

Microbiological Quality and Physico-chemical Properties of Bore-Hole Water from Stored Water Tanks in Selected Hostels in Ifite-Awka, Nigeria

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ABSTRACT: The microbiological and physicochemical properties of bore-hole waters from water reservoirs in selected hostels in ifite-Awka metropolis were evaluated. Five (5) bore-hole water samples from stored-water tanks were evaluated to ascertain the physicochemical parameters, presence and population of different bacterial and fungal groups. Total heterotrophic bacterial (THC) counts ranged from 1.20×10^3 cfu/ml to 6.5×10^3 while the fungal counts spanned from 2.5×10^3 cfu/ml to 8.9×10^3 cfu/ml. Bacteria obtained from the borehole waters include *Salmonella* spp., *Escherichia coli* and *Shigella* spp. *E. coli* was the most prevalent with MPN 380/100 ml of water reported in sample A while *Shigella* sp. was the least prevalent with MPN 130/100 ml of water reported in samples B and E. Fungal isolates obtained include *Aspergillus* species, *Candida* species, *Acremonium* species and *Cladosporium* species. *E. coli* and *Aspergillus* spp. were predominant than other isolates. The pH ranged from 6.65 to 7.47; hardness ranged from 92 mg/l to 156 mg/l and Iron concentration ranges from 0.267 ppm to 0.378 ppm, phosphate contents ranged from 2.375 to 6.125 while Nitrate contents ranged from 1.071 to 6.214. The presence of these organisms in water meant for municipalities indicates faecal contamination. This calls for improved sanitary conditions of reservoir tanks in these locations and beyond.

KEYWORDS: Bore-hole water; faecal coliforms; microbial assessment; physicochemical properties; stored water tanks.

1. Introduction

A large percentage of the population in developing countries, mainly African countries, lacks access to potable water supply. As a result, they are compelled to use untreated water from other sources such as rivers, reservoirs, springs, streams, and groundwater for drinking and other domestic purposes [1]. The citing of a borehole or well requires thorough consideration of several factors, including the location of the borehole or well in relation to surface drainage and groundwater flow. Globally, 20% of irrigation water and 40% of water used in

industry are derived from groundwater [2]. Groundwater serves as a major source of drinking water worldwide and is stored in aquifers. The hydrological recharge of aquifers varies significantly geographically and depends on various factors such as climate, geology, soil type, vegetation, and land use [3]. The presence of total coliform in water samples are therefore, an indication that opportunistic pathogenic bacteria such as *Klebsiella* and *Enterobacter* which can multiply in water environments and pathogenic pathogens such as *Salmonella* spp., *Shigella* spp., *V. cholera*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *E. coli* may be present [4].

The presence of microorganisms in water helps in the estimation of water quality [5]. The fecal coliform group includes other organisms such as *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., which are not exclusively of fecal origin. *E. coli*, on the other hand, is specifically of fecal origin and can be found in birds, humans, and other warm-blooded animals. Fecal coliform bacteria are considered to be a more specific indicator of the presence of fecal matter [6]. Fecal coliforms are generally used to indicate unacceptable microbial water quality and can be used as an indicator in place of *E. coli* [7]. Despite the shortcomings of indicator microorganisms, it is better to use a combination of indicators to obtain a more accurate picture of the microbiological quality of water. In general, each country has its own set of guidelines for drinking water, although most of these guidelines are similar across different countries and utilize the same indicator microorganisms to detect the presence of pathogenic microorganisms [8].

Drinking water and poultry water samples are often contaminated with microorganisms that pose public health risks [9]. The presence of total coliforms in water samples indicates that opportunistic pathogenic bacteria such as *Enterobacter* and *Klebsiella* can multiply in water environments, and pathogenic pathogens such as *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella* spp., *Shigella* spp., *V. cholera*, *E. coli*, and *Yersinia enterocolitica* may be present [10]. Regular monitoring of the quality of drinking water is important because polluted water is unfit for human consumption. To obtain purer and higher-quality water, it should be assessed physicochemically for its metal contents, temperature, color, odor, pH, nitrate, and organic residues [11, 12]. The aim of this research is to examine the microbial quality and physicochemical properties of borehole water samples from stored water tanks in selected hostels in Ifite Awka Metropolis. The objectives of this research was to assess bacterial and fungal contaminants in stored water, isolate and identify bacterial and fungal contaminants from stored water tanks, and evaluate the physicochemical properties of borehole water in stored water tanks in the study area.

2. Materials and Methods

2.1. Study area.

The research was conducted in Ifite, Awka, the capital of Anambra State, located in South East Nigeria, with coordinates of 6.22200N, 7.08210E. The study was limited to selected hostels within the study area. Boreholes belonging to households that primarily rely on borehole water as their main source of drinking and household water were utilized for the study.

2.2. Sample collection.

Stored borehole water samples were randomly collected from five (5) different water storage tanks in selected hostels for the study. To collect the samples, each respective tank was opened and water was allowed to run for the first 2 minutes before sterile sample bottles were opened for collection. The samples were collected into 30 ml sterile screw-capped containers and appropriately labeled. They were then placed in ice packs and immediately transported to the microbiology and chemical laboratories for microbiological and physicochemical analyses, respectively. The selected samples used in this study were designated as A-E.

2.3. Microbiological analyses.

Microbial assessment of the borehole water samples was conducted using methods previously described by [13].

2.3.1 Enumeration of total heterotrophic and coliform Bacteria.

Total heterotrophic bacteria counts were estimated using methods described by [14], while coliform counts were determined using the most probable number (MPN) technique as described by [13].

2.4. Biochemical identification of the isolates.

Different biochemical tests were performed using standard methods previously described by [15]. For bacterial isolates, tests such as coagulase, urease, citrate utilization, sugar fermentation, methyl-red, indole, and motility were conducted. Gram stain techniques were employed for both bacterial isolates and yeast cells. The lactophenol cotton blue stain test was used for identifying mold isolates.

2.5. Physicochemical analyses.

Physicochemical parameters as pH, water hardness, nitrate, phosphate and iron contents were determined using the previous methods of [16, 17].

2.5.1. pH determination.

The Laboratory Hanna model HI991300 pH meter was used in the determination of pH using Electrometric Methods.

2.5.2. Determination of water hardness.

The hardness was determined by introducing 50 cm³ of the respective borehole water samples into a crucible and then adding 1cm³ buffer solution of NH₃ into it. This was then followed by adding 3 drops of Solochrome Black T indicator and the set up swirled vigorously. The set up was then titrated using 0.01EDTA solution until there was color change from red to blue. The total hardness then was calculated thus:

$$\text{Total hardness (mg/CaCO}_3\text{)} = \frac{\text{Volume of titrate} \times 1000}{\text{Volume of samples (cm}^3\text{)}}$$

2.5.3. Nitrate determination.

The nitrate content was determined using a UV spectrophotometer (PD303). Firstly, a porcelain dish was utilized to measure 50 ml of the borehole water sample, which was then placed on a hot water bath to evaporate to dryness. During the process, a glass rod was constantly used to stir the residue after adding 2 ml of phenol disulphonic acid to dissolve it. Subsequently, distilled water and concentrated sodium hydroxide solution were added to make the residue alkaline. The residue was then filtered into a Nessler's tube, and distilled water was gently added to bring the volume up to 50 ml. After color formation, a spectrophotometer was used to measure the absorbance at 410 nm. A standard curve was created by plotting the concentration along the X-axis and the spectrophotometric readings (absorbance) along the Y-axis. The nitrate content was estimated by comparing the absorbance values of the sample with the standard curve, and the results were expressed in units of mg/l.

2.5.4. Phosphate content determination.

The phosphate content was determined using standard methods described by [13]. Initially, a conical flask was used to weigh 100 ml of the sample, which was then homogenized and filtered. Another conical flask was prepared by weighing the same volume of distilled water (used as a control). To both conical flasks, 1 ml of 18 M H₂SO₄ and 0.89 g of ammonium persulfate were added. The mixtures were gently boiled for 1 hour and 30 minutes while maintaining the volume at 35 cm³ with distilled water. After cooling, one drop of phenolphthalein indicator was added to each flask. The solutions were then neutralized to a light pink color using 2 M NaOH solution. The pink color was further neutralized by adding 2 M hydrochloric acid dropwise, and the solutions were made up to 100ml using distilled water. Next, 20 ml of the sample was pipetted into test tubes for the colorimetric analysis. Then, 10 ml of the combined reagent was added to each test tube, shaken, and allowed to stand for 10 minutes before measuring the absorbance at 690 nm using a spectrophotometer. As a reference standard, 20ml of distilled water and 1ml of the reagent were used.

2.5.5. Determination of iron content.

Metal analysis was determined using Varian AA240 Atomic Absorption Spectrophotometer according to previously described method of [17]

2.6. Ethical approval.

Approval was duly obtained from heads of the hostels used for the study before collection of the samples was made.

3. Results and Discussion

3.1. Microbial and biochemical properties of bore-hole waters from stored water tanks.

The Bacterial and fungal counts of isolates from the water samples in hostels in ifite Awka metropolis are shown in table 1. The total bacterial count ranges from 1.78x10⁴ to 3.5x10⁴

cfu/ml, where Sample E had the highest plate count of 3.5×10^3 cfu/ml and Sample B the least count of 1.78×10^4 . Fungal counts ranged from 1.22×10^4 cfu/ml to 4.5×10^4 cfu/ml among the sampled borehole waters.

Table 1. The Bacterial and fungal counts of the isolates from bore-hole water samples.

Samples	Bacterial count (10^4 cfu/ml)	Fungal counts (10^4 cfu/g)
A	2.20	4.50
B	1.78	2.52
C	1.95	3.78
D	2.15	3.12
E	3.50	1.22

The morphological and cultural characteristics of the bacterial isolates are shown in Table 2.

Table 2. Morphological properties of bacterial isolates from the stored water tanks.

Isolates	Elevation	Colour	Texture	Shape	Probable Organism
A	Convex	Dark Metallic Sheen	Smooth	Circular	<i>E. coli</i>
B	Raised	Milky	Smooth	Circular	<i>Salmonella</i> sp.
C	Convex	Dark Metallic Sheen	Smooth	Circular	<i>E. coli</i>
D	Raised	Cream	Smooth	Circular	<i>Shigella</i> sp.
E	Convex	Dark Metallic Sheen	Smooth	Circular	<i>E. coli</i>

The bacterial isolates in this study have intact margins. The isolated bacteria include *E. coli*, *Salmonella species*, and *Shigella species*. The presence of these bacteria in water is consistent with the findings of previous research conducted by [2]. *E. coli* was the most prevalent bacterium isolated in this study, which aligns with the previous research [18, 19], both of which identified *E. coli* as the predominant microorganism in water based on their respective studies.

Table 3. Biochemical properties of bacterial isolates from the bore-hole water.

Isolate SN	Gram reaction	Shape	Indole	Catalase	Voges proskauer	Citrate	Urease	Methyl Red	Sugar Ferm. Test	Fructose	Glucose	Lactose	Probable organism
A	-	Rods	+	+	-	+	+	-	A	A/G	A	A	<i>Salmonella</i> sp.
B	-	Rods	-	+	-	+	+	+	A	A/G	+	+	<i>Shigella</i> sp.
C	-	Rods	+	+	+	+	+	-	A/G	A/G	-	-	<i>E. coli</i>
D	-	Rods	+	+	+	+	+	+	A/G	A/G	-	-	<i>E. coli</i>
E	-	Rods	+	+	+	+	+	+	A/G	A/G	-	-	<i>E. coli</i>

Key: A/G = Acid and Gas production; + = Positive; - = Negative; MR = Methyl Red; A = Acid production

The presence of coliform microorganisms in the samples indicated potential contamination with fecal matter, which aligns with the findings of [20] who detected pathogenic bacteria in water environments.

Table 4. Coliform counts of the bore-hole water samples from the selected hostels

Samples	<i>Salmonella</i> sp. (MPN/100ml)	<i>E. coli</i> (MPN/100ml)	<i>Shigella</i> sp. (MPN/100ml)
A	220	380	160
B	200	320	130
C	190	310	140
D	170	340	160
E	150	370	130

The examined borehole water samples from all selected hostels had counts that exceeded the acceptable limit of 0 MPN values per 100 ml set by the World Health Organization (WHO). Coliform bacteria were detected in significant numbers in all the samples, and their values were reported as most probable numbers (MPN). Bacteria obtained from the borehole waters include *Salmonella* spp., *E. coli* and *Shigella* spp. *E. coli* was the most prevalent, with MPN 380/100 ml of water reported in sample A while *Shigella* sp. was the least prevalent with MPN 130/100 ml of water reported in samples B and E. The isolated fungi belonged to four genera which include *Aspergillus* species, *Candida* species, *Acremonium* species and *Cladosporium* species with a septate cell wall and presence of conidiospores and chlamyospores. Table 5 shows the morphological properties of the fungi obtained from the borehole waters.

Table 5. Morphological properties of fungi from the water samples.

Samples	Nature of hyphae	Spore type	Fungi
A1	Septate uninucleated	Conidiospore	<i>Aspergillus</i> spp.
B2	Septate uninucleated	Conidiospore	<i>Aspergillus</i> spp.
C3	Septate uninucleated	Chlamyospore	<i>Candida lipolytica</i>
D4	Septate uninucleated	Conidiospore	<i>Acremonium</i> spp.
E5	Septate uninucleated	Conidiospore	<i>Cladosporium</i> spp.

Many of the fungi isolated in this study, were previously reported by [20] who isolated *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp. and *Acremonium* spp. from drinking water. In this study Seven (7) genera of microorganisms were obtained. The probable organisms isolated and identified include; *E. coli*, *Shigella* spp., *Salmonella* spp. and *Candida* spp., *Aspergillus* spp., *Acremonium* spp., and *Cladosporium* spp. showing more occurrence of *E. coli* and *Aspergillus* spp. across the isolated samples. The *E. coli* isolated in this research is in agreement with the previous research by [21], who indicated the specie as the major indicator of faecal contamination of water samples.

3.2. Physicochemical properties of the borehole water samples.

Table 6 shows the physico-chemical characteristics of the bore-hole water samples. The pH values ranged from 6.65 to 7.47; Hardness values ranged from 92 mg/l to 156 mg/l and Iron concentration ranges from 0.267 ppm to 0.378 ppm across the borehole waters sampled.

Table 6. Physicochemical properties of the borehole water samples.

Parameters	A	B	C	4	5
Ph	7.16±0.11	6.56±0.16	7.14±0.15	7.47±0.41	6.65±0.14
Hardness (mg/l)	92±0.14	156±0.24	108±0.18	110±0.23	98±0.32
Iron (mg/l)	0.267±0.10	0.378±0.13	0.304±0.12	0.317±0.14	0.289±0.42
Phosphate (mg/l)	3.125±0.14	4±0.14	2.375±0.34	6.125±0.17	4.8±0.36
Nitrate	3.000±0.19	2.500±0.29	2.614±0.42	4.143±0.28	1.071±0.35

Data are mean of replicate determinations ± SD.

Figure 1A shows the comparative assessment of the phosphate contents of the borehole water samples from different hostels' storage tanks. Phosphate contents ranged from 2.375 to 6.125. Figure 1B illustrates the Nitrate concentration of the borehole water samples from different hostels' storage tanks. Nitrate contents ranged from 1.071 to 6.214. Various physicochemical properties obtained in this study viz; pH, Nitrate, Phosphate, Hardness and Iron of the water are in line with the previous works [11, 22].

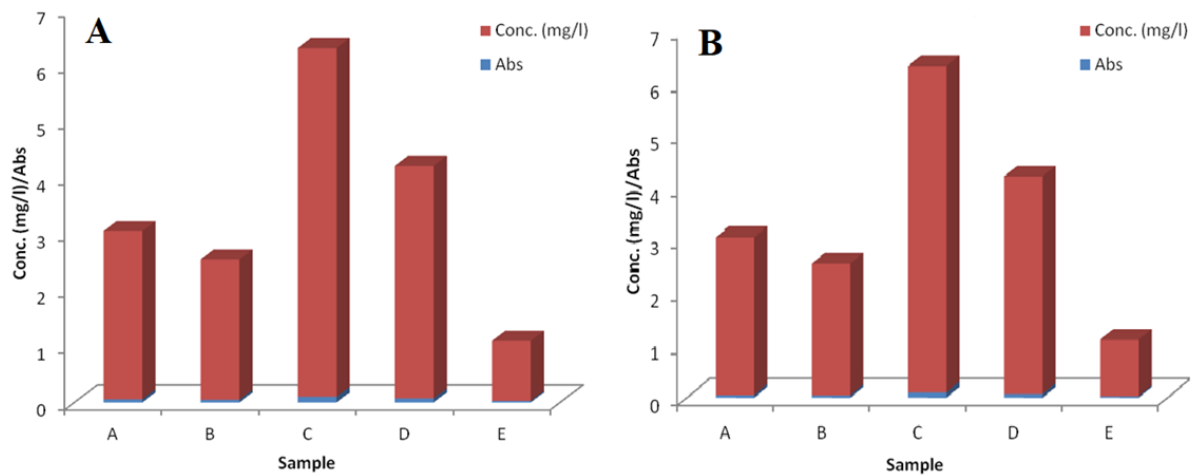


Figure 1. The phosphate content of the water samples (A) and the comparison of nitrate concentrations of the water samples (B).

4. Conclusions

The study successfully identified the presence of microorganisms in borehole waters stored in reservoir tanks of selected hostels. *E. coli* was found to be the most dominant organism isolated from the borehole water samples obtained from the stored water tanks. The presence of these organisms, along with the observed physicochemical properties, indicates fecal contamination in the studied borehole water samples. This contamination suggests the potential presence of pathogenic microorganisms and trace amounts of hardness and heavy metals in the sampled borehole waters. These identified organisms are opportunistic pathogens that can pose risks to individuals, particularly those with underlying health conditions and compromised immune systems. Consequently, there is a pressing need for improved sanitary conditions not only in the reservoir tanks but also in the water sources within these locations.

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Competing Interests

The authors hereby declare that competing interests does not exist regarding the publication of this research.

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