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The Microbiological and Physico-Chemical Analysis of Borehole Waters used by Off-Campus Students of Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria

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Abstract: The microbiological and physico-chemical analysis of the seven borehole waters used by off-campus students of MOUAU were carried out using the Most Probable Number Technique (MPN) for the detection of faecal coliform. Five bacterial species *Enterobacter aerogenes*, *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated together with two fungal specie namely: *Mucor racemosus* and *Aspergillus niger*. The Total Heterotrophic Plate Count (THPC) gave a range of 4.7×10^{4}-1.82×10^{5} cfu mL^{-1} with a mean of 7.89×10^{4} cfu mL^{-1} while the Total Coliform Plate Count (TCPC) gave a range of 4.1×10^{3}-8.6×10^{3} cfu mL^{-1} with mean 6.24×10^{3} cfu. The Presumptive faecal coliform ranged between 0-12 coliform per 100 mL with a mean of 3.57 coliform per 100 mL. Faecal coliform was detected in 57.1% of the water samples. The physico-chemical parameters fell within acceptable limits except for nitrate whose range fell between 65-20 mg L^{-1} above WHO guidelines of 10 mg L^{-1}. The findings show that the water samples except those from AL and AG boreholes did not meet WHO standard for drinking. Thus, the bore-holes water and should be treated before drinking.

Key words: Bacteria, borehole water, contamination, off-campus, students, Nigeria

INTRODUCTION

Water is indispensably and intricately connected to life and without it, there is no life. Good drinking water is not a luxury but one of the most essential amenities of life itself. Water is fundamentally important to all plants, animals and man (Ajewole, 2005). Water is the matrix of life as all biological reactions occur in water and is the most versatile chemical formed within any metabolizing cell. The supply of safe drinking water to all has therefore engaged the attention of many individual, groups, governmental organisation and private. Safe drinking water is the priority of all people.

Water is made up of the two atoms of hydrogen and one of oxygen and because of the unique nature of the binding, water is a solvent for many minerals and can be referred to as a universal solvent. It can exist in three states as liquid, gas (at 100°C) and as solid (at freezing temperature of <4% (Nelson, 2002). Water is significantly unique due to its chemical and physical properties. The need for determining the suitability of water for drinking and bathing purposes has been recognized since 1855 when Snow and Budd related outbreaks of typhoid fever and cholera to water contaminated with faecal waste.

Microorganisms play a major role in water quality and the microorganisms that are concerned with water borne diseases include *Salmonella* sp., *Shigella* sp., *Escherichia coli* and *Vibrio cholerae* (Birmingham *et al*., 1997). These cause typhoid fever and dysentery. Other agents of water borne diseases are protozoan of diarrhoea *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Crytococcus pervum* (Kelly *et al*., 1997) and enteroviruses of various clinical ailments which include poliovirus, Rotavirus, Hepatitis A virus (Hejkal *et al*., 1982) and Hepatitis E virus (Benjelloun *et al*., 1997).

Recently according to the United Nations (UN), about >5 million people die annually first from diseases caused by unsafe drinking water and secondly due to lack of sanitation. It is estimated that >80% of ill health in developing counties are water and sanitation related (Cheesbrough, 2000).

In the year 2000, the estimated global burden of disease associated with poor water supply equalled >2 billion cases of diseases with an annual death toll of 2.2 billion (WHO, 2004). The major problems of safe drinking water are those of availability and quality (Ajewole, 2005). The most dangerous form of water pollution occurs when faeces enter the water supply.

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Many diseases are perpetuated by the faecal-oral route of transmission in which the pathogens are shed only in human faeces. Presence of faecal coliforms like *E. coli* is used as an indicator for the presence of any of water borne pathogens (Chukwura, 2001; Okpokwasili and Akujobi, 1996).

To maintain good health, Cheeshrough (2000) stated that water should be of good quality and quantity meeting local demands while WHO recommended standards of taste, odour and appearance.

Justification: The staff and students of Michael Okpara University of Agriculture Umudike living in off-campuses depend on groundwater (borehole) apart from periods of rainfall (rainy seasons) for most of its domestic uses. The potential for pathogens from human and animal wastes present to contaminate the drinking water is very high. Borehole water is pumped out with the aid of submersible pumping machines into overhead tanks (Olowo, 2005). There has been report of borehole water contamination through many domestic waste water and livestock manure, especially where there was a puncture in the soil layers.

These waste and sewage when deposited near the borehole may travel with percolating rain-water directly into the borehole or may travel along the well-wall or surrounding materials of the drill-hole. The borehole water which may be contaminated in the storage tanks does not undergo any form of treatment before consumption, the possibilities of such contamination justifies this research. This study carried out in 2011 was intended to ascertain the potability status of borehole water in the study area since the water is not given any form of treatment.

MATERIALS AND METHODS

Sample collection: The borehole water samples were aseptically collected from the sources using washed and sterilized glass containers after pumping water sample to waste for 3-5 min. The nozzles or taps of the boreholes were swabbed with cotton wool soaked in 70% (v/v) ethanol and flamed for 2-3 min. The water sample were kept between a temperature of 4-10°C and transported to the laboratory <6 h of collection and analysed within 24 h. A total of 7 water samples were collected from the selected boreholes within the area between the hours of 9 and 10 a.m., when the sampling points were free of students. The sampling protocols described by Claasen (1982) and Barcelona *et al.* (1985) were strictly adhered to during sample collection.

Sampling code:

AG = Angels on Guard lodge
AL = Anglican Lodge
DL = Divine Lodge
DOL = Dominion Lodge
GL = Goshen Lodge
PG = Palm Groove lodge
BC = Bishop's Cott lodge

Media used: All the laboratory glass wares were sterilized by autoclaving at 121°C for 15 min with the media used prepared the previous day according to manufacturer's instructions. The plates were prepared in duplicates and kept in the incubator to check for contamination while uninoculated plates were kept as control.

Enumeration of total heterotrophic bacterial and fungal counts: Total heterotrophic bacterial and fungal in water samples were obtained using spread plate method. A 4-fold serial dilution of 10^{-1} to 10^{-4} of the samples were prepared using peptone water and duplicate 0.1 mL of each dilution was inoculated in to each already prepared plates. For the heterotrophic plates count on nutrient agar (10^{-4}) dilution was used; the third serial dilution (10^{-3}) was used for the total coliform count on MacConkey agar while the first dilution (10^{-1}) was used for the total fungal count on Sabourand dextrose agar. These were incubated at 37°C for 24 h and petri-dishes from dilutions containing between 30 and 300 discrete colonies were counted (Ogbulie *et al.*, 1999).

Identification and characteristics of bacterial isolates: Cultural and microscopic examinations as well as biochemical test and sugar fermentation test were used to identify the pure isolates (Cheesbrough, 2000).

Identification of fungal isolates

Lactophenol cotton blue staining: A drop of 70% alcohol was placed on a microscope slide and the test organism was immersed on the drop of alcohol. Two drops of the lactophenol cotton blue mountant/stain were added before the alcohol dried out. With the cover-slip held between the forefinger and thumb one edge of the mountant was touched and lowered gently avoiding air bubble and then viewed under the microscope.

Coliform test:

Presumptive test: Total and faecal coliform loads were enumerated by multiple tube fermentation tests as described by APHA *et al.* (2005). Coliform count was obtained using the three tube assay of the Most Probable Number (MPN) Technique. Presumptive test was carried

out using McConkey broth. The 1st set of the five tubes had sterile 10 mL double strength broth and the 2nd and 3rd sets had 10 mL single strength broth. All the tubes contained Durham tube before sterilization. The three sets of the tubes received 10, 1 and 0.1 mL of water samples using sterile pipette.

The tubes were incubated at 37°C for 24-48 h for estimation of total coliforms and at 44.5°C for faecal coliforms for 24-48 h and examined for acid and gas production. Acid production was determined by colour change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tubes.

Confirmed test: This test was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube of brilliant green lactose Bile broth withs Durham tubes. The tubes were incubated at 37°C for 24-48 h for total coliform and 44.5°C for faecal coliform and observed for gas production.

Completed test: Completed test was carried out by streaking a loopful of broth from a positive tube on the Eosin Methlylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37°C for the 24-48 h. Colonies developing on EMB agar were further identified as faecal coliforms.

Determination of physico-chemical properties: The physico-chemical parameters measured include pH, temperature, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD), Sulphate (SO_4), Nitrate (NO_3). The metallic ions (K, Mg, Na and Ca) were determined using Atomic Absorption Spectrophotometric (AAS) Method. The DO and BOD were determined by the Winkler's Tritrometric Method while pH and temperature were determined *in situ* using the Jenway HANNA 1910 multipurpose tester. Turbidity and conductivity were both measured *in situ* using turbidometer to determine turbidity and a Mobile Jenway digital conductivity meter to determine conductivity.

RESULTS AND DISCUSSION

Five bacteria namely by *E. coli*, *Enterobacter aerogenes*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and two fungi: *Mucor racemosus* and *Aspergillus niger* were isolated from the seven selected bore-hole water samples analysed in this research (Table 1 and 2). The Total Heterotrophic Plate Counts (THPC) was in the range of 4.7×10^4-1.82×10^5 with borehole DL having the highest value and borehole GL the least.

The Total Coliform Plate Count (TCPC) was in the range of 4.1×10^3-8.6×10^3 with borehole BC and AG having the highest and least values, respectively. The distribution of the microbial groups enumerated in the seven selected boreholes as shown in Table 3. Table 3 shows that *Staphylococcus aurues*, *Aspergillus niger* and *Mucor recemosus* had 100% occurrence in all the boreholes analysed while *Pseudomonas aeruginosa* had 71.4% followed by *E. coli*, *Enterobacter aerogenes* *Streptococcus faecalis* (57.1%), respectively. The result of the MPN tests for occurrence of the presumptive coliforms is shown in Table 3 while Table 4 shows the results of the analysed physico-chemical parameters of the selected borehole water samples. Results show that several of the borehole water samples of MOUAU off-campus hostels were contaminated with both non-faecal and faecal coliform bacteria. From Table 5, *Staphylococcus aureus* was isolated from the seven water

Table 1: Identified isolates

Colonial morphology	Microscopy	Gram stain	Motility	Catalase	Oxidase	Coagulae	Lactose	Manitol	Glucose	Isolated organisms
Colonial smooth and small with convex elevation and opaque	Cocci in chain	+	-	-	+	+	-	A	AG	*Streptococcus faecalis*
Smooth and circular translucent on nutrient agar	Short rods single and separate	-	+	+	-	-	AG	A^g	AG	*Escherichia coli*
Colonies round and smooth, creamy to white colour, dull and soft appearance	Short rods	-	+	+	-	-	AG	AG	AG	*Enterobacter aerogenes*
Colonies shiny and smooth with grey tint in nutrient agar	Small scattered rods	-	+	+	+	-	-	AG	A	*Pseudomonas aeruginosa*
Smooth, large, circular and creaming colonies with outlined edge	Cocci in cluster	+	-	+	-	+	A	A	AG	*Staphylococcus aurues*

+ = Positive; - = Negative; AG = Acid and Gas production; A = Acid production only; A^g = Slight gas and acid production

Table 2: Morphological identification of fungal isolates

Macroscopic characteristics	Microscopic characteristics	Probable isolate
White surface with brown reverse	Filamentous and non-septate without colia thick wall is formed	*Mucor racemosus*
White colonies later turn black	Septate hypae, unbranched of variable length double sterigmata covers	*Aspergillus niger*
Reverse side is brown	the vesicle and forms a radiate head	

samples while *Escherichia coli*, *Streptococcus faecalis*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* were not isolated from all the water samples analyzed. The presence of *E. coli* (an indicator organism) is undesirable in drinking water as it indicates faecal contamination of the water and this was found to be present in 57.1% of the 7 water samples tested (Table 5). *Escherichia coli* is known to cause many enteric diseases such as traveller's diarrhoea and other forms of diarrhoea (Pandey *et al*., 1999). *Pseudomonas aeruginosa* is opportunistic pathogen responsible for many hospital acquired (nosocomial) infections.

Table 3: Occurrence of presumptive coliform in tube of the MPN

Samples	50 mL	10 mL	1.0 mL	MPN index per 100 mL
AL	0	0	0	000
		0	0	
		0	0	
		0	0	
		0	0	0 coliform
AG	0	0	0	000
		0	0	
		0	0	
		0	0	
		0	0	0 coliform
DL	1	0	1	123
		1	0	
		0	1	
		0	0	
		1	1	12 coliform
DOL	0	0	0	010
		0	0	
		0	0	
		0	0	
		1	0	0 coliform
PG	0	1	0	022
		0	1	
		0	0	
		0	0	
		1	1	4 coliform
GL	0	0	0	001
		0	0	
		0	0	
		0	0	
		0	1	1 coliform
BC	0	0	1	112
		0	0	
		1	0	
		0	0	
		0	1	7 coliform

The high level of faecal contamination in some of the water samples could be attributed to proximity of some of these boreholes to soak away pits and septic tanks. Apart from having faecal origin, some of these coliforms are natural inhabitants of soil, water, plants, human skin and animal. Some of the selected hostels whose borehole water were analysed are situated in environments close to contamination sources such as refuse dumps soak away pit and septic tanks. The fungi identified in the research are *Mucor racemosus* and *Aspergillus niger* (Table 2). The moulds are normal flora of the soil, terrestrial habitat and fruit plants. Most of the hostel boreholes have fruit trees and plants around them which provide shade for its inhabitants and also serve as source of possible contamination with these organisms which were found to be very close and could be washed below the ground level, thereby leach into the aquifer. The presence of the fungi and bacteria in the water samples can be attributed to the possibility of leakages in the manholes of the boreholes which could have resulted from routine maintenance and repairs carried out more often on these boreholes. Water samples from boreholes AL and from AG source are fit for drinking and domestic purposes because they have faecal coliform counts of zero MPN/100 mL (Table 3). This is in conformity to the set of standard of WHO (1993) which says no water sample should contain faecal coliform in any 100 mL of water sample. The other water samples analysed were contaminated with faecal coliforms, thus they need to be treated before consumption. The Total Heterotrophic Plate Count (THPC) (Table 6) is in the range of 4.7×10^4 to

Table 4: Physico-chemical parameters of the water sample

Parameters	WHO guideline	AL	AG	DL	DOL	PG	GL	BC	Range	Mean
Colour	15TCU	8.000	6.000	6.000	7.000	7.000	8.000	6.000	8.000	6.860
pH	6.5-8.5	6.900	7.200	6.700	6.900	7.000	6.800	7.200	7.200	6.960
Temperature (°C)	12-28	27.000	29.000	26.000	27.000	25.000	28.000	28.000	29.000	27.100
Turbidity	0.5NTU	0.016	0.023	0.007	0.039	0.015	0.003	0.011	0.039	0.016
Salinity	NG	0.200	0.500	0.300	0.300	0.200	0.100	0.200	0.500	0.260
Conductivity ($\mu s\ cm^{-1}$)	250 ($ms\ cm^{-1}$)	212.000	245.000	229.000	250.000	241.000	235.000	240.000	250.000	236.000
Dissolved solids,		0.600	0.900	0.700	1.000	0.700	0.700	0.600	1.000	0.610
Dissolved oxygen		6.000	4.000	7.000	3.000	4.000	6.000	6.000	7.000	5.140
BOD ($mg\ L^{-1}$)		54.000	75.000	83.000	62.000	78.000	71.000	68.000	83.000	70.100
Total solids ($mg\ L^{-1}$)	1000	594.000	585.000	600.000	590.000	603.000	601.000	602.000	603.000	596.430
Total acidity (titratable)	NG	1.500	1.900	1.500	2.000	0.700	1.000	2.100	2.100	1.530
Total hardness ($g\ cu^{-1}\ m$) ppm	0-5	0.200	0.300	0.200	0.400	0.200	0.400	0.300	0.400	0.290
Sodium ($mg\ L^{-1}$)	200	171.000	193.000	201.000	186.000	200.000	169.000	197.000	201.000	188.000
Calcium	100	85.000	96.000	88.000	78.000	77.000	86.000	93.000	96.000	86.100
Sulphate ($mg\ L^{-1}$)	400	332.000	295.000	287.000	305.000	270.000	262.000	260.000	332.000	287.300
Nitrate ($g\ cu^{-1}\ m$)($mg\ L^{-1}$)	10	42.000	35.000	65.000	50.000	20.000	49.000	27.000	65.000	41.100
Chloride ($mg\ L^{-1}$)	250	220.000	209.000	250.000	240.000	260.000	240.000	248.000	260.000	238.100

Borehole water sample coded: AL AG, DL, DOL, PG, GL and BC; NG = No Guidelines

Table 5: Distribution of bacteria and fungi in the selected borehole waters

Isolates	No. of boreholes examined	No. of boreholes present	Occurrence (%)
Escherichia coli	7	4	57.1
Staphylococcus aurues	7	7	100.0
Streptococcus faecalis	7	4	57.1
Enterobacter aerogenes	7	4	57.1
Pseudomonas aeruginosa	7	5	71.4
Aspergillus niger	7	7	100.0
Mucor recemosus	7	7	100.0

Table 6: Total viable bacterial count (cfu mL^{-1})

Code	Heterotrophic plate counts (cfu mL^{-1})	Total coliform plate counts (cfu mL^{-1})
AL	7.80×10^{4}	5.90×10^{3}
AG	4.90×10^{4}	4.10×10^{3}
DL	1.82×10^{5}	8.10×10^{3}
DOL	5.00×10^{4}	4.20×10^{3}
PG	5.40×10^{4}	6.90×10^{3}
GL	4.70×10^{4}	5.90×10^{3}
BC	9.20×10^{4}	8.60×10^{3}
Range	4.70×10^{4}-1.82×10^{5}	4.10×10^{3}-8.6×10^{3}
Mean	7.89×10^{4}	6.24×10^{3}

1.82×10^{5} cfu mL^{-1} and this is higher than the stipulated guideline of WHO (1,000 cfu mL^{-1}) and NAFDAC (2004) (100 cfu mL^{-1}). The THPC is significantly high in borehole DL (1.82×10^{5} cfu mL^{-1}) well above the mean value of 78.86×10^{4} cfu mL^{-1}. This is because DL is the oldest hostel borehole of the seven boreholes (>14 years old). A high contamination of the reservoir tanks and distribution system or several repairs in the drill holes or manholes may have accounted for the high counts of aerobic bacteria. The Total Coliform Plate Count (TCPC) from Table 3 gave a range of 4.1×10^{3}-8.6×10^{3} cfu mL^{-1} and a mean of 62.43×10^{3} cfu mL^{-1} for the seven boreholes tested. The highest counts were obtained in DL (8.1×10^{3} cfu m^{-1}) and BC (8.6×10^{3} cfu m^{-1}). These could be regarded as non-faecal since the resultant faecal coliform counts for these boreholes gave presumptive count of 12 coliforms for DL and 7 coliforms for BC per 100 mL, respectively (Table 3).

The result of the presumptive coliform in Table 5 indicates a coliform contamination in two boreholes (DL = 12 and BC = 7, coliform per 100 mL). Borehole DL is located <20 m to an old septic tank while BC is situated on an environment which used to be a refuse dump before the establishment of a hostel borehole. The proximity of this borehole is against the NAFDAC (2004)'s stipulated minimum distance of 30 m. These findings are of great importance because they affect the health of the consumer by these microorganisms. The introduction of coliforms into the borehole may have resulted from the environment during drilling. The physico-chemical parameters of the seven boreholes were compared with regard to WHO guidelines for drinking water and the result of the samples tested fell within WHO permissible limits with the exception of nitrate concentrations which were higher than the specifications (Table 4). The guidelines for nitrate in potable water is 10 (g cu^{-1} m) (mg L^{-1}). However, its concentration range fell between 65-20 mg L^{-1} for the analysed samples of water with a mean concentration of 41.1 mg L^{-1}. Nitrates are known to occur in ground water in high amounts. Due to its potential toxicity and widespread occurrence, it is regulated. Its toxic effects in infants have been demonstrated. The right potential anthropogenic activity aiding the high concentration of nitrate in water is not clear since there is no active agricultural activity which could supply nitrogenous compounds that might be washed into the boreholes. High concentration of nitrates in groundwater of shallow aquifer beneath areas of extensive development (Obi and Okocha, 2007) could be a possible explanation for the high convention level of nitrate in the analysed boreholes. Moreover, nitrates occur naturally in mineral deposits (generally sodium or potassium nitrate) in soil, seawater, freshwater system, the biota and atmosphere.

It is of great relief to note that the boreholes AL and AG are of better bacteriological and physico-chemical qualities than all the analysed boreholes. Apart from being the newest of the seven analysed boreholes, they are situated in an area with no history of any environmental risk factors. The soak away pit and septic tanks in the hostesl are positioned according to NAFDAC (2004)'s stipulated minimum distance of 30 m. Their physico-chemical parameters (temperature) gave a little bit higher result of 29°C which can be attributed to climatic factor (Radiation) within the environment and have also shown to have no effect on the aesthetic value of the boreholes water samples.

CONCLUSION

The location of waste and refuse dumps near boreholes and indiscriminate situation of boreholes in areas without proper environmental conditions suitable for distribution network of the water pipelines to the students' hostels contributed to level of the contamination identified in this research. Therefore, the treatment of boreholes water supplies before consumption is highly recommended. Continuous consumption of this water by students could lead to an increase risk level of outbreak of water borne diseases on the university off-campus hostels.

RECOMMENDATIONS

The university authorities, the private hostel developers and the government should map out more refined public health measure to mitigate or minimize the

occurrence of major water borne diseases outbreak on the campus and also try to reduce the high level of contamination such as nitrate which can only be removed from water by very expensive method such as Reverse Osmosis (RO) and Ultra Filtration (UF).

The private hostel developers (owners) are also advised to adopt cost-effective methods of water treatment like the WHO (2004) stipulated method of flushing the entire water system (including the storage tanks and distribution pipes) with chlorinated water (having up to 40 ppm free residual chlorine) for 30 min and followed by overnight soaking of the entire system with super chlorinated water. Proper health education on the student should be conducted to enlighten them on the effects of drinking contaminated water on health. Constant monitoring of the hostel boreholes water supplies should be taken up by the appropriate local authority to ensure the maintenance of a good drinking water quality within and outside Michael Okpara University of Agriculture Umudike off-campus hostels.

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