Coliform Profile and Physicochemical Analyses of Well Water Samples in Oluyole Local Government Area, Ibadan, Nigeria

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Abstract: Outbreak of disease as well as epidemics could occur in a population where water consumed is contaminated with faecal organisms. This study was carried out to assess the coliform profile and determine physicochemical properties of well water samples of six different sites in Oluyole Local Government area in Ibadan, Nigeria. Samples were plated on nutrient agar, MacConkey and eosine methylene blue agar. Gram staining of the isolates was done. IMVIC test on isolates obtained were also carried out. Physicochemical analyses of samples were also carried out. The results showed that most of the physical and chemical values were within limits of the acceptable guideline of the World Health Organization (WHO) for potable water. The well water samples were colorless, odorless and tasteless. While pH ranged between 6.0 and 7.3, hardness ranged from 0.0 – 2.0 and temperature ranged from 31°C to 32 °C. Nickel and Lead were not detected in all the water samples but Copper was detected in samples B and F and not in samples A, C, D and E. Coliform bacteria were not found in any of the well water samples and the microbial isolates were Gram positive. This implies the potability of all the well water sampled.

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1. Introduction

Water makes up 60% of our body weight. In the United States of America, public water supplies are disinfected with chlorine which kills pathogenic microorganisms by oxidation. Most bottled water are disinfected with ozone which leaves no odor or flavor in the water (Bettelheim *et al.*, 2010). Water bodies in Nigeria are polluted by municipal, industrial and agricultural wastes. Water being an excellent carrier of pathogens can cause infections and invariably diseases (Ramasarma, 2012). Drinking water should be free of pathogenic microorganisms (Kapil, 2013). In the United States, boiling and filtration have been recommended to campers and hikers who drink water from streams (Greenberg and Gold, 1999).

Members n the groups Bacteria, Chlamydiae, Rickettsiae, Mycoplasma, Viruses and Fungi are recognized harmful pathogenic microorganisms (Crowley, 2007).

The current lack of pipe-borne water has made the inhabitants of Ibadan, Nigeria resort to use of well, stream and bottled waters for consumption. In the present study, wells from Oluyole Local Government area, Ibadan, Nigeria were sampled for the presence of coliforms and heavy metals in an attempt to determine the potability of water in the selected locality.

2. Materials and Methods

Materials

In the course of analyzing the water samples, the following were used:

Reagents: Kovac's reagent, Gram staining reagents, Phenolphthalein, Sodium thiosulphate, Methyl purple, Buffer l0 solution, DPD rapid No l tablet, 80% ethanol, Ethylene diamine tetra acetic acid (EDTA) and solochrome indicator.

Methods

Study Area

This research study was carried out in Oluyole Local Government area, Ibadan, Nigeria. Water samples were obtained from wells in six different locations of the local government.

Study samples

From each well, samples were collected in sterile bottles and brought to the laboratory for testing within l2 hours of collection. Precautions were taken to prevent accidental contamination of water during collection and transportation to the laboratory. Water samples were transported to the laboratory in ice packs. Some physical parameters were determined at the point of collection.

Description of water sample

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

The water in the sterile containers was allowed to settle for 30 minutes and the sedimentation rate of the particle was recorded.

Determination of Physicochemical Parameters

Temperature

The temperature was taken using a mercury bulb thermometer. The thermometer was sterilized by cleaning with a cotton wool soaked in ethanol. The thermometer was then dipped to about 15cm below the surface of water for 3 minutes and the temperature was recorded.

Hydrogen ion concentration (pH)

This test was carried out using the water testing kit model WA-2015. The pH electrode was connected to the meter and the electrode was dipped into the water sample to be tested and the pH was read and recorded from the meter.

Dissolved oxygen (DO)

The test for Dissolved Oxygen was carried out using the water testing kit model WA-2015. The DO electrode was connected to the meter and the electrode was dipped into the water sample to be tested and the DO was read and recorded from the meter.

Electrical conductivity (CD)

The electrical conductivity was carried out using the water testing kit model WA-2015. The CD electrode was connected to the meter and the electrode was dipped into the water sample to be tested and the CD was read and recorded from the meter.

Redox potential

The redox potential was determined using the water testing kit model WA-2015. The ORP electrode was connected to the meter and the electrode was dipped into the water sample to be tested and the ORP was read and recorded from the meter.

Alkalinity test

Approximately 100ml of raw water to be tested was measured into a conical flask (250ml), 3 drops of phenolphthalein was added and colour change was observed and titrated against 0.02N sulphuric acid (H_2SO_4). The colour change was noted which is P value (Partial alkalinity). Drops of sodium thiosulphate ("T" indicator solution) was added to neutralize any excess acid in the water. 2-3 drops of methyl purple ("M" indicator) was added and titration was continued until the colour finally changed from blue to pink to give the N value (Final alkalinity).

Total alkalinity was calculated using the formula 2P-N.

Where P = partial alkalinity

N = final alkalinity

Standard value = 2-7mg/l

Hardness test

Approximately 50ml of water sample was measured and 5ml of buffer 10 solution was added then 2 drops of solochrome indicator. The mixture was titrated against EDTA.

Calculation for hardness

$$\text{Hardness} = \frac{0.01 \text{ X titre value X } CaCO_3 \text{ X } 1000}{50\text{ml (water sample)}}$$

Where 0.01 = number of moles of EDTA

$CaC0_3$=100. (Molar mass)

1000 = standard

Residual chlorine test

The test was carried out using DPD No 1 chlorine tablet. One tablet was crushed and dissolved in a test tube containing 2ml of each water sample. No colour change was observed.

Test for heavy metals

Metals such as Nickel, Cobalt and Lead were tested for in each of the water samples using Atomic Absorption Spectrophotometer. The test was carried out at Fatlab Nigerian Company Ibadan, Nigeria and results were recorded accordingly.

Culture Media

The culture media used were Nutrient agar, Eosine methylene blue agar, MacConkey agar, Simmons citrate agar, peptone water and glucose. Each of them was prepared according to manufacturer's specifications.

Isolation of Coliforms

MacConkey agar and Eosine methylene blue agar were used for coliform test using the membrane filter technique: The forceps was flamed and used to pick the sterile membrane filter and placed on the filter support. The furmel was then placed on the filter support. 100ml of each water sample to be tested was poured into the funnel. The vacuum pump was turned on and each of the samples was drawn completely. The vacuum was then turned off. The funnel was removed and the membrane filter with its trapped bacteria was picked with a sterile forceps and thereafter placed on the surface of the solid media prepared (MacConkey and Eosine methylene blue agar). They were then incubated at 37°C for 48 hours.

Gram staining of isolates

A smear from each culture was made on a microscope slide using a sterile inoculating loop. This was heat-fixed by passing the slide over a Bunsen burner flame. Each slide was then flooded with crystal violet solution which was allowed to 'stay for 30 seconds. Each slide was then rinsed with distilled water and then flooded with Lugol's iodine solution which was allowed to stay for 1 minute. The iodine was rinsed off with distilled water and the specimen was decolourized using 85% alcohol. Each slide was then rinsed with distilled water and the specimen was

counterstained with carbol fuschin which was allowed to stain for 20 seconds. Each slide was then rinsed with distilled water, blotted dry and examined under oil immersion objective lens (X l00 magnification) of the microscope.

Biochemical Tests

Indole test

Some organisms are able to hydrolyze the amino acid Tryptophan and one of the end products is lndole. Isolates were each inoculated into the broth from a young agar cultures and was incubated at 37°C for 3days. 0.5ml Kovac's indole reagent was then added to each universal bottle of the test culture. The bottles were shaken gently and thereafter allowed to stand. There was no change in colour hence recorded as negative.

Methyl red test

Methyl red test determines whether the microorganism performs mixed acid fermentation when supplied with glucose. Test isolates were each inoculated into the medium (peptone water and glucose) from a young agar cultures and was incubated at 370C for 3 days. After, 5 drops of the indicator (methyl red solution) was added to the culture. A red colour developed. This indicated that test was positive. In some, a yellow colour developed. This indicates that the test was negative.

Voges-Proskauer test

Test isolates were each inoculated into the medium (peptone water and glucose) from a young agar cultures and was incubated at 37°C for 3 days. lml of alpha-naphthol solution and 0.5ml of potassium hydroxide was added. The bottles were then shaken. No colour change to red. This indicated a negative result. This test is used to detect acetoin in a bacterial broth culture.

Citrate utilization test

This test shows the ability of the organism to utilize citrate. The slant of simmon citrate agar was aseptically inoculated by streaking a 24hours old culture of the test organism over the surface using a sterile wire loop. This was then incubated at 37^0C for up to 3 days. A positive result was indicated by the colour change of the medium from green to blue while a negative result was indicated by no colour change of the medium.

Control for test

The sterility of each batch medium was confirmed by incubating two uninoculated plates and bottles along with the inoculated test. Any uninoculated bottle and plate that showed evidence of microbial growth was discarded because it shows that the plates or bottles were contaminated.

3. Results

Table 1: Physical parameters of well water from six locations in Oluyole Local Government Area, Ibadan, Nigeria

	Samples					
Parameters	**A**	**B**	**C**	**D**	**E**	**F**
Colour	Colourless	Colourless	Colourless	Colourless	Colourless	Colourless
Appearance	S/S	S/ S	S/S	S/S	S/S	S/S
Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless

S/S: Suspended solids present

Table 2: Chemical parameters of well water from six locations in Oluyole Local Government Area

	Samples						
Parameters	**A**	**B**	**C**	**D**	**E**	**F**	
pH	6.9	7.3	6.6	6.0	6.6	6.4	
Temperature (^{0}C)	32	31	31	31	31	31	
Total alkalinity (mg/L)	35	47	15	10	25	24	
Total hardness (mg/L)	2.0	2.0	2.0	2.0	2.0	2.0	**Chlorine**
(mg/L)	Nil	Nil	Nil	Nil	Nil	Nil	
Dissolved oxygen (mg/L)	6.4	6.4	5.5	7.0	6.4	6.7	
Conductivity (mg/L)	0.9	0.9	0.4	0.4	0.3	0.1	
Redox potential (mg/L)	2.4	2.5	2.5	2.2	2.4	2.5	

*Each value is a mean of triplicate readings

A total of six different well samples were analyzed and named A-F respectively. The samples were collected from wells that were used every day. Table 1 shows the results of physical analyses. These include odour, taste, appearance and colour. All water samples collected were odorless, tasteless and colourless. Table 2 shows the results of chemical analyses. These include pH, alkalinity, hardness, chlorine, dissolved oxygen, conductivity, and temperature. Temperature values ranged between 31^{O}C and 32^{O}C. The pH values of all water samples were between 6.0 and 7.0 which met the standard for potable water (WHO, 2005). Table 3 shows the result of heavy metals. These include Copper, Lead and Nickel. Lead and Nickel were not detected in all the water samples analyzed. However, samples B and F each contained 0.1mg/L of copper. Copper was not detected in sample A, C, D and E.

Table 3: Test for heavy metals of well water from six locations in Oluyole Local Government Area

	Samples					
Parameters	**A**	**B**	**C**	**D**	**E**	**F**
Lead	Nil	Nil	Nil	Nil	Nil	Nil
Nickel (mg/L)	Nil	Nil	Nil	Nil	Nil	Nil
Copper (mg/L)	Nil	0.01	Nil	Nil	Nil	0.01

Table 4: Biochemical tests for coliform

Sample	Indole	Methyl-red	Voges-Proskauer	Citrate	Gas formation	Inference
A	-	+	-	-	-	No coliform
B	-	-	-	-	-	No coliform
C	-	-	-	-	-	No coliform
D	-	+	-	-	-	No coliform
E	-	-	-	-	-	No coliform
F	-	-	-	+	-	No coliform

+ indicates Positive
- indicates Negative

Table 5: Gram reaction of microbial isolates from samples

Sample	Gram reaction
A	+
B	+
C	+
D	+
E	+
F	+

Table 4 shows result of biochemical tests. Isolates from samples A, B, C, D, E and F tested negative for indole as well as for Voges Proskauer. Isolates from samples A, B, C, D and E tested negative for citrate while sample F tested positive.

Isolates from samples A and B tested positive for methyl red, while samples B, C, E and F tested negative. There was no gas formation in samples A, B, C, D, E and F.

Table 5 shows Gram reaction of microbial isolates from samples A, B, C, D, E. The isolates were Gram positive.

4. Discussions, Conclusions and Recommendations

Discussions

The pH values of the water samples ranged between 6.0 and 7.3. This conforms with the range reported by Okonko et al. (2008). Also according to Edema et al., (2001), the pH of natural water ranges from 6.3 and 8.3. By international standard the pH of potable water is between 6.5 arid 8.5.

The temperature range of 29^0C-30^0C reported in this study is comparable to that reported by other researchers in a similar study. For instance, Okonko et al (2008) reported a temperature range of 28^0C-30^0C of natural water as influenced by intensity of sunlight.

The total hardness of each of all the heavy metals such as lead and nickel were not detected in any of the samples but 0.0lmg/L of copper was detected in each of the samples B and F. Copper has the maximum acceptable concentration of 1.5mg/L (WHO, 2004). Little copper is essential for good health but too much can be harmful. ingesting large amount of copper compounds can cause death through the failure of the nervous system, liver and kidney (Mohod and Dhote, 2013).

Underground water generally should not contain faecal organisms or pathogenic microorganisms of any kind (Kapil, 2013). Results obtained confirm that all the well water samples were devoid of coliform bacteria.

Conclusions

Outbreak of disease as well as epidemics could occur in a population due to consumption of water contaminated with faecal organisms. This is because most of the diseases which prevail in developing countries during shortage of water are infectious diseases caused by bacteria and other pathogenic microorganisms arising from water contaminated with faecal matter.

Coliform bacteria were not detected in each of the well water samples being analyzed and most of the physicochemical parameters met the World Health Organization standard for drinking water. Therefore all the water in the wells sampled are safe for drinking and for domestic purposes.

Recommendations

(i). Well water can be treated before consumption by treating with chlorine and lime.

(ii). Boiling water before drinking would also kill some pathogenic organisms since some of them cannot withstand l00^{o}C.

(iii). The society should be educated on water-borne diseases and infections.

(iv). The rule of washing of hands before and after using of toilets must be obeyed. Also the washing of hands before and after eating must be observed.

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