6°59'51" E); O3 (4°50'30" N, 6°59'52" E)
Nkpolu	N1 (4°51'52" N, 6°58'25" E); N2 (4°51'52" N, 6°58'33" E); N3 (4°51'34" N, 6°58'44" E)
Mgbu-adu	M1 (4°50'48" N, 6°59'28" E); M2 (4°50'52" N, 6°59'25" E); M3 (4°51'25" N, 6°59'20" E)
Mgbu-Esilaru	E1 (4°51'18" N, 6°58'60" E); E2 (4°51'16" N, 6°58'53" E); E3 (4°51'52" N, 6°58'58" E)

SC: Study communities, SPC: Sample point code

Sample Collection

Groundwater samples were collected in September 2023, during the wet season in Rivers State, Nigeria. Three water samples were collected from each sampling point, totaling 36 samples. Samples were collected with the aid of appropriately labeled disinfected water bottles. Collected samples were transported to the Department of Microbiology, Rivers State University laboratory, where bacteriological and physicochemical analyses were carried out on them.

Physicochemical Analysis of the Samples

The groundwater samples were analyzed for electrical conductivity (EC), salinity, pH, turbidity, nitrate, phosphate, iron, lead, and zinc. EC, salinity, turbidity, and pH were determined using their respective electronic hand-held meters; nitrate using a modification of the Indophenol blue method (Tzolla *et al.*, 2010); phosphate using the ascorbic acid method (APHA, 1992); iron, lead, and zinc using atomic absorption spectroscopy (Nalatambi, 2009).

Bacteriological Analysis of the Samples

The samples were analyzed for the presence and population of coliform bacteria, total heterotrophic bacteria (THB), *Vibrio* species, *Salmonella* species, and *Shigella* species. The presence and populations of *Salmonella* and *Shigella* species were determined using

Salmonella-Shigella agar (SSA), *Vibrio* species using thiosulfate citrate bile-salts sucrose agar (TCBSA), and THB using nutrient agar (NA). The agar media were prepared according to the manufacturers' specifications. Aliquot of 1 ml of the samples were transferred into 9 ml sterile normal saline to obtain 10^{-1} dilution, then 0.1 ml of the undiluted samples were spread onto the plates of NA, SSA, and TCBSA; 0.1 ml of the 10^{-1} diluted samples were spread inoculated only on NA. Inoculated plates were incubated at 37°C for 24 hours, while inoculated SSA and TCBSA plates were incubated at 37°C for 48 hours. After incubation, colonies on NA plates were counted and used to calculate the THB population; colorless colonies and colonies with black centers on SSA were counted and used to calculate *Shigella* and *Salmonella* populations, respectively; yellow colonies on TCBSA were counted and used to calculate the *Vibrio* population.

The presence and population of coliforms were determined using the 5-tube variant of the multiple-tube fermentation technique. MacConkey broth was used as the fermentation medium. For each sample, double-strength and single-strength MacConkey broth were prepared. Exactly 5 ml of the double-strength broth was dispensed into 5 test tubes labeled DS; 9 ml of the single-strength broth into 5 test tubes labeled SS1; and 9.9 ml of the single-strength broth into 5 test tubes labeled SS0.1. Durham tubes were placed in all the test tubes, and the tubes were sterilized in an autoclave at 15 PSI, 121 °C, for 15 minutes. After sterilization and cooling, the 5 tubes labeled DS were inoculated with replicate sample volumes of 10 ml, the 5 SS1 tubes with replicate sample volumes of 1 ml, and the 5 SS0.1 tubes with replicate sample volumes of 0.1 ml. All the inoculated tubes were incubated at 37 °C for 48 hours. After incubation, the tubes were observed for color change and gas production. The numbers of positive tubes were appropriately recorded, and the records were used to obtain an estimate of the coliform population with the aid of the 5-Tube MPN table (APHA, 1998).

Isolation and Identification of Bacteria

Bacterial colonies were sub-cultured onto fresh sterile NA plates and coded. Stock cultures on NA slants were prepared from the sub-cultured pure isolates and stored at 4 °C in a refrigerator. Sub-cultured isolates were subjected to Gram staining and microscopic examination and the following biochemical/physicochemical tests: catalase, oxidase, citrate utilization, indole production, Methyl Red, Vogues Proskauer, motility, salt (7 % NaCl) tolerance, starch hydrolysis, and fermentation tests using glucose, lactose, mannitol, and xylose. The tests were carried out as described in Peekate (2022). Stock cultures of identified isolates were also sent for identification through molecular techniques.

Identification of Bacterial Isolates using molecular techniques

DNA Extraction: DNA extraction was done using the ZR bacterial DNA miniprep kit (Zymo Research Co.). An Aliquot of 2 ml of bacterial broth culture was placed in a sterile centrifuge tube, and the lysis solution (750 µL) was added. After about 5 minutes, the tube was centrifuged at 10,000× g for 1 minute; g here is the acceleration due to gravity (9.81 m/s^2). After centrifugation, 400 µL of the supernatant was collected and transferred into the Zymo-SpinTM IV Spin Filtertube, and the Filter tube was centrifuge at 7,000 × g for 1 minute. After centrifugation, Bacterial DNA Binding Buffer (1,200 µL) was added to the filtrate, and 800 µL of the resulting mixture was transferred into a Zymo-SpinTM IIC Column in a collection tube and the tube centrifuge at 10,000× g for 1 minute. The flow-through liquid from the tube was placed back into the Zymo-SpinTM IIC Column in the Collection tube, and the tube was centrifuge again at 10,000× g for 1 minute. The flow-through liquid was then discarded, and 200 µL DNA Pre-Wash buffer was added to the Zymo-Spin TM IIC Column in a new Collection tube, and the tube was centrifuge at 10,000× g for 1 minute. About 500 µL Fungal/Bacterial DNA Wash Buffer was added to the Zymo-SpinTM IIC Column in the collection tube, and the tube was centrifuge at 10,000× g for 1 minute. The Zymo-SpinTM IIC Column was then transferred to a clean 1.5 ml micro-centrifuge tube, and 100 µL DNA Elution Buffer was added to the column matrix. The column in the micro-centrifuge tube was then centrifuged at 10,000× g for 30 seconds to elute the DNA.

Amplification of 16SrRNA Genes in Extracted DNA: The 16S rRNA genes of the extracted DNAs were amplified by the Polymerase Chain Reaction (PCR) in an ABI 9700 Applied Biosystems thermal cycler. The PCR mix was made up of 2 µL of the extracted DNA (template), 8.5µL Nuclease water,1 µL of 10 µM forward primer (27F: AGAGTTTGATCMTGGCTCAG),1 µL of 10 µM reverse primer (1525R: AAGGAGGTGWTCCARCCGCA), and 12.5 µL of Taq 2X Master Mix from New England Biolabs (M0270). The Master mix consists of taq polymerase, dNTPs, and MgCl. The reaction mixture was placed in the PCR machine, and the PCR cycling conditions were as follows: temperature of 94 °C and duration of 5 minutes for initial DNA denaturation; temperature of 94

°C and duration of 30 seconds for DNA denaturation; temperature of 56°C and duration of 30 seconds for DNA annealing; temperature of 72 °C and duration of 45 seconds for DNA extension; and temperature of 72 °C and duration of 7 minutes for final DNA extension. The PCR process was carried out in 36 cycles. Some of the PCR products alongside a DNA molecular weight marker were subjected to electrophoresis (voltage: 120 V, duration: 15 minutes) using 1 % agarose gel impregnated with ethidium bromide. After electrophoresis, separated DNA bands were visualized under UV light.

Sequencing and Identification: Amplified 16S rRNA genes were sequenced using the BigDye Terminator v3.1 cycle sequencing kit on a 3500xl sequencer from Applied Biosystems. The obtained DNA sequence was edited using the bioinformatics algorithm Bio-Edit. The DNA sequence was then compared with DNA sequences deposited in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) on the NCBI webpage. The software MEGA X was used for genetic analysis.

Data analysis

Data obtained were analyzed for significance differences using the Analysis of Variance (ANOVA); the confidence interval was 95 % (α = 0.05). Also, a correlation between bacterial population and physicochemical data was determined. ANOVA and correlation were calculated using the statistical package SPSS version 26.

RESULTS

Physicochemical Characteristics of the Groundwater Samples

The physicochemical characteristics of groundwater from Omoi/Orosi, Nkpolu, Mgbuadu, and Mgbu-Esilaru communities in Rumuigbo are presented in Table 2. In the Table, it can be seen that Electrical conductivity (EC), pH, and iron concentration were highest in Omoi/Orosi and lowest in Mgbu-esilaru; salinity was highest in Nkpolu and lowest in Mgbuadu; turbidity was highest in Mgbu-esilaru and Nkpolu and lowest in Omoi/Orosi and Mgbuadu; nitrate concentration was highest in Nkpolu and lowest in Mgbu-esilaru and Mgbuadu; phosphate and zinc concentrations were highest in Mgbuadu and lowest in Nkpolu; and lead concentration was highest in Omoi/Orosi and lowest in Mgbuadu. Significant difference ($P<0.05$) between the EC of groundwater from the different communities; the same was observed for pH, iron concentration, and zinc concentration. There was no significant difference ($P>0.05$) between groundwater salinity from the different communities; the the same was observed for turbidity, nitrate concentration, phosphate concentration, and lead concentration.

Bacterial Population in the Groundwater

The population of total heterotrophic bacteria, *Vibrio* species, *Salmonella* species, *Shigella* species, and total coliform bacteria in groundwater samples from the study communities are presented in Table 3. From the Table, it can be seen that there were no *Vibrio* and *Shigella* in all the groundwater samples; a very low population of coliforms was found in only one sampling point in Mgbu-Esilaru community, giving an average value of 0.7 coliforms/100 ml. *Salmonella* was found in only one sampling point in Mgbu-adu community, giving an average value of 10 CFU/ml. The total heterotrophic bacteria population within the study communities ranged from 180±260 to 340±320 CFU/ml, with the highest in Omoi/Orosi and the lowest in Mgbu-Esilaru. There was no significant difference ($P\geq 0.05$) between THB groundwater populations from the different Rumuigbo communities.

Table 2: Physicochemical Characteristics of Groundwater from the Four Communities in Rumuigbo

PP	Omoi/Orosi	Nkpolu	Mgbuadu	Mgbu-Esilaru	PLPP
EC (µS/cm)	17.08 ± 1.03	5.22 ± 1.07	14.19 ± 1.03	3.74 ± 0.38	1000[a]
Salinity (mg/L)	1.024 ± 0.001	1.66 ± 0.54	1.02 ± 0.01	1.08 ± 0.07	-
.pH	6.95 ± 0.23	6.07 ± 0.05	6.53 ± 0.18	5.94 ± 0.01	6.5 – 8.5[a]
Turbidity (NTU)	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	5[a]
Nitrate (mg/L)	0.50 ± 0.08	0.71 ± 0.02	0.46 ± 0.08	0.46 ± 0.06	50[ab]
Phosphate (mg/L)	2.97 ± 0.15	2.85 ± 0.03	3.34 ± 0.15	2.97 ± 0.06	-
Iron (mg/L)	6.74 ± 0.08	3.02 ± 0.66	5.06 ± 0.75	2.34 ± 0.55	0.3[a]
Lead (mg/L)	0.40 ± 0.14	0.21 ± 0.04	0.18 ± 0.04	0.20 ± 0.02	0.01[ab]
Zinc (mg/L)	3.17 ± 0.28	0.40 ± 0.06	3.91 ± 0.13	1.15 ± 0.17	3.0[a]

PP: Physicochemical parameters, EC: Electrical conductivity; PLPP: Permissible limit of physicochemical parameter; values with superscript "a" are from Standards Organisation of Nigeria (SON, 2015), values with superscript "b" are from the World Health Organization (WHO, 2017).

Table 3: Bacterial Populations in the Groundwater

IC	THB (CFU/ml)	VP (CFU/ml)	SP (CFU/ml)	SG (CFU/ml)	CP (coliforms/100 ml)
Omoi/Orosi	$3.4\pm3.2 \times 10^2$	0	0	0	0
Nkpolu	$2.2\pm2.4 \times 10^2$	0	0	0	0
Mgbu-adu	$2.2\pm1.9 \times 10^2$	0	10±17	0	0
Mgbu-Esilaru	$1.8\pm2.6 \times 10^2$	0	0	0	0.7±1.2

IC: Investigated communities, THB: Total heterotrophic bacteria, VP: *Vibrio* population, SP: *Salmonella* population, SG: *Shigella* population, CP: Coliform population.

Identity of Bacteria isolated from the groundwater

Results of Microscopy, physicochemical, and biochemical tests on the isolated bacteria are presented in Table 4a, b, c & 4d. The output generated from keying in the result patterns into the search dialogue of Advanced Bacterial Identification Software (available at https://www.tgw1916.net/bacteria_logare.html) revealed the identity of the isolated bacteria as *Proteus* spp., *Bacillus* spp., *Micrococcus* spp., *Pseudomonas* spp., *Salmonella* sp., *Erythrobacter* sp., and *Klebsiella aerogenes*. *Salmonella* sp. was present only in groundwater from Mgbuadu community, while *Klebsiella aerogenes* was present only in groundwater from Mgbu-Esilaru community. The identity of some isolates identified through bio-molecular means is shown in Table 5. It can be seen in the Table that only isolate 1O2 was identified as *Proteus* species through physicochemical/biochemical and bio-molecular means.

Table 4a: Morphological and Physicochemical/biochemical characteristics of bacteria in groundwater from Omoi/Orosi

SPC	IC	MP	GS	CT	OX	MT	ST	IP	CU	MR	VP	SH	GF	LF	MF	XF	SB
O1	1O1	R	-	+	-	+	-	-	-	-	+	-	AG	-	-	-	*Proteus* sp.
	1O2	R	-	+	-	+	-	+	+	+	+	-	AG	-	-	A	*Proteus vulgaris*
	1O3	R	+	+	+	+	-	-	-	+	-	+	A	-	-	A	*Bacillus* sp.
	1O4	R	+	+	+	+	+	-	-	+	-	+	A	-	-	-	*Bacillus cereus*
	1O5	R	-	+	-	+	-	-	-	-	-	+	AG	-	-	A	*Proteus* sp.
	1O6	R	+	+	-	-	+	-	-	-	-	+	A	-	-	-	*Bacillus* sp.
	1O7	C	+	+	+	-	-	-	-	-	+	+	AG	A	A	A	*Micrococcus* sp.
O2	2O1	C	+	+	+	-	-	-	-	-	+	+	AG	A	A	A	*Micrococcus* sp.
	2O2	R	+	+	-	-	+	-	-	-	-	+	A	-	-	-	*Bacillus* sp.
	2O3	R	-	+	-	+	-	-	-	-	-	+	AG	-	-	A	*Proteus* sp.
O3	3O1	C	+	+	+	-	-	-	-	-	+	+	AG	A	A	A	*Micrococcus* sp.
	3O2	R	-	+	-	+	+	-	-	+	+	+	AG	-	-	-	*Proteus* sp.
	3O3	R	-	+	-	+	+	-	-	+	-	-	A	-	-	-	*Proteus* sp.
	3O4	R	+	+	+	+	-	-	-	-	-	+	A	-	A	A	*Bacillus* sp.
	3O5	R	-	+	+	-	+	-	+	-	+	-	AG	A	A	A	*Proteus* sp.
	3O6	R	-	+	+	-	+	-	+	-	-	-	A	-	A	A	*Pseudomonas* sp.

SPC: Sample point code, IC: Isolate code, MP: Morphology, R: rods, C: cocci, GS: Gram stain, CT: Catalase, OX: Oxidase, MT: Motility, ST: Salt tolerance, IP: Indole production, CU: Citrate utilization, MR: Methyl red, VP: Voges Proskauer, SH: Starch hydrolysis, GF, LF, MF, XF: Glucose, Lactose, Mannitol, and Xylose fermentation, SB: Suspected bacteria.

Table 4b: Morphological and Physicochemical/biochemical characteristics of bacteria in groundwater from Nkpolu

SPC	IC	MP	GS	CT	OX	MT	ST	IP	CU	MR	VP	SH	GF	LF	MF	XF	SB
N1	1N1	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	1N2	C	+	+	+	-	+	-	+	-	+	-	A	-	-	-	*Micrococcus* sp.
	1N3	R	-	+	+	+	+	-	+	-	-	-	A	-	-	-	*Pseudomonas* sp.
	1N4	R	-	+	-	+	-	-	+	+	+	-	AG	-	-	A	*Proteus* sp.
	1N5	R	+	+	+	+	-	-	-	+	+	+	A	-	-	-	*Bacillus* sp.
N2	2N1	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	2N2	R	-	+	-	+	-	-	+	+	+	-	AG	-	-	A	*Proteus* sp.
	2N3	R	-	+	-	+	+	-	-	+	-	-	AG	-	-	A	*Proteus* sp.
	2N4	C	+	+	+	-	+	-	+	-	+	-	A	-	-	-	*Micrococcus* sp.
N3	3N1	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	3N2	C	+	+	+	-	+	-	+	-	+	-	A	-	-	-	*Micrococcus* sp.
	3N3	R	-	+	-	+	-	-	-	-	-	-	A	-	-	-	*Pseudomonas* sp.
	3N4	R	+	+	-	-	+	-	-	+	-	+	A	-	A	-	*Bacillus* sp.

Table 4c: Morphological and Physicochemical/biochemical characteristics of bacteria in groundwater from Mgbu-adu.

SPC	IC	MP	GS	CT	OX	MT	ST	IP	CU	MR	VP	SH	GF	LF	MF	XF	SB
M1	1M1	R	+	+	-	-	+	-	-	-	-	+	A	-	-	-	*Bacillus* sp.
	1M2	R	-	+	+	+	+	-	+	-	-	-	A	-	A	A	*Pseudomonas* sp.
	1M3	R	-	+	+	+	+	-	+	-	-	-	A	-	-	-	*Pseudomonas* sp.
	1M4	C	+	+	+	-	-	-	-	+	+	+	AG	A	A	A	*Micrococcus* sp.
	1M5	R	-	+	-	+	+	-	+	+	+	-	A	-	-	-	*Proteus* sp.
	1M6	R	+	+	+	+	+	-	-	+	-	+	A	-	-	-	*Bacillus cereus*
M2	2M1	R	+	+	-	-	+	-	-	-	-	+	A	-	-	-	*Bacillus* sp.
	2M2	R	-	+	+	+	+	-	+	-	-	-	A	-	A	A	*Pseudomonas* sp.
	2M3	R	-	+	+	+	+	-	+	-	-	-	A	-	-	-	*Pseudomonas* sp.
	2M4	Ci	+	+	+	-	-	-	-	+	+	+	AG	A	A	A	*Micrococcus* sp.
	2M5	R	-	+	-	+	+	-	+	+	+	-	A	-	-	-	*Proteus* sp.
M3	3M1	R	-	+	-	+	+	-	+	+	-	-	AG	-	-	-	*Salmonella* sp.
	3M2	R	-	+	+	+	+	-	+	-	-	-	A	-	A	A	*Pseudomonas* sp.
	3M3	R	-	+	-	+	+	-	+	+	+	-	A	-	-	A	*Proteus* sp.
	3M4	R	+	+	+	+	-	-	-	-	-	+	A	-	A	A	*Bacillus* sp.
	3M5	R	+	+	-	+	+	-	-	+	+	+	AG	-	-	-	*Bacillus* sp.
	3M6	R	-	+	-	+	-	-	-	-	+	+	A	-	-	-	*Proteus* sp.
	3M7	R	-	+	+	-	+	-	+	-	+	+	A	-	-	-	*Erythrobacter*sp.
	3M8	R	-	+	-	+	+	-	+	-	+	-	A	-	-	-	*Proteus* sp.
	3M9	R	-	+	-	-	+	-	-	-	-	-	A	-	-	-	*Pseudomonas* sp.

Table 4d: Morphological and Physicochemical/biochemical characteristics of bacteria in groundwater from Mgbu-Esilaru

SPC	IC	MP	GS	CT	OX	MT	ST	IP	CU	MR	VP	SH	GF	LF	MF	XF	SB
E1	1E1	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	1E2	R	-	+	-	+	+	-	-	+	-	-	AG	-	-	A	*Proteus* sp.
E2	2E1	R	+	+	-	-	+	-	+	+	-	+	A	-	-	-	*Bacillus* sp.
	2E2	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	2E3	R	-	+	-	-	+	-	+	-	+	-	AG	AG	A	-	*Klebsiellaaerogenes*
E3	3E1	R	+	+	-	-	+	-	+	+	-	+	A	-	-	-	*Bacillus* sp.
	3E2	R	+	+	-	+	+	-	+	+	+	+	A	-	-	-	*Bacillus cereus*
	3E3	R	-	+	-	+	+	-	-	+	-	-	AG	-	-	A	*Proteus* sp.

Table 5: Identity of some bacteria through Physicochemical/biochemical and biomolecular means

Isolate code	As identified through	
	Physicochemical/Biochemical means	Bio-Molecular means
1M6	*Bacillus* sp	*Staphylococcus epidermidis* (76 %)
3M1	*Salmonella* sp	*Staphylococcus aureus* (70 %)
3M7	*Erythrobacter* sp.	*Serratia marcescens* (89 %)
2E3	*Klebsiella aerogenes*	*E. coli* (93 %)
1O2	*Proteus* sp.	*Proteus vulgaris* (98 %)
3M9	*Pseudomonas* sp	*Providencia rettgeri* (81 %)

Correlation between the Bacterial Population and Physicochemical Characteristics of the Groundwater

There was a positive correlation between THB and EC (r = 0.80) in the groundwater sampled from the different communities in Rumuigbo; THB and pH (r = 0.90); THB and iron (r = 0.89); THB and lead (r = 0.95). There was no correlation between THB and salinity (r = -0.25); THB and turbidity (r = -0.67); THB and nitrate (r = -0.04); THB and phosphate (r = -0.11); THB and zinc (r = 0.47).

DISCUSSION

This study investigated the physicochemical and bacteriological characteristics of groundwater in communities of Rumuigbo in Obio-Akpor Local Government Area (LGA), Rivers State, Nigeria. The results of the physicochemical analyses revealed that electrical conductivity (EC), turbidity, and nitrate of groundwater in all the communities were lower than the permissible limits provided by the Standards Organisation of Nigeria (SON) and the World Health Organization (WHO). The salinity of groundwater in all the communities was lower than the salinity of a freshwater river (7.19±0.04 mg/L) in the lower Niger Delta region of Nigeria (Woke & Umesi, 2018). The pH of the groundwater in Nkpolu and Mgbu-Esilaru was slightly acidic and outside the permissible limits provided by SON; the pH in Omoi/Orosi and Mgbuadu was within the permissible limits. Iron and lead concentrations in groundwater from all the communities exceeded the permissible limits provided by SON and WHO. Zinc concentration in groundwater from Omoi/Orosi and Mgbuadu communities was higher than the permissible limit provided by SON; groundwater from Nkpolu and Mgbu-Esilaru communities was lower than the permissible limit. In a similar study carried out by Ugbaja & Otokunefor (2015) on groundwater from some communities in Obio-Akpor LGA, of which Rumuigbo is a subset, the pH of groundwater in some of the communities was acidic and outside the permissible limits provided by SON, while of groundwater in the others it was within the limits. Turbidity, zinc, and iron concentrations in groundwater in the communities were lower than the limits provided by SON; nitrate concentration was below the limit provided by SON and WHO. Lead concentrations in some of the communities were at the limit provided by SON and WHO, while in groundwater from the others, it was above the limits. In another similar study carried out by Nwankwoala (2014) on groundwater in major areas in Port Harcourt City and some communities in Obio-Akpor LGA, groundwater from Rumuigbo was found to have acidic pH outside the permissible limits provided by SON; EC value and iron concentration lower than the permissible limits provided by SON, salinity of 50.0 mg/L; and nitrate concentration lower than the permissible limits provided by SON and WHO. What was observed in the works of Ugbaja & Otokunefor (2015) and Nwankwoala (2014) is similar to the findings in this study, except for salinity. Salinity of 1.02±0.01 to 1.66±0.54 mg/L was observed in this study, whereas salinity of 50.0 mg/L was recorded in the work of Nwankwoala (2014). This discrepancy could be attributed to the sampling season; in this study, sampling was carried out in the wet season. In the work of Nwankwoala (2014), the sampling season was not stated.

The Centers for Disease Control and Prevention (CDC) have specified that heterotrophic plate count levels in potable water should be less than 500 CFU/mL (CDC, 2003); 0 CFU/ml has been reported to have been specified for *Salmonella* in drinking water by the WHO (Wamyil *et al.*, 2023); and not more than 10 CFU/ml (or 1000 CFU/100ml) has been specified for total coliforms in drinking water by SON (SON, 2015). Based on these permissible limits and the bacteriological results obtained, groundwater from the communities may be fit for drinking, except groundwater in the one sampling point in the Mgbu-adu community where *Salmonella* was

isolated. In similar studies (Elenwo *et al.*, 2019; Ugbaja & Otokunefor, 2015) carried out on groundwater from some communities in Obio-Akpor LGA, of which Rumuigbo is a subset, *Vibrio* and *Salmonella* species were not detected in any of the groundwater samples. However, in the work of Ugbaja & Otokunefor (2015), THB and coliform populations of 2 – 274 CFU/ml and 0 to 9.1 coliforms/100 ml, respectively were observed. The observation in these works is similar to the findings in this study, except for coliforms and *Salmonella*; the coliform population (0.7 coliforms/100 ml) recorded in this study was lesser than that recorded in the work of Ugbaja & Otokunefor (2015), and *Salmonella* was found in this study, though at only one sampling point. The lower coliform count may indicate that underground septic tanks and soakaway pits are sited at considerable distances from the groundwater in the communities.

Some of the bacteria isolated in this study are present in groundwater in Obio-Akpor LGA in other related studies. Azuonwu *et al.* (2020) and Ugbaja & Otokunefor (2015) isolated *Proteus* spp., *Pseudomonas* spp., and *Klebsiella* from groundwater in some communities located in Port Harcourt and Obio-Akpor LGA. The presence of these bacteria in groundwater is likely due to underground septic tanks close to groundwater extraction boreholes. The differences between the identity of the bacteria identified through physicochemical/biochemical and biomolecular procedures may be due to errors in any of the procedures. For instance, isolate 2E3 was identified as *Klebsiella aerogenes* through physicochemical/biochemical characterization but as *E. coli* through biomolecular means. *Klebsiella aerogenes* and *E. coli* are both coliforms, and it is likely that during editing of the nucleotide sequence of the 16S rRNA genes, some nucleotides may have been misplaced, thereby making the sequence closely related to the nucleotide sequence of any of the other coliform members.

The correlation results indicate that EC, pH, iron, and lead influenced the bacterial population in the groundwater across the different communities in Rumuigbo, whereas salinity, turbidity, nitrate, phosphate, and zinc did not influence the bacterial population in the groundwater. In a related work carried out by Adesakin *et al.* (2020) in the northern part of Nigeria, there was a correlation between total heterotrophic bacteria counts and pH, phosphate, or nitrate in borehole water; THB correlation with pH is in agreement with the result obtained in this study. The correlation of THB with phosphate or nitrate is in disagreement with the result obtained in this study and could be attributed to the disparity in geographical locations in terms of weather and soil type. In another related work carried out by Traore *et al.* (2023), though there was no correlation between bacterial population and EC, pH, turbidity, iron, or nitrate in another country. No correlation between bacterial population and turbidity or nitrate is in accord with the result obtained in this study; No correlation between bacterial population and EC, pH, or iron is in disagreement with the result obtained in this study and could also be attributed to differences in geographical locations.

CONCLUSION

The physicochemical and bacteriological quality of groundwater in Rumuigbo of Obio-Akpor LGA was assessed in this study. The results revealed that groundwater from some of the communities within Rumuigbo fulfills some of the requirements for potable drinking water as set by the WHO and Standards Organisation of Nigeria but does not meet the standards for iron, lead, zinc, and *Salmonella*. Therefore, groundwater from the communities requires some form of treatment to meet all the requirements for potable drinking water. There was the correlation between THB and pH, which is in agreement with what has been observed in another study, and there was no correlation between THB and turbidity or nitrate, which is also in agreement with what has been observed in another study. This indicates that pH can influence bacterial population in groundwater, whereas turbidity and nitrate do not influence bacterial population in groundwater.

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