



Exploring the Prevalence of Microorganisms in Selected Water Sources in Benue South of Nigeria using Selected Environmental Factors and Microbial Counts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In this study, we assessed the water and microbial qualities of major water sources in nine sites within three Local Government Areas (LGAs-Otukpo, Ohimini and Apa LGAs) in Benue South between January and March, 2023. Water samples were collected monthly for the analyses of selected physico-chemical variables and microbial constituents in the studied sites following

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standard procedures. The results of the physico-chemical variables showed that most of the variables were proportionately higher than the maximum standards by appropriate regulatory bodies. For instance, electrical conductivity (EC) was highest in S2-Upu borehole, Otukpo LGA ($1,181 \pm 285 \mu\text{S}/\text{cm}$) and lowest in S4-Okpokwu River, Ohimini LGA ($19.35 \pm 6.35 \mu\text{S}/\text{cm}$). The concentration of dissolved oxygen (DO) in all the sites showed less aerated water except for S2 ($5.37 \pm 0.34 \text{ mg/l}$) and 5S-Awulema borehole, Ohimini LGA ($5.06 \pm 0.14 \text{ mg/l}$). On the microbial quality, the results of the total coliform counts from the nine sampling sites (water sources) showed that the highest coliform count ($>1,600 \text{ MPN}/100\text{mL}$) was recorded in S1 in January 2023 while the lowest coliform count (12 MPN/100mL) was recorded in S2. The total bacteria viable counts (TVC) detected in the water sources sampled in the three LGAs in showed that the highest bacteria count (204 cfu per mL) was recorded in S3-Depot well, Otukpo LGA in January 2023, and the lowest bacteria count (36 cfu per mL) was recorded in S2 in January 2023. *Escherichia coli* was the most prevalent bacteria as it occurred in all the sites sampled except S6- Ijami well, Ohimini LGA and S9- Amoke well, Apa LGA. Our finding based on the concentration of pollution indicating physico-chemical variables (e.g., EC, BOD_5 , and COD) showed the water sources studied in the three LGAs in Benue South were relatively perturbed. This was also confirmed by the prevalence of microbial loads which in most cases exceeded the minimum standard set by WHO for drinking water.

Keywords: Microbial quality; water sources; Benue South; Nigeria.

1. INTRODUCTION

Water is the most important natural resource on earth and every living organism depend on water in one way or the other [1]. Among the existing living organisms in need of water for their survival, humans are the most dependent on water, as they need water for their day-to-day activities. Access to portable water supply is a serious constraint to over one billion people globally [2]. The scarcity or shortage of potable water for humans resulted from overpopulation, urbanization, industrialization, and agricultural activities [3]. Furthermore, climate change and water-related disasters (e.g. flooding) are also contributing factors to the shortage of clean water [1]. Most rural dwellers depend on rivers and streams for their drinking water and other domestic uses, hence when there is flooding for instance, the rivers and streams water will be turbid making the water unfit for human consumption and other use. The absence of disasters such as flooding does not rule out the possibility of rivers, streams, and other sources of water being unfit for drinking.

Naturally, most sources of water contain a certain proportion of microorganisms which the human system can contain in the event they consume such water [4]. However, an aggravated proportion of microorganisms and other pathogens and chemical substances in high concentration which result in water-borne diseases can be harmful to humans when they consume such untreated water [4-5]. Untreated water results in incidences of several water-

borne diseases including cholera, diarrhea, typhoid, giardiasis and amoebiasis, and this is more pronounced in rural locations where access to treated water is scarce [6]. Most water-borne diseases are very fatal, as they can lead to death in the case that the diseases are attended to timely and correctly, and this poses a serious public health challenge. To assuage diseases posed by contaminated water, portable and quality sources of water must be available to humans of all level for consumption.

The supply of portable water to various households for drinking and other domestic uses is key to reducing the public health challenges that may results from contaminated water [7]. To achieve this, water harvested from different sources should be properly treated before supplying same to various households for consumption. Hence, in this study, we assessed the water and microbial qualities of major water sources in three Local Government Areas (LGAs) in Benue South, Nigeria in a bid to ascertain the quality of water the locals within the studies areas consume. This is to enable us provide useful recommendations to the appropriate regulatory bodies to resolve the challenges posed by poor water quality in various water sources.

2. MATERIALS AND METHODS

2.1 Study Area/Sampling Sites and Duration of the Study

Nine sampling sites were marked out in three Local Government Areas (LGAs) within Benue

South Senatorial District. The LGAs include; Otukpo, Ohimini, and Apa (Fig. 1). The geographical locations of the three sampling LGAs are: Otukpo ($7^{\circ} 11' 35''$ N and $8^{\circ} 47'$ E), Ohimini ($7^{\circ} 10' 12.973''$ N, $7^{\circ} 47' 60''$ E), and Apa ($7^{\circ} 37' 30''$ N $7^{\circ} 52' 16''$ E). The area is typified by a tropical climate and a wet and dry season. Wet season spans from March to September and the dry season spans from October to February. The area is in the

guinea savanna belt of Nigeria characterized with shrubs, grasses and trees, and patches of forested areas.

Sampling was carried out for a period of two months (January and March 2023). At each LGA, three most common water sources (rivers, boreholes, and wells) were selected for the study.

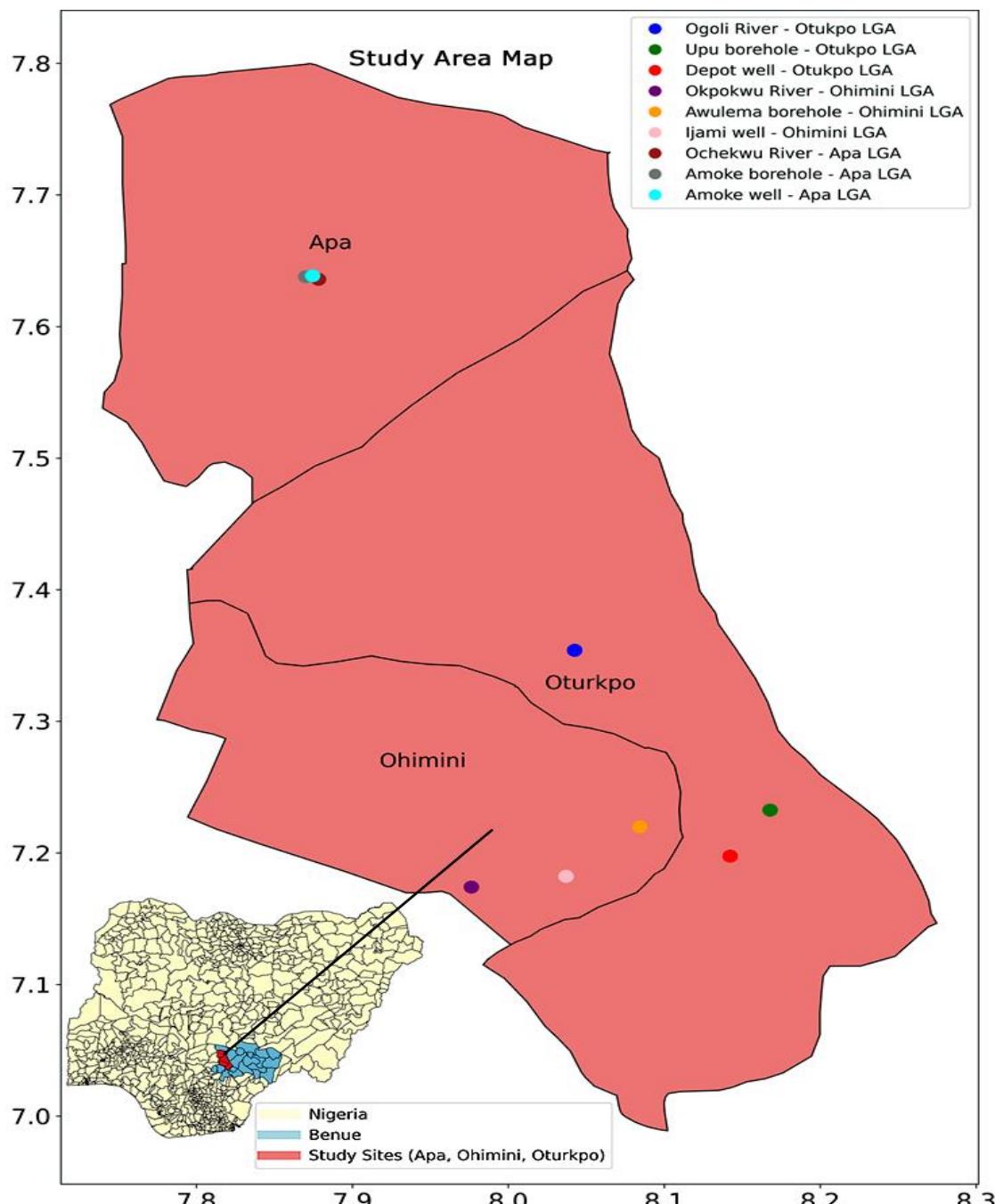


Fig. 1. Map of the study area showing the sampling locations

2.2 Water Sampling Procedures

Water samples were collected at each sampling site using bottled water containers which have not been used. At every sampling expedition, three packs (36 bottles) of bottled water were purchased and the water in the bottles was emptied before use on the field. At each sampling site, one bottle was filled with surface water for microbial analyses, then the remaining three for physico-chemical variables analyses.

2.2.1 Physico-chemical variables analyses

Some physico-chemical variables such as water temperature, electrical conductivity (EC), pH, total suspended solids (TSS), total dissolved solids (TDS) were measured in situ at each sampling site using a multi-probe meter. At each sampling site, a separate bottle was filled with water and covered with black cellophane to avoid the biochemical reaction of microorganisms for the analysis of biochemical oxygen demand (BOD_5). Further, two separate bottles were filled with water, one for dissolved oxygen (DO) which was fixed with Winkler solution A (manganese II sulphate) and B (potassium iodide) to trap the on-site dissolved oxygen before analysis at the laboratory and the other for analysis of other variables including TSS, turbidity and chemical oxygen demand (COD). At the laboratory, DO and BOD_5 were analysed following APHA [8] procedures while COD and TSS were measured using multiprobe meter and turbidity was measured using a turbidometer.

2.3 Bacteriological Analysis

To estimate the TVC we used aliquots of the collected water samples for microbial analysis following established procedures as has been described in the work of Ugbaja and Otokunwor [9] with minor modifications. We diluted water samples in sterile 0.85% normal saline at a ratio of 1:9 and plated the duplicate on a nutrient agar (NA) produced by Oxoid Ltd., Basingstoke in the UK. The agar plates were incubated at 25°C for a period of 24 hours. Bacterial colonies unit (cfu/mL) number was calculated as follows: number of colonies/plate X dilution factor of 10^2 .

Further, multiple-tube fermentation method was employed for the presumptive coliform test, adapting Leong et al. [1] procedures with slight modification. We used differential mediums for

the isolation of the coliform. Series of five broth tubes were used- the first series contains and they were inoculated with 10, 1 and 0.1 mLs of water at ratio 5:5:5. All tubes were observed for 24 hours for the first instance, then 48 hours at the second instance at a temperature of 37°C. The presumptive tests were deemed positive for coliform if the acid and gas were produced in Durham tubes.

2.3.1 Confirmed and completed tests

Presumptive test was confirmed by transferring a loopful of culture from a positive tube from the presumptive test into a tube of brilliant green lactose bile broth with Durham tubes. The tubes were observed at 24 hours, then at 48 hours at a temperature of 37°C for total coliform observed for gas production. Further, a loopful of broth from a positive tube was streaked on an eosin methylene blue (EMB) agar plate for pure colonies and incubated at 37°C for 24 hours and then for 48 hours. Colonies showing visible green metallic sheen were observed, and thereafter, the most probable number (MPN) per 100 mL of water was tabulated.

2.4 Data Analysis

Datasets from both the field and laboratory were first recorded in a Microsoft Excel sheet. All statistical significances were conducted at 0.05 (95%) level of significance. We performed descriptive statistics (mean \pm standard error) on the physico-chemical variables. After this, a one-way analysis of variance (ANOVA) was performed on the physico-chemical variables to determine their level of significance among the sites sampled for the two months (January and March 2023). Most probable number (MPN), total viable counts (TVC) and the prevalence of microorganisms were presented in tables. Palaeontological Statistical Package (PAST) and Microsoft Excel 2010 were used to perform all data analyses [10].

3. RESULTS AND DISCUSSION

3.1 Physico-Chemical Variables

We explored the water and microbial qualities of selected water sources in three LGAs in Benue South Senatorial District of Nigeria, and we presented our findings and discussion. Table 1 shows the mean \pm standard errors of physico-chemical variables in the three LGAs studied...

Water temperature was highest in S2 ($30.1\pm4.5^{\circ}\text{C}$) and lowest in S1($23.89\pm1.89^{\circ}\text{C}$). Analysis of variance (ANOVA) revealed that there was no significant difference in water temperature among the sites sampled ($F=0.92$; $P>0.05$). The pH of the sites sampled was between slightly acidic and slightly alkaline. The highest mean pH (7.51 ± 0.21) value was recorded in S2, and it was the only site that was slightly alkaline. The lowest pH (6.17 ± 0.17) in S5, and was more acidic than the remaining sites. ANOVA performed showed that there was no significant difference in pH among the sites sampled ($F=3.83$; $P>0.05$). Electrical conductivity (EC) was highest in S2 ($1,181\pm285 \mu\text{S}/\text{cm}$) and lowest in S4 ($19.35\pm6.35 \mu\text{S}/\text{cm}$). ANOVA performed showed that there was a significant difference in EC concentration among the sites sampled ($F=15.53$; $P<0.000099$). Similar ranges of water temperature, pH and EC have been recorded by other studies [11-12]. The high temperature values recorded in the studied month is not unexpected as temperature values are usually high in the dry season compared to wet season. Further, the pH values of S2 and S5 that are alkaline and slightly acidic, respectively may be occasioned by the type of human influence occurring in the area. For instance, S5 is within a residential area and majority of the locals do their laundry activities there as well as use fire woods for their cooking. Burning of fire woods may increase the carbon dioxide in the atmosphere, and the mixing of carbon dioxide to hydrogen leads to acid rain [13].

The concentration of dissolved oxygen (DO) in all the sites showed less aerated water except for S2 ($5.37\pm0.34 \text{ mg/l}$) and S5 ($5.06\pm0.14 \text{ mg/l}$). ANOVA performed showed that there is a significant difference among the sites sampled in the course of the study ($F= 9.56$; $P<0.05$). The biochemical oxygen demand (BOD_5) results showed that the sites sampled were relatively perturbed. The highest BOD_5 was recorded in S5 ($2.03\pm0.17 \text{ mg/l}$) and the lowest value was in S1 ($1.08\pm0.52 \text{ mg/l}$). ANOVA showed that there is no significant difference in BOD_5 concentrations among the sites sampled ($F=0.42$; $P>0.05$). The values of DO and BOD_5 recorded showed the fast-deteriorating states of the drinking waters in the study area. Low DO and high BOD_5 have been reported by most water sources in Nigeria and elsewhere [12,14-15]. COD was highest in S2 ($2.5\pm0.2 \text{ mg/l}$) and

lowest in S6 ($0.97\pm0.34 \text{ mg/l}$). ANOVA performed showed that there was no significant difference in COD concentrations among the sites sampled ($F=0.79$; $P>0.05$). Total dissolved solids (TDS) were highest in S2 ($743.5\pm12.5 \text{ mg/l}$) and lowest in S4 ($9.5\pm3.5 \text{ mg/l}$). There was a very high significant difference in TDS concentration among the sites sampled ($F= 1277$; $P=2.84\text{E-}10$). Total suspended solids (TSS) were highest in S8 ($98.5\pm42 \text{ mg/l}$) and the lowest in S2 ($4.61\pm0.60 \text{ mg/l}$). There was a significant difference in TSS concentration among the sites sampled ($F=4.14$; $P<0.043$). Turbidity was highest in S8 ($72.65\pm25.85 \text{ NTU}$) and the lowest in S5 ($9.3\pm0.3 \text{ NTU}$). There was a significant difference among the sites sampled ($F= 4.83$; $P<0.029$). Nyantakyi et al. [16] had reported similar trend in the concentrations of COD, TDS and TSS of a water system in Tano, Ghana. They stressed that lack of water treatment and uncontrolled discharges from nearby catchments results in the increased concentration of these environmental variables. In the current study, waste discharges and indiscriminate human influences were noted in some of the sampled sites (e.g., S1 and S7). Further, most of the water sources we sampled are not treated including the boreholes which would have undergone some degree of treatment regularly to maintain the quality of the water.

3.2 Microbial Quality

3.2.1 Coliform counts: Most Probable Number (MPN)

The coliform counts from the nine sampling sites (water sources) in the three LGAs studied in Benue South are presented in Table 2 below. The highest coliform count ($>1,600 \text{ MPN}/100\text{mL}$) was recorded in S1 (Ogoli River) in Otukpo LGA in January 2023 while the lowest coliform count ($12\text{MPN}/100\text{mL}$) was recorded in S2. It has been reported that high level of coliform counts is an indication of contaminated water resulting from increased faecal materials and other harmful bacteria and this has adverse effect on water quality [17-18]. Overall, the results of the coliform counts in the nine sites sampled in January 2023 and seven sites in March 2023 exceeded the minimum standard for coliform bacteria in water ($3 \text{ coliform}/100\text{mL}$) by the World Health Organisation [19].

Table 1. Summary (mean+standard error) of physico-chemical variables in the three local government areas (LGAs) in Benue South, Benue State, Nigeria

Physico-chemical variables	S1	S2	S3	S4	S5	S6	S7	S8	S9	ANOVA	
										F-value	P-value
Water temperature (°C)	23.39±1.89	30.1±4.5	-	25.1±3.4	28.35±0.35	28.45±1.65	27.65±0.85	29.3±2.1	-	0.92	0.53
pH	6.71±0.1	7.51±0.21	-	6.62±0.28	6.17±0.17	6.43±0.31	6.24±0.12	6.66±0.31	-	3.83	0.051
EC (µS/cm)	52.12±15.12	1,181±285	-	19.35±6.35	538.5±26.5	216.6±26.4	54.49±30.82	24.685±1.02	15.53	0.00099	
DO (mg/l)	4.08±0.12	5.37±0.34	-	4.59±0.27	5.06±0.14	4.415±0.39	4.605±0.41	2.46±0.34	-	9.56	0.0044
BOD ₅ (mg/l)	1.08±0.52	1.36±0.34	-	1.96±0.14	2.03±0.17	1.09±0.11	1.40±0.33	1.44±1.36	-	0.42	0.844
COD (mg/l)	1.67±1.43	2.5±0.2	-	1.99±0.16	1.45±0.25	0.97±0.34	1.13±0.21	1.54±0.27	-	0.79	0.61
TDS (mg/l)	19.5±1.5	743.5±12.5	-	9.5±3.5	271.5±8.5	15±3	13.9±2.4	109±12	1277	2.84E10	
TSS (mg/l)	8.85±2.35	4.61±0.60	-	8.95±3.15	11.2±2.3	15.1±6.5	18.12±2.92	98.5±42.5	-	4.14	0.043
Turbidity (NTU)	23.6±8.4	12.3±0.8	-	11.1±0.7	9.3±0.3	10.27±1.23	33.45±4.75	72.65±25.85	-	4.83	0.029

Note: Sites codes: S1= Ogoli River-Otukpo LGA, S2=Upu borehole- Otukpo LGA, S3= Depot well- Otukpo LGA, S4=Okpokwu River-Ohimini LGA, S5=Awulema borehole- Ohimini LGA, S6= Ijami well- Ohimini LGA, S7= Ochekwu River-Apa LGA, S8= Amoke borehole- Apa LGA, S9= Amoke well- Apa LGA

Table 2. The most probable number (MPN per 100 mL) for the five tubes methods for the presumptive test analysis of the total coliform bacteria in selected water sources in the three Local Government Areas (LGAs) studied in Benue South, Benue State, Nigeria

Sampling sites	January 2023		March 2023		WHO, [18] Standard (Count per 100mL)
	Number of positive tubes	MPN per 100mL	Number of positive tubes	MPN per 100mL	
S1	5<5~5	>1600	2<3~4	>1400	3
S2	2~2~1	12	3~2~3	89	3
S3	5~4~2	542	-	-	3
S4	5~4~4	345	5~5~4	219	3
S5	5~4~5	426	5~4~3	621	3
S6	4~1~2	26	4~3~1	102	3
S7	5~4~1	172	3~2~1	75	3
S8	5~3~2	141	4~2~4	321	3
S9	5~1~3	84	-	-	3

Table 3. Values of total bacteria count (cfu per mL) in selected water sources in the three Local Government Areas (LGAs) studied in Benue South, Benue State, Nigeria

Sampling sites	January 2023	March 2023	WHO, [18] Standard (cfu per mL)
	TVC (total viable count) (cfu per mL)	TVC (total viable count) (cfu per mL)	
S1	156	Nill	100
S2	36	124	100
S3	204	-	100
S4	136	Nill	100
S5	134	82	100
S6	74	88	100
S7	66	72	100
S8	116	118	100
S9	128	-	100

Table 4. Prevalence of bacteria in the sampled sites of the present study area

Sampling sites	Bacteria composition	March 2023
	January 2023	
S1	<i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Proteus</i> spp.	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>K.</i> spp., <i>Proteus</i> spp.
S2	<i>B.</i> spp.	<i>P.</i> spp., <i>B.</i> spp.
S3	<i>E. coli</i> , <i>P.</i> spp., <i>B.</i> spp, <i>Shigella</i> spp., <i>Staphylococcus</i> spp.,	-
S4	<i>E. coli</i> , <i>K.</i> spp., <i>Shigella</i> spp.	<i>E. coli</i> , <i>Shigella</i> spp.
S5	<i>E. coli</i> , <i>B.</i> spp., <i>P.</i> spp., <i>Staphylococcus</i> spp.	<i>E. coli</i> , <i>B.</i> spp., <i>Staphylococcus</i> spp.
S6	<i>B.</i> spp. <i>Staphylococcus</i> spp.	<i>K</i> spp., <i>Staphylococcus</i> spp. <i>P.</i> spp.
S7	<i>Shigella</i> spp., <i>B.</i> spp., <i>Staphylococcus</i> spp., <i>E. coli</i>	<i>B.</i> spp., <i>Shigella</i> spp., <i>E. coli</i>
S8	<i>K.</i> spp., <i>P.</i> spp., <i>E. coli</i> , <i>B.</i> spp.	<i>Shigella</i> spp., <i>Staphylococcus</i> spp., <i>P.</i> spp., <i>E. coli</i> , <i>K.</i> spp.,
S9	<i>B.</i> spp. <i>Staphylococcus</i> spp.	-

3.2.2 Total viable counts: membrane filtration method

The TVC detected in water sources sampled in the three LGAs in Benue South are presented in Table 3 above. The highest bacteria count (204 cfu per mL) was recorded in S3 in January 2023, and the lowest bacteria count (36 cfu per mL) was recorded in S2 in January 2023. No bacteria counts were recorded in S1 and S4 in March 2023. The variation in TVC may be due to environmental changes resulting from increased temperature and precipitation which can boost the survival rates of coliform bacteria [20]. For most of the sites sampled, bacteria count exceeded the minimum standard of 100 cfu per mL by WHO for drinking water except for S2, S6, and S7 in January 2023 and S5, S6, and S7 as well as S1 and S4 were non-bacteria counts were recorded. As the TVC in most sites exceeded the WHO standard, it is incumbent on the appropriate water regulatory bodies in Nigeria to address this concern which may lead to increase in diseases rate resulting from contamination of water sources by bacteria. Amuah et al. [21] in Ghana and Udooh et al. [22] in Nigeria had earlier suggested similar action to be taken to avoid consumption of contaminated water most especially by locals who do not have the means to purchase treated water.

3.3 Prevalence of Microorganisms in the Study Area

Table 4 above shows the prevalence of bacteria in the studied sites. *Escherichia coli* was the most prevalent bacteria as it occurred in all the sites sampled except in S6 and S9. This was immediately followed by *Staphylococcus* spp. and *Bacillus* spp. *Shigella* spp. and *Klebsiella* spp. were the least prevalent among the nine sites sampled in the three LGAs in Benue South. More bacteria composition was recorded in January 2023 compared to March 2023.

4. CONCLUSION

Our finding based on the concentration of pollution indicating physico-chemical variables (e.g., EC, BOD₅, and COD) showed the water sources studied in the three LGAs in Benue South were relatively perturbed. This was also confirmed by the prevalence of microbial loads which in most cases exceeded the minimum standard set by WHO [19] for drinking water. Sadly, from our investigation, most of the locals

depend on these water sources for their day-to-day activities. This calls for serious attention by the water regulatory agencies in Nigeria to ameliorate the impact of these pollutants in water sources around the study area and the entire Benue State to forestall the incidence of diseases caused by microbial loads and deteriorating water quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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