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Full Length Research Paper

Evaluation of the bacteriological and physicochemical quality of water supplies in Nsukka, Southeast, Nigeria

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The bacteriological and physicochemical quality of various water samples from bore hole, dug well and spring, collected from ten different locations within Nsukka were determined. Total viable and coliform counts were evaluated using the standard plate count method and the most probable number (MPN) technique, respectively. The physicochemical parameters were analyzed using standard methods. The mean total bacteria count of the water samples ranged as follows: bore hole (0.92×10^4 to 1.41×10^4) cfu/ml, well water (1.80×10^4 to 2.40×10^4) cfu/ml and spring water (0.78×10^4 to 1.06×10^4) cfu/ml. The mean total coliform count of the samples in (MPN/100 ml) ranged as follows: bore hole (10 to 15), well water (14 to 18) and spring water (8 to 10). The isolated and identified bacteria were *Enterobacter* spp., *Alcaligenes* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus aureus* and *Bacillus* sp. The physicochemical values of the water samples ranged as follows: pH (5.6 to 6.4), dissolved oxygen (DO) (5.4 to 6.4), biochemical oxygen demand (BOD) (10.0 to 20.4), chloride (1.6 to 2.3) mg L^{-1}, total hardness (48.6 to 68.0) mg L^{-1}, total dissolved solids (6.3 to 9.7) mg L^{-1}, sulphate (2.0 to 3.4) mg L^{-1} and nitrate (1.2 to 4.1) mg L^{-1}. The water supply sources in the present study have good physicochemical attributes for human consumption but the presence of *E. coli* and other potential enteric pathogens indicated faecal matter contamination of the water implying that they are not suitable for human consumption.

Key words: Drinking water, bacteriological and physicochemical quality, coliforms.

INTRODUCTION

The quality of drinking water is very essential for public health. A primary concern of people living in developing countries is that of obtaining clean drinking water. In many African and Asian countries, most of the large cities utilize tap water, but many millions of people in peri-urban communities and rural areas are dependent on surface and groundwater. The situation is not different in Nigeria, particularly in the rural areas. The provision of clean and safe drinking water is one of the major infrastructure problems for the urban and peri-urban areas. Many households in urban areas of Enugu state have access to treated piped water, which is received directly in their homes or at community standpipes. However, this is not the case in most rural communities. The microbiological quality of drinking water is of serious concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness is well documented in different countries (Payment, 1997). It has been reported by Isaac-Renton et al. (1996) that sporadic cases of waterborne intestinal illnesses will not be detected or, if detected, may not be recognized as water related. Hunter (1997) reported that waterborne diseases might account for one third of the intestinal infections world-wide. A total number of 1.2 million water related cases of illness has been reported by Hunter and Syed (2001), while Pruss et al. (2002) estimated that water, sanitation and hygiene were responsible for 4.0% of all deaths and 5.7% of the total disease burden occurring worldwide.

Contamination of water and food with fecal bacteria is, and remains, a common and persistent problem,

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impacting public health, local and national economies (Stewart et al., 2007). Water-related diseases are the major cause of mortality and morbidity worldwide. Among these, diarrheal diseases are estimated to cause 1.8 million deaths each year, mostly in developing countries (WHO, 2004). Improved water supply and proper sanitation can reduce the occurrence of these diseases. However, outbreaks of water and food-borne diseases still often occur, even in developed countries. In the United States, 76 million cases of food borne illnesses occur every year resulting in 325000 hospitalizations and 5000 deaths (CDC, 2005). Pathogenic agents causing these diseases include the enteric bacteria (diarrhegenic *E. coli*, *Salmonella*, *Shigella* and *Campylobacter*), viruses (norovirus, hepatitis A) and protozoa (*Cryptosporidium* and *Giardia*) (Mead and Slutsker, 1999). An outbreak of *E. coli* O157:H7, caused by spinach in the U.S. and Canada, was reported in 2006 (CDC, 2006). The spinach was most likely contaminated by irrigation water in California.

The occurrence of water borne illnesses has both economic and social impacts. Consequently, monitoring the levels of contamination and the prevention of disease outbreaks is important from both economic and public health perspectives. Moreover, the need to assess the microbiological quality of water has become imperative because it has a direct effect on the health of individuals. The recognition of the connection between pollution and the need to protect human health, recreation and fisheries production led to the early development of water quality regulations and monitoring methods (Jenkins et al., 1996; USEPA, 2007). The use of certain bacteria as indicators of potential presence of pathogenic micro-organisms in water is the standard means of assessing the microbiological quality of a water body (Payment et al., 1997; El-Abagy et al., 1999; El-Taweel and Shaban, 2001). This study examined water samples taken from ten different sources of domestic water supplies in parts of Nsukka, Enugu state, for microbiological and some physicochemical properties, in order to evaluate the quality of water from such supplies.

MATERIALS AND METHODS

Sampling area

The sampling area, Nsukka, has an urban setting and is the site of the University of Nigeria, the first indigenous Nigerian university and one of Nigeria's largest universities. Nsukka is a town and Local Government Area in Enugu state, South-East, Nigeria. Currently, the town has a number of Federal parastatals in the University, such as National Biotechnology Development Agency, Centre for Basic Space Science, and the Energy Research Centre. Nsukka, with an estimated population of 400, 000, has the inhabitants rely on wells, boreholes and pipe borne water supply.

Collection of samples

A total of 30 water samples were collected from ten different locations. The locations were chosen at random from different parts of Nsukka town and the University community. The locations were four boreholes, four dug wells and two springs and were respectively designated as BH1, BH2, BH3, BH4, WW1, WW2, WW3, WW4, SP1 and SP2. The collections were made for three consecutive months of March, April and May. The water samples were aseptically collected, in duplicates, in sterile containers, transported to the laboratory and analyzed within 4 h of collection. The well mouth is covered with a lid, which is removed and replaced during and after drawing (fetching) from it.

Enumeration, isolation and identification of aerobic heterotrophic bacteria

One milliliter (1 ml) of each water sample was transferred into 9 ml of normal saline and further dilutions were made. Aliquots (0.1 ml) of the appropriate dilutions of the serially diluted samples were then inoculated onto nutrient agar plates in duplicates. The spread plate method using a sterile bent glass rod was used. Inoculated plates were incubated at 37°C for 24 h. Discrete colonies that developed on each plate were counted, their average calculated and recorded as total heterotrophic counts of aerobic bacteria in cfu/ml. Morphologically different colonies were aseptically collected, inoculated onto freshly prepared agar plates and incubated at 37°C overnight to obtain pure isolates. Purified isolates were inoculated onto nutrient agar slants and incubated at 37°C overnight and stored in the refrigerator as stock cultures for further biochemical tests. The obtained isolates were characterized and identified using the schemes of Barrow and Feltham (1993) and Holt et al. (1994).

Analysis of water samples for coliform and faecal coliforms

The Most probable number (MPN) technique for coliform and total coliform was used for the water analysis. The procedure, which involved the use of three dilutions (10, 1, and 0.1 ml) of each sample, was adapted from APHA (1998). Results were expressed as the most probable number per 100 ml (MPN 100 m l^{-1}).

Physicochemical analysis of water samples

Each water sample was analyzed for pH, dissolved oxygen (DO), total hardness, total dissolved solids (TDS), biochemical oxygen demand (BOD), NO_3^-, SO_4^{2-}, and Cl^-. The pH was determined using a pH meter (Mettle Delta-340, England), after the meter has been duly calibrated with standard buffers of pH 4.0, 7.0 and 9.0. Total hardness was determined by titrimetry; total dissolved solid was determined by gravimetric method; chloride was determined by argentometric titration; sulphate was determined by titrimetry and nitrate was determined colorimetrically by Spectronic-20 (Gallenkamp, UK) as described by AOAC (1990).

Statistical analysis

Determinations were done in duplicates. Data were subjected to analysis of variance and the least significant difference (LSD) test was used to separate differences between means at 5% probability level.

RESULTS

Bacteriological examination

The bacteriological quality of the water samples

Table 1. Total counts and most probable numbers of indicator bacteria.

Sample	Total count (cfu/ml) (× 10^4)	MPN/100 ml	
		Total coliform	Faecal coliform
BH1	1.41	10	9
BH2	0.92	14	13
BH3	1.20	15	10
BH4	1.12	11	12
WW1	2.40	18	22
WW2	1.80	16	14
WW3	2.30	18	12
WW4	2.10	14	16
SP1	0.78	8	12
SP2	1.06	10	8
WHO guidelines[a]	1.0 x 10^2	0	0

BH: Borehole water; WW: Well water; SP: Spring water, [a]WHO (1996).

Table 2. Bacterial isolates obtained from water samples.

Bacterial isolate	Frequency number (%)	Water sample									
		BH1	BH2	BH3	BH4	WW1	WW2	WW3	WW4	SP1	SP2
Enterobacter spp.	4 (19.0)	-	-	+	-	-	+	+	+	-	-
Alcaligenes spp.	3 (14.3)	+	-	-	+	-	-	-	-	+	-
E. coli	3 (14.3)	+	-	-	-	+	-	-	-	-	+
Proteus spp.	3 (14.3)	-	+	+	-	-	-	+	-	-	-
Klebsiella spp	2 (9.5)	-	+	-	-	+	-	-	-	-	-
Pseudomonas aeruginosa	2 (9.5)	-	-	-	-	-	+	+	-	-	-
Acinetobacter spp.	2 (9.5)	-	-	-	+	-	-	-	-	-	+
Staphylococcus aureus	1 (4.8)	-	-	-	-	-	-	-	+	-	-
Bacillus sp.	1 (4.8)	-	-	-	-	-	+	-	-	-	-

BH: Borehole water; WW: Well water; SP: Spring water.

evaluated in the present study is shown in Table 1. Microbial indicator organisms were present in all the water samples from the different locations of the study irrespective of the time of sampling (Table 1). The results are compared with permissible limits of the World Health Organization (WHO, 1996). In borehole water samples, at the different locations, the range of the average numbers of total viable heterotrophic bacterial counts was between 0.92×10^4 and 1.41×10^4 cfu/ml. The mean total counts for well water samples ranged between 1.80×10^4 and 2.40×10^4, while for spring the mean total counts were 0.78×10^4 and 1.06×10^4, respectively. The results show very high microbial counts for the various drinking water samples when compared with the WHO standard of 1.0×10^2 cfu/ml. Analysis of variance on the data obtained showed that there was significant difference ($p \leq 0.05$) in total aerobic heterotrophic bacterial count between the various water samples. The results of the estimation of total coliform and faecal coliform bacteria counts in the water samples using the MPN technique are as also shown in Table 1. Analysis of variance on the data obtained showed that there was significant difference ($p \leq 0.05$) between the various drinking water samples in both the total coliform MPN and in faecal coliform bacteria.

Isolation and identification of bacteria

The numbers and percentages of isolated bacteria from the different water samples are shown in Table 2. The results show that nine different bacterial genera were isolated with *Enterobacter* spp. as the dominant bacteria with a frequency occurrence of 19%. The other eight genera of bacteria isolated and percentages of their occurrence were: *Alcaligenes* spp. (14.3%), *Escherichia coli* (14.3%), *Proteus* spp. (14.3%), *Klebsiella* spp. (9.5%), *Pseudomonas aeruginosa* (9.5%), *Acinetobacter* spp. (9.5%), *Staphylococcus aureus* (4.8%) and *Bacillus*

Table 3. Physicochemocal properties of water samples.

Parameter	Water sample										
	WHO guidelines[a]	BH1	BH2	BH3	BH4	WW1	WW2	WW3	WW4	SP1	SP2
pH	6.5-8.5	6.0	6.2	5.8	6.1	6.4	5.9	5.6	6.0	5.6	6.2
Total hardness (mg/l)	-	52.3	55.6	54.6	56.1	64.3	66.2	68.0	64.6	48.6	46.3
DO (mg/l)	7.5	5.6	5.9	5.4	6.1	6.4	5.8	6.0	6.1	5.4	5.6
BOD (mg/l)	10.0	12.0	10.2	12.5	14.0	18.6	20.4	20.0	18.6	10.0	12.0
Total dissolved solid (mg/l)	-	11.2	9.4	9.7	8.4	8.0	6.8	8.5	7.2	6.8	6.3
Nitrate (mg/l)	50	1.4	3.2	2.8	2.3	1.4	4.1	2.6	3.3	1.2	2.2
Sulphate (mg/l)	500	3.4	2.8	3.0	3.2	2.4	2.6	2.8	2.4	2.2	2.0
Chloride (mg/l)	250	2.1	2.0	1.6	1.6	1.8	2.0	2.2	2.3	1.6	1.5

BH: Borehole water; WW: Well water; SP: Spring water, [a]WHO (1996).

spp. (4.8%). Of the nine bacterial genera isolated from the different water samples, six were obtained from the borehole samples, six from well water samples and only three genera from spring water samples.

Physicochemical analysis of water samples

Table 3 shows the results of analysis for some physicochemical properties of the water samples, namely, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total hardness, total dissolved solids (TDS), nitrate, sulphate and chloride. The results show that the pH of all the water samples, irrespective of source, was within a narrow range which was between 5.6 and 6.4. All the pH values fall within the acid range and showed overlapping tendencies. The well water samples showed higher total hardness (64.3 to 68.0 mg/L) levels than the borehole and spring water samples (BH: 52.3 to 56.1 and SP: 46.3 to 48.6), respectively. The total hardness for well water samples ranged from 64.3 to 68.0 mg/l which was significantly higher ($p \leq 0.05$) than the values for borehole and spring water samples, respectively. Similar pattern of results were also obtained for BOD. However, the TDS, DO, nitrate, sulphate and chloride values of all the water samples are within the standards recommended by WHO (1996).

DISCUSSION

The present study showed the presence of aerobic heterotrophic bacteria, coliform and faecal coliform in the various drinking water samples. The study has therefore revealed the bacteriological quality of drinking water samples in Nsukka. The total aerobic heterotrophic counts in the different water samples appeared very high. It is therefore desirable to disinfect all drinking water supplies before consumption or use. The presence of such bacteria as *Pseudomonas aeruginosa* and *E. coli* is of significant value in determining the extent of water pollution. Pathogens such as *Klebsiella* and *Staphylococcus aureus* were also isolated. Environmental bacteria such as *Acinetobacter* and *Bacillus* spp., which are mostly saprophytic in origin were isolated from some of the waters. It was also found that all the water samples were positive for coliform MPN, showing high contamination and risk to public health. All the counts obtained for the total heterotrophic bacteria, total coliform and faecal coliform were high and far above WHO (1996) recommended guidelines. Similar results for domestic water supplies have been reported by Okonko et al. (2008).

The detection of faecal coliform indicates feacal pollution of the water supplies. The presence of faecal indicators such as *E. coli* and enteric pathogens such as *Enterobacter* indicated that the various water sources are polluted with faecal matter. Since coliform is indicative of feacal contamination, the implication is that all the water samples studied in Nsukka were of very poor sanitary conditions, which is in agreement with the report of Banwart (2004). It has also been observed by Edema et al. (2001), that many brands of water sold in Abeokuta did not meet WHO microbiological standards. The coliform is an indicator bacterial group that is used to evaluate the quality of drinking water, and any presence of coliforms indicates contact of the water with sewage or inadequate treatment or post treatment contamination. In un-piped (well) water supplies, sometimes up to 10 coliforms/100 ml are allowed as standards for tropical countries, but this should not occur repeatedly; if occurrence is frequent and sanitary condition cannot be improved, an alternative source must be found, if possible (WHO, 1981). Feacal contamination of drinking water has been reported to have very serious health implications (Banwart, 2004). Guidelines (WHO, 1993) for presumptive coliform test for both treated and untreated water samples is 0/100 ml, but in occasional untreated water samples, 3/100 ml are allowed on the condition that these would not be detected in consecutive water samples. In order to protect public health and ensure that water is safe for public use, any water intended for drinking, treated or untreated, piped or un-piped must meet certain microbiological standards. Thus, the direct consumption of water from these various sources could contribute to the spread of many infectious diseases.

The pH values obtained in this study showed the water samples to be slightly acidic and below the lower permissible limit recommended by WHO (1996). It may be reasonably presumed that low pH of the waters is likely caused by organic contamination. Udom et al. (2002) recorded similar low pH, though slightly higher (6.9) than the values for water samples in this study. They attributed the low pH to the abundance of organic matter in the overlying soils. Decomposition of organic matter leads to a decrease in pH (acidity). The levels of organic contamination in the soil would vary ideally, with depth of the soil strata; that is, decreasing from the top. Total dissolved solid is a measure of the level of dissolved solid in water and it influences the taste of drinking water. All the water supply samples studied met the recommended WHO guidelines in terms of physical characteristics. Oyeku et al. (2001) and Nwosu and Ogueke (2004) made similar observations in the physical characteristics of sachet water sold in Lagos and Owerri metropolis, respectively.

Water hardness is the traditional measure of the capacity of water to react with soap; hard water requires considerably more soap to produce lather. It is not caused by a single substance but by a variety of dissolved polyvalent metallic ions, predominantly calcium and magnesium cations, although other cations, for example, barium, iron, manganese, strontium and zinc, also contribute (WHO, 1996). Total hardness data obtained in this study indicate that the waters are soft and suitable for domestic uses. The waters will foam easily with soap. According to the study of Sharma et al. (2004), the direct effect of hardness on human health is yet to be proven scientifically. The levels of sulphate and nitrate recorded are also very low compared to WHO limits. This means that the sulphate and nitrate levels of the water samples are not injurious to health. High sulphate concentration causes gastrointestinal irritation. Excess nitrate in drinking water causes infantile methaemoglobinaemia, which acts on hemoglobin in children, leading to poor oxygen uptake at the cellular level. A direct relationship between high incidence of stomach cancer and the prolonged intake of nitrate-rich drinking water has been shown (Sharma et al., 2004). Chloride values obtained are quite lower than the WHO limit of 250 mg/l. Concentrations of chloride above 250 mg/l make drinking water impalatable by imparting salty taste, and may harm metallic pipes. The values of DO concentration obtained are within the WHO limits. Sources of oxygen in water include the atmosphere, as by-product of photosynthesis and through hydro-mechanical input (that is, surface agitation). According to UNESCO/WHO/UNEP (1992), DO is of much more limited use as an indicator of pollution in groundwater, and is not useful for evaluating the use of groundwater for normal purposes.

Conclusion

On the basis of the physicochemical parameters examined in this study, the water supply sources are suitable for domestic uses and drinking as at the time of investigation. However, the low pH recorded for all the water samples falls outside WHO permissible limit, and raises serious health concern. Microbiologically, the water samples are not of adequate quality. The presence of heterotrophic bacteria and coliforms in all the waters assessed imply that consumers of such waters are vulnerable to the risk of infection. From the standpoint of both microbiological and physicochemical parameters examined, the quality of the waters for use is in the order: spring > borehole > well.

REFERENCES

AOAC (1990). Association of Official Analytical Chemists, Washington, DC. , 15th edn.

APHA (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.

Banwart GJ (2004). Basic Food Microbiology, 2nd ed. Chapman & Hall Inc., New York, p. 751.

Barrow GI, Feltham RKA (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edition. Cambridge University Press, Cambridge.

CDC (2005). Foodborne illness. Frequently asked questions (Online). http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/foodborne_illness_FAQ.pdf

CDC (2006). Multi-state outbreak of E. coli O157:H7 infections from spinach. (Online). http://www.cdc.gov/foodborne/ecolispinach/

Edema MO, Omemu AM, Fapetu OM (2001). Microbiology and physicochemical analysis of different sources of drinking water in Abeokuta, Nigeria. Nig. J. Microbiol., 15(1): 57-61.

El-Abagy MM, Shaban AM, El-Taweel GE (1999). Efficiency of water treatment systems in production of drinking water at Beni-Suif water treatment plant. Egypt. J. Appl. Sci., 14: 23-33.

El-Taweel GE, Shaban AM (2001). Microbiological quality of drinking water at eight water treatment plants. Int. J. Environ. Health Res., 11: 285-290.

Holt JG, Krieg RN, Sneath AHP, Staley TJ, Williams TS (1994). Bergey's Manual of Determinative Bacteriology 9th Edition. (International Edition).

Hunter PR (1997). Waterborne Diseases Epidemiology and Ecology. 1st Edn, John Wiley and Sons, Chichester, United Kingdom.

Hunter PR, Syed Q (2001). Community surveys of self-reported diarrhea can dramatically overestimate in size of outbreaks of waterborne cryptosporidiosis. Water Sci. Technol., 43: 27-30.

Isaac-Renton J, Moorhead W, Ross A (1996). Longitudinal studies of *Giardia* contamination in two adjacent community drinking waters supplies: cyst levels, parasite viability and health impact. Appl. Microbiol., 62: 47-54.

Jenkins P, Southern T, Truesdale V, Jeary A (1996). Waters. Watts S, Halliwell L (Eds.). Essential environmental science. Methods and Techniques London: Routledge, pp. 336-350.

Mead PS, Slutsker L (1999). Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Nwosu JN, Ogueke CC (2004). Evaluation of sachet water samples in Owerri metropolis. Nig. Food J., 22: 164-170.

Okonko IO, Adejoye OD, Ogunnusi TA, Fajobi EA, Shittu OB (2008). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos

State, Nigeria. Afr. J. Biotechnol., 7(5): 617-621.
Oyeku OM, Omowumi OT, Kupoluyi CF, Toye EO (2001). Wholesomeness studies of water produced and sold in plastic sachets (Pure Water) in Lagos metropolis. Nig. Food J., 19: 63-69.
Payment P (19970. Epidemiology of endemic gastrointestinal and respiratory diseases-incidence, fraction attributable to tap water and cost to society. Water Sci. Technol., 35: 7-10.
Payment P, Siemiatycki J, Richardson L, Renaud G, Franco E, Prevost AM (1997). Prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. Int. J. Environ. Health Res., 7: 5-8.
Pruss A, Kay D, Fewtrell L, Bartram L (2002). Estimating the burden of disease due to water, sanitation and hygiene at global level. Environ. Health Perspect., 110: 537 542.
Sharma SK, Vijendra S, Chandel CPS (2004). Ground water pollution and evaluation of physico-chemical properties of groundwater. Environ. Ecol., 22 (2): 319-324
Stewart J, Santo-Domingo JW, Wade TJ (2007). Fecal pollution, public health and microbial source tracking. In Santo-Domingo JW, Sadowsky MJ (Eds). Microbial Source Tracking. ASM Press, Washington D.C., pp. 1-32.
Udom GJ, Ushie FA, Esu EO (2002). A geochemical survey of groundwater in Khana and Gokana local government area of Rivers State, Nigeria. J. Appl. Sci. Environ. Manage., 6(1): 53-59.
UNESCO/WHO/UNEP (1992). Water quality assessment – A guide to use of biota, sediments and water in environmental monitoring.
USEPA (2007). Recent recommended water quality criteria. United States Environmental Protection Agency, http://www.epa.gov/waterscience/criteria/wqcriteria.html.
WHO (1981). Drinking Water and Sanitation, 1981–1990: A way to Health, pp. 1-56.
WHO (1993). Guidelines for drinking water quality, Vol. 1, Recommendations. Second edition,World Health Organization, Geneva, pp. 188.
WHO (1996). Guidelines for Drinking Water Quality. Health Criteria and other Supporting Information. 2nd Edition, WHO, Geneva, p. 271.
WHO (2004). Water, sanitation and hygiene links to health: facts and figures. World Health Organization, Geneva, Switzerland. (Online). http://www.who.int/water_sanitation_health/factsfigures2005.pdf.