



## **Bacteriological and Physicochemical Analysis of Groundwater in Selected Communities in Obio Akpor, Rivers State, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between the authors. Author TVO designed the study, reviewed the protocol of study and helped in execution and successful completion of the study and reviewed the manuscript for submission. Author VCU wrote the protocol of study, performed laboratory experiments, managed the literature searches, data compilation and wrote the manuscript. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** This research work is aimed at assessing the quality of groundwater in Rumuekini, Rumuosi, Aluu communities in Obio-Akpor, Rivers State, Nigeria.

**Methodology:** Bacteriological and physicochemical analyses were carried out on selected borehole water in Obio-akpor local government area, Rivers state and its environs. Twenty seven samples were obtained randomly from the following locations/communities namely: Rumuekini (nine samples), Rumuosi (nine samples) and Aluu (nine samples). In the study, total heterotrophic bacteria and coliforms were enumerated using the membrane filtration and multiple tube fermentation methods, respectively. The isolates were identified based on cultural, morphological characteristics and a battery of biochemical tests. Selected samples were also subjected to physicochemical analysis of parameters like pH and electrical conductivity which were measured in situ using the pH meter and the conductivity meter respectively. The temperature of the samples was also measured in situ using a thermometer. The nitrate, sulphate and chloride content of the

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samples were determined using the spectrophotometric and argentometric methods respectively. Biological Oxygen Demand and Chemical Oxygen Demand were measured using the Winkler (respirometric) and titrimetric methods respectively; while the heavy metals' content of the samples were determined using the atomic absorption spectrophotometer.

**Results:** In this study, the total heterotrophic bacteria count for the samples ranged from 0.02 to  $2.74 \times 10^2$  cfu/ml and were quite high compared to E.P.A and W.H.O standards of  $1.0 \times 10^2$  cfu/ml for total heterotrophic bacteria. The results obtained from this study showed that coliform bacteria were present in forty percent of the samples analysed with counts ranging from 0MPN/100 ml to 9.1 MPN/100 ml. These values were much higher than the W.H.O. standards of zero coliform/100 ml. *Enterobacter aerogenes* was the most common coliform isolated while *Escherichia coli* was isolated from only one of samples obtained from Aluu community. Pathogens like *Salmonella* and *Vibrio* were not detected in any of the samples. The physicochemical analysis of borehole water samples obtained from the three locations revealed the following range values: pH (5.8-7.5), temperature (25-29°C), turbidity (0-0.8 NTU), Nitrate (1.32-7.72 mg/l), Sulphate (0.43-14.8 mg/l), B.O.D (1.8-4.4) etc. The concentration of zinc in the sampling locations ranged from 0.01 to 0.34 mg/l while that of lead and iron ranged from 0.01 to 0.06 and 0.03 to 0.25 mg/l respectively.

**Conclusion:** The investigation in this study suggests that the borehole water samples were within W.H.O specified limits for the physicochemical parameters apart from pH that is below limit (5.8) for one of the samples obtained from Rumuekini; The status of water in some of these locations using microbiological, and physicochemical parameters as indicators can be said to be of acceptable limit for human consumption. However, the results obtained from this study showed that forty percent of the samples analysed had total coliforms (i.e. do not confirm with W.H.O standards); this may be as a result of improper construction/drilling of the boreholes or contamination of the storage tanks. There is need for the treatment of water from some of these boreholes in order to reduce the risk of infection.

**Keywords:** Coliforms; physicochemical analysis; total heterotrophic bacteria; W.H.O.

## 1. INTRODUCTION

Water is one of the earth's most precious resources. Although water is essential for human survival, many are denied access to sufficient potable drinking water supply and sufficient water to maintain basic hygiene. Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers and open wells. The majority of these are in Asia and sub-Saharan Africa. Furthermore, 2.4 billion people lack adequate sanitation worldwide [1]. In developing countries, thousands of children under five years die every day due to drinking contaminated water [1]. Thus, lack of safe drinking water supply, basic sanitation and hygienic practices are associated with high morbidity and mortality from excreta related diseases. Water-borne pathogens infect around 250 million people each year resulting in 10 to 20 million deaths world-wide. An estimated 80% of all illness in developing countries is related to water and sanitation and 15% of all child deaths under the age of five years in developing countries result from diarrhoeal diseases [2].

The lack of safe drinking water and adequate sanitation measures could also lead to a number

of diseases such as dysentery, salmonellosis, shigellosis and typhoid, and every year millions of lives are claimed in developing countries [3].

The evaluation of potable water supplies for coliform bacteria is important in determining the sanitary quality of drinking water. High levels of coliform counts indicate a contaminated source, inadequate treatment or post-treatment deficiencies in drinking water [3]. Many developing countries suffer from either chronic shortages of freshwater or the readily accessible water resources are heavily polluted [4].

Microbiological health risks remain associated with many aspects of water uses, including drinking water in developing countries [5], irrigation reuse of treated wastewater and recreational water contact [6]. It has been reported that drinking water supplies have a long history of association with a wide spectrum of microbial infections [6]. The primary goal of water quality management from a health perspective is to ensure that consumers are not exposed to doses of pathogens that are likely to cause diseases. Protection of water sources and treatment of water supplies have greatly reduced the incidence of these diseases in developed

countries [5,6]. To attain safe water supply for various communities, an understanding of water that is microbiologically and chemically certified safe is therefore imperative. Above all, to ensure that the microbiological characteristics of drinking water are satisfactory for human consumption, the National Agency for Food and Drugs Administration and Control in association with the World Health Organization [1] recommended that the acceptable limit of coliforms in potable water for human consumption should be zero coliforms per 100 ml. Many people in rural and urban communities rely on ground water as a source of drinking water and other purposes.

Therefore this study focused on quality assessment of groundwater at Obio-akpor with a view to ascertain its suitability for domestic purposes.

## 2. LOCATION OF STUDY

Rumuekini lies approximately between latitude 4°53'N and longitude 6°57'E in Obio-Akpor L.G.A, Rivers State. Rumuosi lies between latitude 4°51.05'N and longitude 6°57'E in Obio-Akpor L.G.A, Rivers State. Aluu which is in Ikwerre L.G.A but has a common boundary with ObioAkpor at the Northern part of it lies approximately within the range of 4°54.05N north and longitude 6°59'E in Obio-Akpor L.G.A.

### 2.1 Sources and Collection of Water Samples

Cotton wool soaked in 70% ethanol was used to sterilize the nozzle of the tap of the storage tanks from which borehole water samples were collected randomly from the points in each of the sampling areas viz: Rumuekini (nine samples), Rumuosi (nine samples) and Aluu (nine samples). The tap was allowed to run for two minutes before sterile screw capped plastic bottle was carefully uncapped and filled with water and recapped. Water samples were transported to the laboratory in a cooler with ice for analysis within three hours of collection.

### 2.2 Bacteriological Analysis

Total Heterotrophic bacteria and total coliform counts were enumerated using the membrane filtration technique (100 ml of water sample was filtered using 0.45 mm pore size membrane) and the Multiple tube fermentation method respectively; as described by [7]. The Isolates

were further identified using standard biochemical procedures.

### 2.3 Isolation of Enteric Pathogens

Selenite F broth and Salmonella Shigella Agar are the enrichment broth and selective medium respectively for the enumeration of *Salmonella* and *Shigella*. One hundred millilitres of each water sample was introduced into sterile 250ml conical flasks containing an already sterilized (by boiling for 10 minutes) and cooled, 100ml double strength selenite F broth; the broth containing the sample was incubated at room temperature for 12-18 hours. Then, 0.1 ml of the incubated sample was aseptically pipetted on already prepared SSA medium. The inoculum was spread with a sterile bent glass rod and incubated in an inverted position at 37°C. After 16-24 hrs, the plates were observed for *Salmonella* growth. Alkaline peptone water and Thiosulphate Citrate Bile Salt agar are enrichment broth and selective medium, respectively for the enumeration of *Vibrio*.

Ten millilitres of each water sample was pipetted into tubes containing already sterilized and cooled alkaline peptone water; the broth containing the sample was incubated at room temperature for 7-8 hours. Then, 0.1ml of the incubated sample was aseptically pipetted on already prepared TCBS medium. The inoculum was spread with a sterile bent glass rod and incubated in an inverted form at 37°C. After a 24 hour period, the plates were observed for the formation of visible colonies.

### 2.4 Physicochemical Analysis

The physicochemical tests included the determination of temperature, turbidity, pH, electrical conductivity, salinity, sulphate, chloride and nitrate content, Biological oxygen demand, chemical oxygen demand and some heavy metals using the methods described by [7].

## 3. RESULTS AND DISCUSSION

### 3.1 Results

The results obtained from microbiological and physicochemical analysis of ground water in the three locations are presented in Tables 1-3; the concentration of heavy metal analysed are presented on Fig. 1. Tables 1 shows the bacteriological counts of the borehole water

samples obtained from the three locations Aluu, Rumuekini and Rumuosi. The total heterotrophic bacteria count of samples in the three locations ranged from 0.02 to 2.74 cfu/ml while the total coliform counts ranged from 0 to 9.1 MPN/100 ml. *Salmonella* and *Vibrio* were not isolated from any of the samples. Table 2 shows the biochemical identification of the isolates (*Escherichia coli* and *Enterobacter aerogenes*) based on cultural, morphological characteristics and biochemical tests. Table 3 shows the result of the physicochemical analysis of borehole water samples obtained from the three localities; the results of the parameters analysed revealed the following range values: pH (5.8-7.5), temperature (25-29°C), turbidity (0-0.8 NTU), Nitrate (1.32-7.72 mg/l), Sulphate (0.43-14.8 mg/l), B.O.D (1.8-4.4) etc. Fig. 1 is a clustered chart which shows the concentration of heavy metals (zinc, lead and iron) analysed; the concentration of zinc in the sampling locations ranged from 0.01 to 0.34 mg/l while that of lead and iron ranged from 0.01 to 0.06 and 0.03 to 0.25 mg/l respectively. Plate 1 reveals the growth of total heterotrophic bacteria of one of groundwater samples on the membrane.

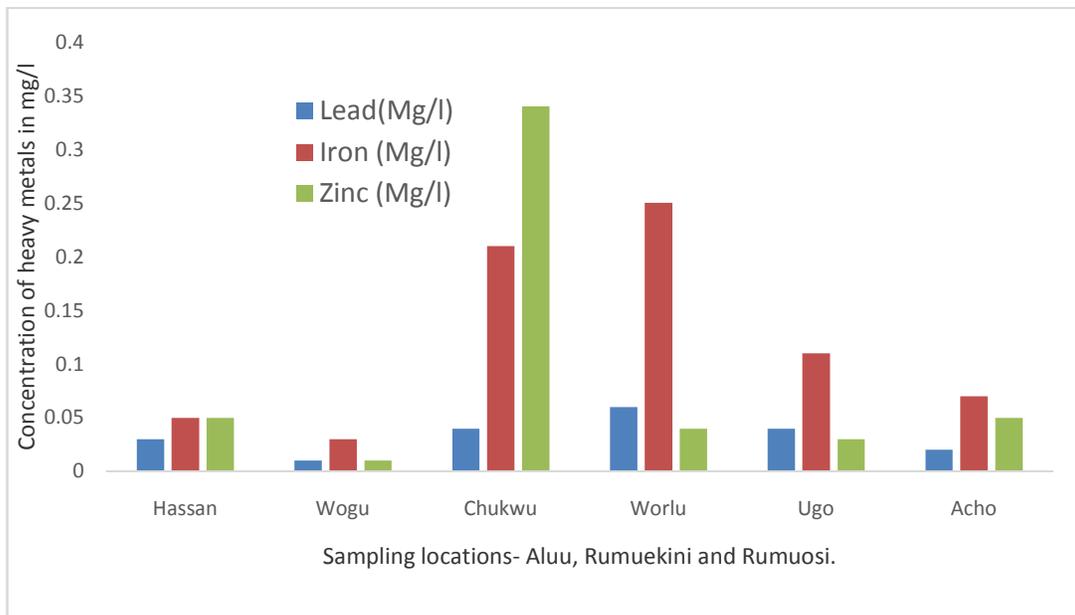
contaminated [9]. Ground water is found to be contaminated due to improper construction, shallowness, and various human activities around the well [10]. In this study, the total heterotrophic counts for some of the samples were quite high compared to E.P.A and W.H.O standards of  $1.0 \times 10^2$  for total heterotrophic bacteria while total coliform count was also quite high in comparison with W.H.O and E.P.A standards of zero per 100 ml for total coliforms.



**Plate 1. Total heterotrophic bacteria growth on the membrane filter**

### 3.2 Discussion

Generally, groundwater is believed to be purest (8) because of the purification processes it went through while percolating through the subsurface. However, it can also be



**Fig. 1. Concentration of heavy metals in the three sampling locations**

**Table 1. Bacteriological counts of groundwater samples in the three locations (Rumuekini, Rumuosi, and Aluu)**

Sampling site	Rumuekini			Rumuosi			Aluu				
	Location	THB(x 10 <sup>2</sup> cfu/ml)	TC(MPN /100 ml)	Sampling site	Location	THB(x 10 <sup>2</sup> cfu/ml)	TC(MPN /100 ml)	Sampling site	Location	THB( x 10 <sup>2</sup> cfu/ml)	TC(MPN /100 ml)
Worlu	Rumuari	0.42	1.81	Ugo	Okwurozu	0.84	3.92	A.Hassan	Omuokiri	0.34	4.02
Nwoke	Rumuari	2.74	0	Emenike	Okwurozu	0.29	0	Wali	Omuchiolu	2.56	1.99
Anita	Rumuorlu	2.56	0	Stainless	Okwurozu	0.64	0	Wegbu	Omuchiolu	0.84	4.50
Nwikobe	Rumuegom	0.45	1.81	Mekele	Rumumba	0.36	1.99	Car Wash	Omuchiolu	0.18	0
Jumbo	Rumuorlu	0.95	1.99	Igwe	Rumumba	0.92	6.7	Kenglori	Omahunwo	0.78	0
J. house	Rumuorlu	0.11	0	Ogondah	Rumumba	2.65	9.1	Wogu	Omahunwo	0.41	1.99
Chukwu	Rumuorlu	0.28	0	Acho	R/chinwere	0.52	1.80	Peters	Mbodo	0.09	0
R. market	R/awechor	0.36	0	Amadi	R/chinwere	1.82	0	Ovundah	Mbodo	0.30	3.98
Uche	Rumuodom	0.98	6.85	R/igwe	R/chinwere	0.65	0	E.Amadi	Mbodo	1.01	0

**Table 2. Identification of isolates using morphological characteristics and biochemical test**

Colony characterization	Gram reaction	MR	VP	Indole	Citrate	Motility	Sucrose	Lactose	Glucose	Probable genera
Small, metallic green sheen, mucoid colonies.	GN rod	+	-	+	-	+	A/G	A/G	A/G	<i>Escherichia coli</i>
Large, dark purple, mucoid colonies with a dark centre	GN rod	-	+	-	+	+	A/G	A/G	A/G	<i>Enterobacter aerogenes</i>
Large, pink, mucoid colonies with a dark centre	GN rod	-	+	-	+	+	A/G	A/G	A/G	<i>Enterobacter aerogenes</i>

**Table 3. Physicochemical properties of borehole water samples in the three locations**

Parameters	Hassan	(Aluu) Wogu	Wegbu	Chukwu	(Rumuekini) Worlu	Uche	Ogondah	(Rumuosi) Ugo	Acho
Ph	6.7	6.8	7.0	6.4	5.8	7.2	6.9	7.1	7.5
Temperature(°C)	28	25	27	27	28	27	28	28	29
Conductivity(us/cm)	55	64	44	39	46	54	42	50	76
Alkalinity	1.80	1.20	1.40	2.40	1.60	1.30	1.62	1.60	1.20
Turbidity(NTU)	0.2	0.1	0.0	0.3	0.8	0.2	0.2	0.0	0.5
B.O.D	2.5	4.0	4.4	2.8	4.1	3.1	3.8	1.8	3.1
C.O.D	3.0	4.1	3.6	4.8	7.2	3.7	4.3	4.0	5.2
Sulphate(Mg/l)	0.71	0.65	0.54	0.68	0.68	0.43	0.84	8.2	14.8
Nitrate (Mg/l)	1.41	1.32	2.41	1.42	1.57	2.54	1.64	7.24	7.72
Salinity	27.01	32.04	24.04	25.27	44.76	15.20	24.20	18.05	18.05
Chloride (Mg/l)	12.00	24.20	12.60	14.00	24.80	14.01	16.05	10.00	12.50

The total heterotrophic count ranged from  $0.02 \times 10^2$  to  $2.74 \times 10^2$  cfu/ml; Agbabiaka and Sule [11] obtained a range of zero to  $2.3 \times 10^2$  cfu/ml from the analysis of boreholes carried out in Ilorin Metropolis. Erah et al. [12] in a study conducted on the quality of ground water in Benin City, Nigeria found unacceptable levels of aerobic bacteria and fungi present in borehole water of Teboga District of Benin City. In another similar work, Eniola et al. [13] obtained a range of  $5.0 \times 10^2$  to  $7.0 \times 10^2$  cfu/ml for stored borehole water samples. The high total heterotrophic count obtained from this study is indicative of the presence of high organic and dissolved salts in the water. The primary sources of these bacteria in water are animal and human wastes. These sources of bacterial contamination include surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria [9].

The total coliform counts of the samples analysed ranged from 0 MPN/100 ml to 9.10 MPN/100 ml; the total coliform contents of the samples analysed by Agbabiaka and Sule [11] ranged from zero to 16 MPN of coliform/ 100ml which is quite similar to that obtained in this study. WHO [1] specified that potable drinking water should be devoid of total coliform in any given sample. It is note-worthy to mention that 60% of the borehole water samples have zero total coliform counts. Results of total coliforms obtained in this study is similar to that of Rogbesan et al. [14] who also obtained total coliform outside the range allowed by WHO. The samples obtained from Omuokiri, Omuchiolu and Omahunwo in Aluu community recorded high total coliform counts ranging from 1.99 MPN/100 ml to 4.50 MPN/100 ml which does not conform to W.H.O standard of zero per 100 ml. However, some of the samples obtained from Rumuorlu, Rumuari, Rumuawechor and Rumuegom in Rumuekini community recorded zero MPN/100 ml and hence met W.H.O and E.P.A standards of zero MPN/100ml with the exception of the samples obtained from Rumuorlu (Mr. Jumbo's Compound) and Rumuegom in which their total coliform count were 1.81 MPN/100 ml. Futhermore, water samples obtained from Okwurozu, Rumumba and Rumuchinwere in Rumuosi community recorded Total coliform counts ranging from 1.80 MPN/100ml to 9.10 MPN/100 ml and do not conform with WHO standard. *Escherichia coli* was isolated from one of the samples in Aluu community; its source

may have been from the soil/environment [15]. *Enterobacter aerogenes* mostly isolated from the borehole water samples but in amounts less than  $10^4$  are examples of non-faecal coliforms and can be found in vegetation and soil which serves as sources by which pathogens enter the water [16]. The British Standard Institute specified that counts greater than  $10^4$  is considered unsatisfactory for *Enterobacter* spp. however, pathogens like *Salmonella* and *Vibrio* were not detected in the samples.

Furthermore, the investigation in this study suggests that the borehole water samples were within the World Health Organization specified limits for the physicochemical parameters apart from pH that is below limit for Rumuari borehole water sample. The pH of the samples ranged from 5.8 to 7.5; this pH range is close to neutrality and would allow the growth of most bacterial species. Eniola et al. [13] obtained similar pH ranges of 6.54 – 7.80 and 6.54 to 7.90 for borehole water samples stored indoor and outdoor in containers of different colours. The temperature values (25-29°C) found in this study are comparable to temperature ranges reported by other workers [9]. Alkalinity values of 1.8 to 2.4 are much lower than W.H.O standard of 500 mg/l in drinking water [1]; no health implication has been identified with such alkalinity values. The turbidity of water depends on the quantity of solid matter present in the suspended state. It is a measure of light emitting properties of water and the test is used to indicate the quality of waste discharge with respect to colloidal matter [17]. The turbidity values of the samples ranged from 0 to 0.8 Nephelometric turbidity units and were within the Environmental protection agency acceptable limits which may be an indication of no/fewer number of planktons, clay particles and disease causing microorganisms [17]; at no time should turbidity go above 5 Nephelometric turbidity units for acceptability [1]. The iron content of the water samples were within the EPA standard of 0.3 mg/l [1] so were other heavy metals analysed such as Zinc and Lead. Chloride is present in appreciable amounts in all natural water. Concentration varies from few milligrams to several thousand milligrams per litre. High concentration of Chloride may cause corrosivity of the pipes and impaired taste [18]. The chloride content recommended by EPA and WHO is 250 mg/l, this is in agreement with the chloride content of all the water samples analysed. Biological Oxygen Demand (B.O.D) is a measure of the oxygen in the water that is required by the aerobic organisms. Rivers with low B.O.D have

low nutrient levels; therefore, much of the oxygen remains in the water. Unpolluted, natural waters should have a B.O.D of 5 mgL<sup>-1</sup> or less [17]. The Biological Oxygen Demand values of the samples ranged from 1.8 to 4.4 mg/l and these B.O.D values show that there was little biological activity in the samples thus safe for consumption since high B.O.D values suggests a high number of heterotrophic bacteria in the environment [17].

#### 4. CONCLUSION

This work was carried out to determine the quality of borehole water in selected communities in Obio-Akpor, Rivers State viz: Rumuekini, Rumuosi and Aluu. The status of water in some of these locations using microbiological and physicochemical parameters as indicators can be said to be of acceptable limit for human consumption. However, the results obtained from this study showed that forty percent of the samples analysed had total coliforms (i.e. do not conform with W.H.O standards); this may be as a result of improper construction/drilling of the boreholes or contamination of the storage tanks and there is need for the treatment of some of these water boreholes (especially those in Rumuosi and Aluu) by the borehole owners and also by simple treatment methods such as boiling by the consumers. Water quality analysis should be carried out on all the boreholes in the communities at least once every two years. This will ensure that incidences of contamination are noticed earlier for remedial action to be taken. The communities should not compromise on their sanitary practices as a dirty environment could serve as source by which groundwater gets contaminated.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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