Bacteriological Quality Assessment of Water Sources in Otukpo Metropolis of Zone C, Benue State, Nigeria

*1Yandev D, 2Abakpa GO, 3Ujoh AJ, and 4Agboola O.O.

*1(a), 2, 3Department of Microbiology, Federal University of Health Sciences, Otukpo, Nigeria.

Abstract

The present study carried out a bacteriological quality assessment of water sources in Otukpo metropolis of zone C, Benue State, Nigeria. Water samples were collected in triplicates from five different sources (Miracle water, River Ewulo, Water board, Okpokwu River and Ochito Ghana River) labeled A-E respectively. Standard bacteriological analyses were carried out. Results were compared with the WHO standard guideline for potable water. A total of eight (8) species of bacteria were found. Miracle water had the least average number of bacterial species (1.3 species) while Ochito Ghana water had the highest number of 4.3 species. Number of bacterial species found in water sample was location dependent (P<0.05). Klebsiella spp was the most prevalent (60%) while Micrococcus spp., Shigella spp. and E. coli each had a prevalence of 40%. Total heterotrophic count and total coliform count (in cfu/ml) exceeded WHO permissible limits for potable water in all water sources, although bacterial load significantly varied from sample to sample (P<0.05). Thus, Ochito Ghana water was heavily loaded with bacterial contaminants. Analysis of the first sample of Ochito water (the most contaminated sample) showed MPN of >1600/100ml. In direct plate count of this sample, THC was 252.0x10³ cfu/ml while TCC= 140.0±8.0 x10³ cfu/ml. Also membrane filtration method gave THC of 291.0±7.0x10³ cfu/ml while TCC was 108.0±4.0 x103 cfu/ml. River Ewulo was second highest in terms of bacterial contamination. All water samples were assigned "unsatisfactory" status using WHO classification of MPN indices where MPN values are >10/100ml. However, water board noozle 1 sample 1had MPN of 9/100ml and assumed the" suspicious" status. The high amount of bacterial contaminants found in the various water sources rendered them unsafe for drinking. The only exception is the Water board nozzle 1 sample that contained very minimal bacterial load. The result suggests a need for stakeholders' intervention to prevent waterborne disease outbreaks in the affected areas. Sources of contamination should be critically studied and reported for control measures.

Keywords: Quality assessment, water sources, contaminants, public health

Introduction

Water is essential to the existence of man on earth. Generally, main sources of water are: groundwater, surface water (rivers, streams and ponds), atmospheric water (rain-water, snow) and springs. Rivers are the most important freshwater resource available to the local inhabitants which are either unsafe or difficult to obtain and are severely stressed by poor management (Anhwange et al., 2012) [8]. These make access to clean water a serious problem, in some instances women and children need to walk for hours to fetch ordinary drinking water. The quality of these water bodies vary widely depending on the location and environmental factors (Anhwange et al., 2012) [8]. The major source of groundwater is precipitation that infiltrates the ground and moves through the soil and pore spaces of rocks. Other sources include water infiltrating from lakes and streams, recharge ponds and water treatment systems. As groundwater moves through soil sediments and rocks, many impurities such as disease-causing

micro-organisms are filtered. In developing countries such as Nigeria many water resources are unhealthy because they contain harmful physical, chemical and biological agents (Asen *et al.*, 2019) ^[9]. To maintain good health, water should be safe to drink and meet the local and international standards. To monitor the water resources and ensure sustainability, national and international criteria and guidelines established for water quality are being used (WHO, 2020) ^[27]. In Benue State, despite efforts made by Government to provide potable water to urban and rural areas, a large percentage of the water supply schemes are malfunctioning, forcing consumers to use unprotected sources that pose health hazards (Anhwange *et al.*, 2012) ^[8].

In many studies, cases of water contamination have been reported. It has clearly been established that, pollution of source of domestic water which are surface water is an ongoing problem in most Nigerian communities, especially the government-ignored villages. Currently, 1.1 billion people

^{*1(}b)Department of Microbiology, Water and Public Health Research Group, University Of Nigeria.

⁴Department of Biological Sciences, Federal University of Health Sciences, Otukpo, Nigeria.

lack access to safe water, and 2.6 billion people do not have proper sanitation, primarily in developing countries, and an imbalance exists between rural and urban areas in access to both improved sanitation and safe drinking water supply (Nwafor et al., 2013) [22]. On a global scale, the restricted access to safe water and to improved sanitation causes 1.6 million deaths per year; more than 99% thereof occur in the developing world. Nine out of ten incidents affect children, and 50% of childhood deaths happen in sub-Saharan Africa. The easily preventable diarrheal diseases caused by unsafe water and lack of sanitation and hygiene contribute to 6.1% of all health-related deaths; one report estimates that unsafe water is responsible for 15% to 30% of gastrointestinal diseases. The main acute disease risk associated with drinking water in developing and transition countries is due to wellknown viruses, bacteria, and protozoa, which spread via the fecal oral route (Ishaku and Ezeigbo, 2010) [16]. Today's hygiene concept relies on the detection of such indicators as a hygienic drinking water quality parameter, and the enteric bacterium E. coli is used worldwide as an indicator of possible fecal contamination (Ebob, 2019) [11]. In addition, the general microbiological state of water is assessed by counting the total number of colony-forming microbes growing on a nutrient agar plate (the heterotrophic plate count, HPC). As the HPC method largely underestimates the number of heterotrophic microbial cells present in a water sample, the HPC was omitted from the recent lists of hygiene parameters of WHO, the European Union, and the United States (WHO, 2020) [27].

Otukpo community is the second largest town in Benue State located in the middle belt region of Nigeria with an estimated population of 359, 600 as at 2016 with an annual population growth rate of 3%. Access to drinking water is one of the major challenges of the Idoma people as it is in many parts of Africa. While there are many reports on the quality of surface and underground water in many parts of Benue State (Akaahan et al., 2016; Shar et al., 2021) [6, 24], there is insufficient data on the overall quality and safety of drinking water from different sources available to people in Otukpo LGA and by extension, Benue State at large. The residents are faced with challenges of water crisis as in the case of other places in Nigeria where access to portable water is a big problem (Ishaku and Ezeigbo, 2010; Ahile et al., 2015) [16, 5]. In the study area, people rely on different sources of water available to them such as river, lakes, well and borehole (Ahile et al., 2015) [5]. Many people fetch water from cracked rock called miracle water because it does not dry up. The Otukpo water board is also overs stretched with the burden of water supply to meet the demand of the people. To safeguard the health of the residents, it is imperative that the quality of the water from these sources be ascertained in line with WHO recommendation. The present study carried out a bacteriological quality assessment of water sources in Otukpo metropolis of zone C, Benue State, Nigeria.

Materials and Methods The Study Area

The study area is Otukpo metropolis, the headquarter of the Idoma people. Otukpo LGA is one of the 23 LGAs of Benue State located in the middle belt axis of Nigeria. The geographic coordinate is 7'11'35N-8'8'47E within an area of 1,385 km². It had a population of 266, 411 people as at 2006 census (NPC 2006) with an annual population change of 3%. It was projected as 359, 600 people as at 2016 (NPC, 2016) [21]

Sample Collection

Water samples were collected in triplicates from 5 main sources of drinking water using sterilized plastic containers with rubber cork. The five water sources are: Miracle water, River Ewulo, Water board, Okpokwu River and Ochito Ghana River as labeled A-E respectively. A total of 15 water samples were collected and packaged in separate sterile bag. They were transported to the laboratory for bacteriological analysis. The sampling points and identification number are given in table 1.

Table 1: Water sources and sampling points

Water Sample ID	Sources/sampling points		
A1	Miracle water sample 1		
A2	Miracle water sample 2		
A3	Miracle water sample 3		
B1	River Ewulo upper stream		
B2	River Ewulo middle stream		
В3	River Ewulo down stream		
C1	Water board public nozzle 1		
C2	Water board public nozzle 2		
C3	Water board public nozzle 3		
D1	Okpokwu river sample 1		
D2	Okpokwu river sample 2		
D3	Okpokwu river sample 3		
E1	Ochito Ghana sample 1		
E2	Ochito Ghana sample		
E3	Ochito Ghana sample 3		

Sample Inoculation

Sample inoculation was done by adding 1ml of water suspension on nutrient agar, MacConkey agar and *Salmonella-Shigella* agar (SSA). Incubation was done at 37°C for 24hours (Abdullahi *et al.*, 2010)^[1].

Cultural and Biochemical Characterization

Morphological observations were recorded on the culture media. These include the colour, shape and outline of the colony as well as shape of each bacterium. Motility test was done by adding a drop of peptone water on a glass slide containing bacterial colony covered with a slip and viewed under the microscope with high power objective lens (Cheesbrough, 2006) [10]. Discreet colonies were sub-cultured on Nutrient agar plate for biochemical test (Hedderwick *et al.*, 2010) [14]. Identification of bacteria species was done using standard microbiological procedures for each of the following biochemical tests: Gram staining, catalase, citrate, urease, indole and hydrogen sulphide tests (Cheesbrough, 2006) [10]. All identified isolates were recorded per water sample.

Direct Plate Count and Membrane Filtration Methods

Serial dilution, pour plates techniques and incubation (37°C for 24 hours) methods employed (Cheesbrough, 2006) $^{[10]}$. Visible colonies on the plates were counted using Colony Counter and Membrane Filtration methods (Metricel® Black PES 0.45µm membrane disc filter). Total Heterotrophic Counts (THC) and Total Coliform Count (TCC) were recorded in cful/ml (colony forming unit per mililitre (Cheesbrough, 2006) $^{[10]}$.

Most Probable Number (MPN) Water Testing

The methods of Cheesbrough (2006) [10] and Adeiza *et al.* (2018) [2] were used. In the presumptive test, five and ten tubes of double and single strength MCA (McConkey agar) respectively were taken from each water sample. Exactly 10ml and 1ml of water sample were added to each of the five and ten tubes respectively while 0.1ml was added to the remaining five tubes of single strength MCA broth. Incubation was done at 37°C for 24 hours. Number of positive tubes was compared. In the confirmatory test, a loopful of medium was transferred from each of the fermented tube in the presumptive test to

- i) 3ml of lactose broth in a sterile tube
- ii) An agar slant
- iii) 3ml of tryptone water.

The lactose broth was incubated at 37°C for 24-48 hours and inspected for gas formation. Gram's stain was added to the agar slant to check for Gram negative bacilli without spore formation. Tryptone water was incubated at 44.5°C for 18-24 hours followed by addition of 0.1ml Kovac's reagent and observed for red colour formation to indicate indole positive reaction. In the completed test, a loopful from lactose broth tube was streaked into Eosin methylene blue agar and incubated at 44.5°C for 24 hours. The values of MPN were estimated per 100ml of water sample (Adeiza *et al.*, 2018) [2].

Data Analysis

Data collected were analysed on the Minitab (17.0) software for descriptive statistical tools. One way ANOVA and Chisquare tests were applied at 95% confidence limit ($P \le 0.05$). The WHO standard permissible limit served as a reference guide for water quality parameters.

Results

Table 2 gives the cultural and biochemical identities of the bacterial isolates. A total of eight (8) species of bacteria were identified including: *Bacillus* spp., *Staphylococcus* spp, *Klebsiella* spp., *Escherichia coli*, *Micrococcus* spp., *Proteus* spp., *Enterobacter* spp. and *Shigella* spp. In terms of distribution (Table 3), number of contaminants ranged from 1 contaminant (in Miracle water sample 2 and 3) to 5 contaminants in Ochito Ghana sample 1. There were no bacterial contaminants found in River Ewulo downstream sample. Miracle water had the least average number of bacterial species (1.3 species) in while Ochito Ghana water

had the highest number of 4.3 species (Figure 2). It was observed that the number of bacterial species was location dependent (P<0.05). *Klebsiella* spp was the most frequently occurring contaminant where it was found in 60% of samples. *Micrococcus* spp., *Shigella* spp. and *E. coli* each had a percentage occurrence of 40% while other species fell below 30%. Therefore, level of contamination significantly depend on the type of bacteria ($\chi^2 = 42.65$, P<0.05).

Table 4 gives the estimates of bacterial load in water samples using two methods: Direct Plate Count (DPC) and Membrane Filtration (MF) methods. All values of THC (total heterotrophic count) and TCC (total coliform count) exceeded WHO permissible limits for potable water. In DPC method, THC significantly varied from 40.0±4.0 x 10³ cfu/ml (Water board public nozzle 3) to 252.0±4.0 x 10³ cfu/ml (Ochito Ghana sample 3) as against <500 cfu/ml regulatory limit. Also, TCC was between 9.0±1.0 x 10³ cfu/ml (Water board public nozzle 3) and 140.0±8.0 x 10³ cfu/ml (Ochito Ghana sample 1) as against standard zero (0) limit of coliform for potable water. In MF method, THC ranged from 48.0±0.0 x 10^3 cfu/ml (Water board public nozzle 3) to $291.0\pm7.0x$ 10^3 cfu/ml (Ochito Ghana sample 1). Also, TCC ranged from 10.5 ± 1.5 cfu/ml (sample C3) and 108.0 ± 4.0 x 10^3 cfu/ml (Ochito Ghana sample 1). Variation in level of bacterial load is shown in Figures 3 and 4. In the two methods used, THC was higher than TCC but complimentary. From the graphs, water samples from Miracle water and Water board contained the lowest amount of bacterial load whereas samples from Ochito Ghana water were heavily loaded with heterotrophic and coliform bacteria, followed by samples from River Ewulo. Bacterial load was location dependent (P<0.05).

The above outcome was supported by the presumptive, confirmatory and completed MPN tests where activities of bacteria such as gas formation, indole production and growth with green metallic sheen were mostly observed in Ochito Ghana and Ewulo upper and middle stream (Table 5). Therefore, sample Ochito Ghana water sample 1 had the highest MPN of >1600/100ml followed by 900/100ml in samples 2 and3. In this study, all water samples were assigned "unsatisfactory" status using WHO classification of MPN indices for samples whose MPN values are >10/100ml. The only exception was Water board public nozzle 1 that had MPN value of 9/100ml thus assigned the "suspicious" status. There was no water sample considered as "satisfactory" as none met the WHO permissible limit of 2.2/100ml MPN value.

Table 2: Cultural and Biochemical	Characterization of Bacterial Isolates
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Colony colour	Colony shape	Elevation	Morphology	Gram'staining	Motility test	Catalase test	Citrate test	Urease test	Indole test	Bacteria
White	Circular	Flat	Rod	+	+	+	+	-	-	Bacillus spp.
Cream	Circular	Raised	Cocci	+	-	+	+	-	-	Staphylococcus spp.
Mucoid pink	Circular	Raised	Rod	-	-	+	+	+	-	Klebsiella spp.
Pink	Regular	Raised	Rod	-		+	-	-	+	Escherichia coli
Cream	Regular	Raised	Cocci	+	-	+	-	-	-	Micrococcus spp.
Pale	Regular	Raised	Rod	-	-	-	+	+	-	Proteus spp.
Pink	Regular	Raised	Rod	-		+	+	+	-	Enterobacter spp.
Pale	Circular	Raised	Rod	-	-	+	-	-	-	Shigella spp.

Table 3: Distribution of bacterial contaminants in water samples

Water Sample ID	Klebsiella spp.	Micrococcus spp.	E. coli	Staphylococcus spp.	Proteus spp.	Enterobacter spp.	Shigella spp.	Bacillus spp.	Contaminants
A1	+	+							2

A2			+						1
A3			+						1
B1	+			+					2
B2			+		+	+			3
В3									0
C1	+	+							2
C2	+	+							2
С3	+	+			+				3
D1	+			+			+	+	4
D2	+	+					+		3
D3		+		+	+		+		4
E1	+		+			+	+	+	5
E2			+			+	+	+	4
E3	+		+		+		+		4
Frequency	9	6	6	3	4	3	6	3	
Relative proportion	60%	40%	40%	20%	26.7%	20%	40%	20%	

 χ^2 (Distribution of contaminants and water sample) = 42.65, P=0.00 (P<0.05)

A1-A3= Miracle water sample 1-3; B1=River Ewulo upper stream; B2= River Ewulo middle stream; B3= River Ewulo

downstream; C1= Water board public nozzle 1; C2= Water board public nozzle 2; C3= Water board public nozzle 3; D1-D3= Okpokwu river sample 1-3; E1-E3= Ochito Ghana sample 1-3.

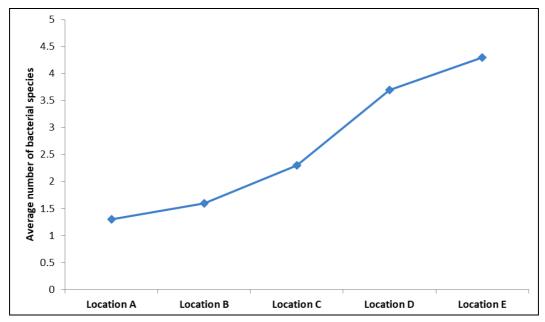


Fig 1: Number of bacterial species found in water sources

Key: Location A= Miracle water; **Location B**= River Ewulo;

Location C= Water board; Location D= Okpokwu river; Location E= Ochito Ghana

Table 4: Bacterial load using Direct Plate Count and Membrane Filtration methods

Sample ID	Direct Plate	Count (DPC)	Membrane Fi	ltration (MF)
	THC (10 ³ cfu/ml)	TCC (10 ³ cfu/ml)	THC (10 ³ cfu/ml)	TCC (10 ³ cfu/ml)
A1	92.0±4.0	34.0±2.0	187.0±11.0	30.0±2.0
A2	76.0±0.0	12.0±0.0	122.0±6.0	11.5±3.5
A3	94.0±2.0	15.5±0.5	168.0±4.0	24.0±2.0
B1	120±4.0	44.0±0.0	204.0±12.0	54.5±1.5
B2	124±0.0	54.0±2.0	220.0±8.0	68.0±4.0
В3	118±2.0	32.0±0.0	186.0±2.0	46.0±2.0
C1	60.0±4.0	23.5±1.5	68.0±4.0	29.0±3.0
C2	46.0±2.0	11.5±0.5	62.0±6.0	16.0±0.0

C3	40.0±4.0	9.0±1.0	48.0±0.0	10.5±1.5	
D1	80.0±4.0 18.5±2.5		120.0±4.0	32.5±0.5	
D2	66.0±2.0	16.5±1.5	110.0±2.0	26.0±2.0	
D3	84.4±4.0	12.0±0.0	130.0±2.0	29.0±3.0	
E1	252.0±00	140.0 ± 8.0	291.0±7.0	108.0±4.0	
E2	240.0±4.0	106±2.0	274.0±2.0	94.0±2.0	
E3	252.0±4.0	92.0±4.0	290.0±2.0	106.0±2.0	
WHO	< 500	0	< 500	0	

Key:

A1-A3= Miracle water sample 1-3; B1=River Ewulo upper stream; B2= River Ewulo middle stream; B3= River Ewulo down stream; C1= Water board public nozzle 1; C2= Water

board public nozzle 2; C3= Water board public nozzle 3; D1-D3= Okpokwu river sample 1-3; E1-E3= Ochito Ghana sample 1-3

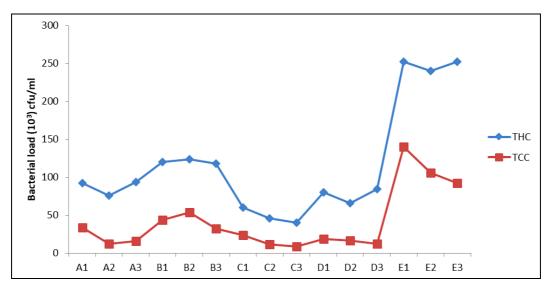


Fig 2: Varied level of THC and TCC of bacteria using Direct Plate Count

 χ^2 (Total heterotrophic count and location) = 85.74, P=0.00 (P<0.05)

 χ^2 (Total coliform count and location) = 51.5, P=0.00 (P<0.05)

Legend:

THC= Total heterotrophic count

TCC= Total coliform count

A1-A3= Miracle water sample 1-3; B1=River Ewulo upper stream; B2= River Ewulo middle stream; B3= River Ewulo downstream; C1= Water board public nozzle 1; C2= Water board public nozzle 2; C3= Water board public nozzle 3; D1-D3= Okpokwu river sample 1-3; E1-E3= Ochito Ghana sample 1-3.

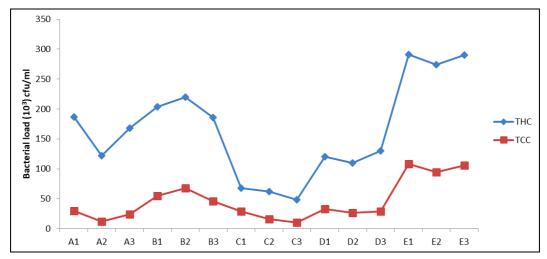


Fig 3: Varied level of THC and TCC of bacteria using Membrane Filtration

 χ^2 (Total heterotrophic count and location) = 76.8, P=0.00 (P<0.05)

 χ^2 (Total coliform count and location) = 49.6, P=0.00 (P<0.05)

Legend:

THC= Total heterotrophic count

TCC= Total coliform count

A1-A3= Miracle water sample 1-3; B1=River Ewulo upper stream; B2= River Ewulo middle stream; B3= River Ewulo down stream; C1= Water board public nozzle 1; C2= Water

board public nozzle 2; C3= Water board public nozzle 3; D1-D3= Okpokwu river sample 1-3; E1-E3= Ochito Ghana sample 1-3

Table 5: Most Probable Number (MPN) of bacteria in water samples

Water Sample ID	Gas formation	Gram –ve Bacilli	Indole production	Growth with green metallic sheen	Combination of positive tubes	MPN/100ml	Status
A1	-	-	-	-	3-2-1	17	Unsatisfactory
A2	-	-	-	-	3-0-1	14	Unsatisfactory
A3	-	-	-	-	3-2-1	17	Unsatisfactory
B1	+	-	-	+	5-3-1	110	Unsatisfactory
B2	+	+	+	+	5-3-3	170	Unsatisfactory
В3	-	-	-	-	4-4-0	33	Unsatisfactory
C1	-	-	-	-	2-2-2	9	Suspicious
C2	-	-	-	-	2-3-0	12	Unsatisfactory
C3	-	-	-	-	2-3-0	12	Unsatisfactory
D1	-	-	-	-	4-4-0	33	Unsatisfactory
D2	-	-	-	-	4-3-1	27	Unsatisfactory
D3	-	-	-	-	3-2-0	14	Unsatisfactory
E1	+	+	+	+	5-5-5	>1600	Unsatisfactory
E2	+	+	+	+	5-5-3	900	Unsatisfactory
E3	+	+	+	+	5-5-3	900	Unsatisfactory
WHO limit						2.2	Satisfactory

WHO Classification

0= excellent; 1-3= satisfactory; 4-10= suspicious; Above 10-unsatisfactory

Legend: A1-A3= Miracle water sample 1-3; B1=River Ewulo upper stream; B2= River Ewulo middle stream; B3= River Ewulo down stream; C1= Water board public nozzle 1; C2= Water board public nozzle 2; C3= Water board public nozzle 3; D1-D3= Okpokwu river sample 1-3; E1-E3= Ochito Ghana sample 1-3

Discussion

The eight species of bacteria reported in this work are clinically important from the public health point of view and therefore cannot be overlooked. They have been implicated in causing gastroenteritis, diarrhoea and food poisoning among other diseases. Also, cases of multi drug resistance by these pathogens have been reported (Jacob and Cohen, 2016; Feglo and Sakyi, 2012; Hernández-Cortez et al., 2017; Jamal et al., 2018; Mir et al., 2018) [17, 13, 15, 19]. Meanwhile, WHO has clearly defined pure and safe drinking water of an area as any 100 water samples without pathogens (WHO, 2020) [27]. These pathogens were previously reported to cause water related disease outbreaks with many casualties across many communities in Nigeria (Abdullahi et al., 2010; Shobowale et al., 2016, Obioma et al., 2017; Adeiza et al., 2018) [1, 25, 23, 2]. The presence of E. coli and Shigella was an indication of faecal contamination of water sources and it is the most frequently occurring etiologic agent of diarrhea (Elum et al., 2022) [22]. Bacillus, Staphylococcus and Proteus had been reported to possess toxins capable of causing food poisoning (Elum et al., 2022) [22]. Klebisella was among the most frequently reported enterogenic pathogens associated with opportunistic infections in immuno-compromised individuals as a common microbiota of the respiratory tract (Obioma et al., 2017) [23]. A study carried out by Tanimu et al. (2011) [26] linked the occurrence of contaminants found in reservoirs supplying drinking water to the people of Kaduna South to the

wastes generated along the supply chain. In terms of bacteriological assessment, Ochito Ghana water was the most contaminated followed by River Ewulo.

The WHO standard does not permit any viable pathogenic bacterium cell to be present in drinking water because if the water is allowed to stand for more days, such a pathogen could multiply rapidly over time to increase the load. This position aligns with other reports stating that bacterial load in drinking is a function of time taken to store such samples in any conducive environment (Amer and Abdel-Gawad, 2012) [7]. The presence of faecal coliforms in the water samples is indicative of water borne diarrheahenic bacteria. In the two methods used, THC was higher than TCC but complimentary. WHO set regulatory limits for total heterotrophic count that must exceed 500 cfu/ml while total coliform count was set at zero by exceeding standard limits, the affected water sources are unsafe for drinking. Result was consistent with the work of Agyo et al. (2020) [4] who carried out a bacteriological quality of water in private wells and boreholes in Makurdi Metropolis, Benue State, Nigeria.

The general public are advised to ensure regular treatment and sterilization of water fetched from these sources before consumption. Water tankers should be trained on public health safety, hygiene and regulatory requirement in the water production cycle. Measures should be put in place to keep drinking water free from bacterial contaminants. All water sources used by the public should be monitored to ensure strict compliance with standard guideline to prevent occurrence of water borne diseases. This view is topical among stakeholders as strategies to prevent enteric diseases (Monney *et al.*, 2014; Adesegun *et al.*, 2020) [20, 3]. The above strategies are in line with the position of the WHO and UN on drinking water supply (WHO, 2020) [27].

Conclusion and Recommendation

A total of eight (8) pathogenic species of bacteria were found in water samples. Total heterotrophic count and total coliform count (in cfu/ml) and MPN value (in 100ml) exceeded WHO permissible limits for potable water in all samples, although bacterial load significantly varied from sample to sample (P<0.05). In terms of bacteriological assessment, Ochito Ghana water was the most contaminated followed by River Ewulo. The high amount of bacterial contaminants found in the various water sources rendered them unsafe for drinking. The only exception is the Water board nozzle 1 sample that contained very minimal bacterial load. The result suggests a need for stakeholders' intervention to prevent waterborne disease outbreaks in the affected areas. Sources of contamination should be critically studied and reported for control measures.

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