Bacteriological and physico-chemical analysis of well water samples in Ibadan South-East Local Government, Ibadan, Nigeria

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Abstract: Provision of safe drinking water is one of the main purposes for community development and improvement. Having a healthy community is related to the safety of drinking water in that community. This study was conducted to assess the levels of physico-chemical and bacteriological water quality in ten different wells in Ibadan South East Local Government. The results show that most of the physical and chemical values of sampled water were within the acceptable guide-line limits of the World Health Organization (WHO) for potable water. The well water were colourless with pH range of between 6.3-7.0; hardness ranged between l2- 32mg/l; and temperature was 30°C for all water samples analyzed. Nickel, cobalt, cadmum, lead and chromium were not detected in all the water samples, however, the bacteriological quality of the samples was poor rendering them unsafe for human consumption unless treated. Total coliform ranged between 2-6 cfu/ml and total viable bacterial counts ranged between 1.7-5.73 cfu/ml. Such microbial contamination pose a threat to well water quality and could lead to an increase in risk level of outbreak of water borne diseases in Ibadan South East Local Government area.
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Key words: safe drinking water; Ibadan South East Local government; total coliform; total viable bacterial count

1. Introduction

Water is vital to our existence in life. It exists in gaseous, liquid or solid state. It is the most abundant compound on earth's surface, covering about 70% of the planet's surface (Nester *et al.*, 2004). According to Taylor (2005), diseases contacted through drinking water accounts for approximately five million pediatric deaths annually. Potable drinking water should be colourless, tasteless, free of smell, without organic matter or suspended particles, of neutral pH, soft and not hard (WHO, 2005).

2. Materials and Methods

2.1 Materials

Reagents: Kovac's reagent, Gram stain reagents, Phenolphtalein, Hydrosulplguric acid, Sodium thiosulphate, Methyl purple, Buffer 10 solution, Solochrome indicator, EDTA, DPD Rapid No 1 tablet, ethanol (95%), glasswares: beaker (100ml, 250ml, 500ml), Conical flask (250ml, 500ml, 1000m1), Durham tubes, media: Nutrient Agar (Britania Laboratories), MacConkey Agar, Salmonella-Shigella Agar, Peptone water by and Starch Agar all by Lab M Limited, Simmon's citrate Agar (Biomark Laboratories), Eosine Methylene Blue and Nitrate peptone water (Lab M Limited).

Apparatus and Equipment: Atomic Absorption Spectrophotometer model A-Ana1yst 200, Microscope (binocular) (Olympus), Autoclave (YMSO), Radiometer acid base analyzer (Model-MK-2), Incubator (Gallenkamp), Weighing balance (Mettler-Toledo), Spectrophotometer (Jenway 632OD) and Mercury bulb.

2.2 Study Area

This research work was carried out in Ibadan South East Local Government area. Water samples were obtained from ten different wells from different locations under the Local Government.

2.3 Study Sample

From each well, water samples were collected into sterile bottles and brought to the Laboratory for testing within 12 hours of collection. Precautions were taken to prevent accidental contamination of the water during collection and transportation to the Laboratory. Water samples were transported to the laboratory in ice packs in a bucket. Some physical parameters were determined at the point of collection.

2.4 Description of Water Samples

The physical appearance of the water samples was noted. These include colour, odour and

turbidity. The water in the sterile containers were allowed to settle for a period of time and the sedimentation rate of the particles were noted.

2.5 Determination of Physico-chemical Parameters

2.5.l Temperature

Temperature was determined using a mercury bulb thermometer. Before use, the Thermometer was cleaned with cotton wool soaked in ethanol. It was dipped to about 15cm below the surface of water for 3 minutes and the temperature was read.

2.5.2 Hydrogen ion concentration

The pH meter used was calibrated with standard buffer of pH 7.0. The pH of samples was measured by placing the glass electrode in the samples and then the results were read on the pH scale.

2.5.3 Alkalinity test

100ml of water sample was measured into a conical flask, 2-3 drops of phenolphthalein were added. Colour change was observed and titrated against 0.02N Sulphuric acid (H_2SO_4). The colour change was noted which is the P value (Partial alkalinity), 2 drops of sodium thiosulphate ("T" indicator solution) were added to neutralize any excess acid in the water. 2-3 drops of methyl purple ("M" indicator solution) was added and titration continued until the colour finally changed from blue to pink to give the final alkalinity (N value). Total alkalinity was calculated with the formula 2P – N. Where P is partial alkalinity; N is final alkalinity and Standard value = 2-7 mg/l.

2.5.4 Miscellaneous tests

Other tests carried out were water hardness, chlorine test and test for heavy metals.

2.6 Culture Media

The culture media used include Nutrient agar, Eosin methylene blue agar, MacConkey agar, Salmonella-Shigella agar. Each of them was prepared according to the manufacturers' specifications.

2.6.1 Nutrient agar

31 grams of nutrient agar was dissolved in a conical flask in a litre of distilled water and mixed well. It was then heated with frequent stirring by boiling until it homogenized completely. The whole flask was wrapped with aluminium foil then sterilized by autoclaving at l21°C for l5 minutes at l5lb/sq inch. The medium was allowed to cool to 50°C before dispensing into petri dishes where it solidified. The medium was used for bacterial characterization, cultural and colonial appearance and determination of the total viable bacteria.

2.7 Isolation of Microorganisms

1ml of each well water sample was serially diluted: lml from dilution 10^{-1}, 10^{-3} and 10^{-5} was inoculated on sterile Nutrient agar and Salrnonella-Shigella agar respectively. The plates were incubated at 37°C for 24hr, after which visible colonies were counted and results were expresses in colony forming unit per mililitre (cfu/ml) (Hans, 2002). MacConkey agar and Eosine Methylene blue agar respectively were used for coliform test using membrane filters. Membrane filter technique was carried out using 100ml of each of the water samples which was passed through membrane filters. The filter with its trapped bacteria was transferred to the surface of solid medium and incubated at 37°C for 24hours (Hans, 2002).

2.8 Characterization and Identification of Isolates

Procedures were carried out according to standard techniques. Bacteria isolation was carried out using streak and pour-plate technique. Isolation of pure culture was completed by streaking on Nutrient agar media plates. Morphological identification was carried out using the Gram staining procedure and examined under X l00 objective of a light microscope. Sub culturing on solid agar was done to maintain viability. Characterization of isolates and identification of the isolate were carried out on the basis of their cultural characteristics on agar plates (Hans, 2002).

2.8.1 Cultural characterization

Sterile wire loop was used to aseptically pick from each bacteria colony and streaked on nutrient agar plates. The plates were incubated at 37°C for 24hr. The cultural characteristics of each isolate were determined by visually observing the elevation, size of colony, surface opacity, colour, edge, pigmentation and consistency of colony.

2.8.2 Morphological characterization

A heat fixed smear of each isolate was prepared on a clean plain slide. Observation was made under oil immersion objective (X 100) of a light microscope. Observed shapes and structures were recorded.

2.8.3 Biochemical characterization

Gram's staining of the isolates was carried out under appropriate conditions to view the nature of bacteria cell wall and classify the isolates.

3. Results

A total of ten different well samples were

analyzed and named A-J respectively. Water samples were collected from wells that were used everyday. Table 1 shows the results of the physiochemical tests that is odour, colour, appearance, pH, alkalnity, hardness, chlorine and temperature of samples.

All samples collected were odourless and tasteless and their temperature was 30°C. Metals such as Nickel, Cobalt, Cadmium, Lead and Chromium were not detected in all the water samples analyzed. Sample A, C, F, G, H and J contained few particles while sample B and I contained suspended solid. Sample D also contained suspected solid and appeared cloudy while sample D contained cloudy particles.

The pH values of all the water samples was between 6.3 and 7.0 which meets the standard for drinking potable water according to the World Health Organization.

Values of total alkalinity of samples A, B, C, D, E, F, G and H were in the normal range. Samples I and J had lower values of 1.8 and 1.0 respectively which is lesser than normal value for total alkalinity (Table 1).

Table 1: Physico-chemical values and composition of the well water samples analysed

	Samples									
Values and constituent	A	B	C	D	E	F	G	H	I	J
Colour	Colouless	Colourless	turbid	Turbid	turbid	Colourless	turbid	colourless	Turbid	colourless
Appearance	Few particles	Suspended solid	Few particles	Cloudy with suspended solid	Cloudy particles	Few particles	Few particle	Few particles	Suspended solid	Few particles
Odour	Odourless	Odourless	Odourless	Odourless	odourless	odourless	odourless	odourles	Odourless	odourless
Taste	tasteless	tasteless	tasteless	Tasteless	tasteless	tasteless	tasteless	tasteless	Tasteless	tasteless
Ph	6.6	6.3	6.0	7.0	6.5	6.5	6.7	6.5	6.4	6.4
Temperature	30	30	30	30	30	30	30	30	30	30
Total alkanity	2	3	3	2.5	2.5	2	2	2.3	1.8	1.0
Total hardness (mg/l)	30	32	14	20	16	16	16	12	18	14
Total chloride (mg/l)	0.5	0.6	NIL	0.5	NIL	NIL	NIL	NIL	NIL	0.2
Nickel (mg/l)	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Cobalt(mg/l)	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Cadmium (mg/l)	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Lead	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Chromium (mg/l)	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Table 2 shows the result of bacteria load of well water samples analyzed. A total of 3.33 x $l0^6$ cfu/ml were isolated from sample A; 2.9 x $l0^6$ cfu/ml from sample B; 5.73 x $l0^6$ cfu/ml from sample C; 2.0 x 10^6 cfu/ml from sample D; 1.93 x $l0^6$ cfu/ml from sample E; 3.2 x $l0^6$ cfu/ml from sample F; 2.63 x $l0^6$ cfu/ml from sample G; 2.6 x $l0^6$ cfu/ml from sample H; I l .7 x $l0^6$ cfu/ml from sample I; and 3.5 x $l0^6$ cfu/ml from sample J (Table 2). Sample B has the highest bacteria load while sample I had the lowest bacteria load count.

The isolate were initially differentiated on the basis of their cultural and morphological studies after which they were subjected to various biochemical tests.

Table 2: Bacteria load of well water samples

S/N	Sample Name	Cultural Appearance and Colonial Description	Bacterial Load (cfu/ml)
1	A	Creamy circular, golden yellow circular, white circular with lobate edge	3.3×10^6
2	B	Creamy circular (swarm), golden yellow circular, pink circular, creamy circular with dentate edge, creamy rhizoid, irregular creamy shape.	2.9×10^6
3	C	Creamy circular, whitish rhizoid,yellow circular, pink circular, creamy circular with dentate edge, creamy rhizoid, irregular creamy shape.	5.73×10^6
4	D	Whitish circular, orange circular, irregular creamy shape, creamy circular(swarm), brownish rhizoid, creamy with lobate edge, creamy circular with undulated edge, creamy fimbrate yellow circular	2.0×10^6
5	E	Yellow circular, creamy rhizoid, creamy circular (swarm), green circular, irregular creamy shape, irregular yellow shape.	1.93×10^6
6	F	Yellow circular, orange circular, creamy circular, whitish circular, irregular creamy shape, pink circular, creamy rhizoid.	3.2×10^6
7	G	Creamy circular with undulated edge, light yellow circular, orange circular, irregular creamy shape, creamy rhizoid, creamy circular, whitish circular, creamy fimbrate.	2.63×10^6
8	H	Irregular whitish shape, golden yellow circular, creamy circular, creamy rhizoid, irregular creamy shape, light yellow circular, whitish circular, white fimbrate	2.6×10^6
9	I	Creamy circular with lobate edge, golden yellow circular, orange circular, irregular creamy shape, creamy circular, creamy rhizoid	1.7×10^6
10	J	Light yellow circular shape, creamy rhizoid, creamy circular shape, irregular creamy shape, creamy fimbrate, orange circular	3.5×10^6

Table 3 shows the result of the coliform of water samples. Sample A, B, C, E, I and J had no coliform count because they tested negative for the coliform test. Sample D had coliform count of 4×10^6 cfu/ml; Sample F had a coliform count of 6×10^6 cfu.ml; Sample G had a coliform count of 2×10^6 cfu/ml; and sample H a coliform count of $2\ 7 \times 10^6$ cfu/ml (Table 3).

Table 3: Coliform count of well water samples

S/N	Samples Name	Coliform Count (cfu/ml)
1	A	NIL
2	B	NIL
3	C	NIL
4	D	$4x10^6$
5	E	NIL
6	F	$6x10^6$
7	G	$2x10^6$
8	H	$2x10^6$
9	I	NIL
10	J	NIL

The probable organisms, from biochemical characterization, were *Microccus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Mycobacteria* and *Enterobacter aerogenes* (Table 4). Table 5 represents the microorganisms in each well water sample.

Table 4: Biochemical characteristics of isolates

Gluce Reaction	Lactose	Glucose	Sucrose	Manitol	Maltose	Starch Hydrolysis	Indole	Catalase	Citrate Utilization	Nitrate Reduction	Probable Bacteria
+	-	-	-	-	-	+	-	+	+	-	*Micrococcus luteus*
-	+	+	+	+	+	+	+	-	-	+	*Escherichia coli*
-	-	+	+	-	-	-	-	+	+	+	*Pseudomonas aeruginosa*
+	+	+	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
+	+	+	+	+	+	+	+	+	+	+	*Bacillus substilis*
-	+	+	+	+	+	+	-	-	+	+	*Klebsiella aerogenes*
+	+	+	-	+	+	+	+	+	+	+	*Mycobacteria*
-	+	+	+	+	-	+	-	+	+		*Enterobacter aerogenes*

Key:

+ = Positive

- = Negative

Table 5: Occurrence of bacterial isolates in the analyzed well water sample

S/N	Sample names	*Micrococcus luteus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus substilis*	*Klebsiella aerogenes*	*mycobacteria*	*Enterobacter aerogenes*
1	A	+	-	-	+	+	-	+	-
2	B	+	-	+	+	+	-	+	-
3	C	+	+	+	+	+	-	+	-
4	D	+	-	+	+	+	+	+	+
5	E	+	+	+	+	+	+	+	-
6	F	+	+	+	+	+	+	+	+
7	G	+	+	+	+	+	+	+	+
8	H	+	+	+	+	+	+	+	+
9	I	+	-	+	+	+	+	+	-
10	J	+	-	+	+	+	+	-	-

Key:

+ = Positive

- = Negative

4. Discussion

In our study, the pH values of water samples under investigation were within the acceptable range (6.3-7.0). This conforms with the pH range reported by Okonko *et al*. (2008). According to Edema *et al*., (2001), the pH of the most natural waters range from 6.3-8.3. The pH of water is extremely important physicochemical factor. The fluctuations in optimum pH range may lead to an increase or decrease in the toxicity of chemicals in water bodies (Ali, 1991; Okonko *et al*., 2008).

The temperature 30°C reported in this study is comparable to the temperature reported by other researchers in similar studies. For instance Alabaster and Lloyd (1980) reported a temperature range of 26 to 30°C in their study. A temperature of 30°C of water samples as observed in this research is believed to have been influenced by the intensity of the sun (Mulusky, 1974; Okonko *et al*., 2008).

The total bacteria count for some of the water samples were generally high, exceeding the recommended standard limit for water (FAO, 1997). Several factors are responsible for the presence of bacteria in well water. The presence of septic tanks as well as refuse dumps in close proximity with wells is sometimes responsible for coliform load in such well water (Adejuwon *et al.,* 2010). Some of the wells were uncovered. The isolated bacteria species were identified to be same with those commonly encountered in water and aquatic environments (Nester *et al.*, 2001). These identified isolates include *Bacillus subtilis, Staphyloccus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa, Micrococcus luteus, Escherichia coli, Mycobacteria, Klebsiella aerogenes*.

The presence of enteric bacteria including *Escherichia coli*, *Klebsiella aerogenes* and *Enterobacter aerogenes* as reported in this study are indicators of faecal contamination as a result of unhygienic handling of the well water and unhygienic environment. The greatest hygiene danger associated with water used for drinking purposes, food processing and human consumption is contamination by human excrement (Edema *et al*., 2001; Okonko *et al*., 2008). Underground water generally should not contain faecal organisms or pathogenic microorganism of any kind. Result obtained confirms the presence of bacteria in the well water sample because of the location of the well which might be close to septic tanks or soak away which created room for seepage of microorganisms into the well and poor drainage system. The need for microbial assessment of water for domestic purpose should always be emphasized to reduce possible infection due to contamination.The presence of *Micrococcus luteus* and *Enterobacter aerogenes* reported in this study has also been earlier reported by Umeh *et al*., (2005) in their study conducted on the bacteriological quantity and safety of water in Akwa, Nigeria using membrane filtration method.

Bacterial growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumers. Most of the organisms found in these water samples are those commonly found in soil and water (Nester *et al.*, 2001). *Staphylococcus aureus*, a nosocomial pathogen of public health concern and significance may contaminate water samples through improper handlers and fetchers of such wells (Nester *et al.*, 2001).

4.1 Conclusion

Outbreak of disease as well as epidemics could occur in a population through consumption of water contaminated with feacal organisms. The prevention of communicable disease requires that the cycle of disease transmission is interrupted. Depending on the prevailing transmission pathways, different interventions in water supply and sanitation are required. Bacterial diarrhoea and epidemics of typhoid fever and shigellosis are often transmitted through water. These current findings on these unsafe water are grim reminders of the need to address the probable sources of contamination.

4.2 Recommendations

As recommendations:

i.) Government should help members of the public in sinking boreholes in these areas so that the consumption of contaminated well water is reduced and the risk of contacting disease through such wells is minimized;

ii.) Well water can be treated before consumption with chlorine, but under strict guidance to reduce the risk of developing cancer;

iii.) Boiling would kill most organisms and almost all pathogenic bacteria since most of them are mesophiles and would not withstand high temperature;

iv.) Awareness is also an important component of health education on water borne infections in a society. People should be educated on personal hygiene. Consumers of well water should be enlightened on the risk of consumption.

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