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ASSESSMENT OF BACTERIOLOGICAL AND PHYSICOCHEMICAL STATUS OF WELL WATERS IN EMENE ENUGU, NIGERIA

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ABSTRACT

Well water is a source of drinking water for many rural as well as sub-urban dwellers but the pollution of this water by bacteriological and chemical contaminants is of public health concern. Hence this study was aimed at accessing the bacteriological quality and physicochemical parameters of well waters located in Emene-Enugu, Nigeria. Water samples were aseptically drawn from ten different wells in Emene-Enugu. Physicochemical parameters such as temperature, pH, turbidity and electrical conductivity were determined using standard methods. Total and fecal coliform counts were also determined using the membrane filtration method. The isolates were also identified using cultural and biochemical tests. Pearson's correlation was used to determine the relationship between the parameters. Temperature of the well waters ranged from 31.5±0.11 to 33.7±0.11ºC while the pH ranged from 6.20±0.01 to 7.40±0.01. Turbidity and conductivity also ranged from 25±0.05 to 150±0.06 NTU and 21±0.58 to 163±0.12µS/cm respectively. Total and fecal coliform counts ranged from 0 to 179±2.31 cfu/100ml and 0 to 58±0.58 cfu/100ml respectively. Bacteria identification revealed the presence of *Enterobacter aerogenes*, *Salmonella enterica*, *Enterococcus faecalis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia* and *Citrobacter freundii*, in the water samples. Turbidity was significantly correlated with temperature, pH and fecal coliform ($p = 0.000$; $r = 0.693$, $p = 0.000$; $r = -0.679$ and $p = 0.000$; $r = -0.655$) respectively. These isolates are potential human pathogens, thus the well waters used in this study are not fit for human consumption and should be properly treated and monitored before domestic use.

Keywords: Total coliform, Fecal coliform, Well waters, Bacteriological, Physicochemical, Emene-Enugu

INTRODUCTION

Water is very essential to human existence and constitutes about 70% of the earth's crust. It can be categorized into atmospheric water in form of rain or snow, surface waters such as rivers, lakes, streams, oceans or sea and ground water such as wells and borehole. Unfortunately, the quality of these waters is impaired by microbial and chemical contaminants through anthropogenic activities. Though natural water is not a good medium for microbial growth but the presence of pollutants in water have aided the growth and proliferation of various pathogenic organisms in natural water bodies. The provision of safe drinking water particularly to developing countries like Nigeria have encountered enormous challenges **(Raji and Ibrahim, 2011)** due to urbanization, industrialization, increase in human population around the world **(Taiwo *et al*., 2020)**, poor sanitation and contamination of drinking water sources **(Alemu *et al*., 2015; De Troyer *et al*., 2016)**. Surface waters are the most polluted due to run off from surrounding land. Ground water may be contaminated through indiscriminate dumping of refuse, leachates from landfill or septic tanks, oil well leakages, mining and industrial activities, among others. Besides, there are grossly inadequate treatment facilities especially in rural communities as well as poor hygiene, thus making them more vulnerable to water related diseases such as typhoid fever, cholera, dysentery, giardiasis, trachoma, schistosomiasis, to mention but a few. Ground water may also be contaminated with dissolved organic and inorganic pollutants which may lead to other health problems to the inhabitants of the community **(Gonfa *et al*., 2019)**.

Water quality testing and sanitation is a critical step in improving municipal water supply in order to minimize the menace of waterborne diseases and other diseases associated with water. It has been a crucial element of the sustainable development goal (SDG) agenda of the United Nations **(UN, 2015)**. Data obtained from water quality testing of a particular drinking water source will assist governmental organizations, stakeholders and policy makers in developing policies geared towards improving domestic water supplies. Such water quality improvement will include water protection, control and abatement of water pollutants to minimize water quality deterioration **(Adah and Abok, 2013; Taiwo *et al*., 2020)**.

Well water equipped with hand pumps is a source of drinking water for many rural communities in Africa **(Bello *et al*., 2013)** including Nigeria. Several homes in Enugu especially the rural communities rely on hand dug well as a source of drinking water and other domestic chores. Many of these hand-dug wells are usually left open or partially covered and water drawn using a rope tied to a bucker or other plastic materials. This makes the water susceptible to both biotic and abiotic contamination. It is therefore pertinent that such water should be monitored, tested for the presence of coliform bacteria; indicator bacteria for the presence of other pathogenic bacteria and certified safe before human consumption. This therefore necessitates this study which was undertaken with the purpose of evaluating the bacteriological quality and physicochemical parameters of well waters located in Emene, Enugu, Nigeria.

MATERIAL AND METHODS

Study area

Emene is a densely populated area located in Enugu East Local Government Area of Enugu State, South Eastern Nigeria. Its geographical coordinates fall within latitude 6º 28' 33" N and longitude 7º 35' 2" E. Enugu is characterized by tropical climate with two distinct seasons; the summer (rainy season) and winter (dry season). The monthly temperature ranges between 24.3ºC to 29.0ºC with an annual rainfall of 1717mm and an average of 71.93 hours of sunshine per month. Akanu Ibiam International Airport is located in Emene Enugu, 7.85km from Enugu town.

Sample collection

Water samples were aseptically collected from 10 different wells located at Emene Enugu, using sterile glass bottles labeled A to J. A strong thread was attached to the neck of each bottle and gently lowered into the wells; the opened bottles were allowed to sink below the water to ensure that the bottles were completely filled and that no air bubble was trapped. The bottles were then gently pulled out of the wells without allowing them to touch the sides of the wells and their caps carefully replaced immediately. Replicate samples labeled for physicochemical analysis were also aseptically collected. Samples were transported to the laboratory for immediate analysis.

Physicochemical analysis

The physicochemical parameters such as water temperature, pH, electrical conductivity and turbidity were determined immediately using standard methods **(APHA, 2001)** after standardizing the equipment's. Temperature was measured using a handheld mercury-in-glass bulb thermometer. The pH was determined by electrometric method using a pH meter, Hanna model H1991300. Turbidity was determined using Labtech turbidity meter model Aq4500 and conductivity measured using the electrical conductivity meter. Resistivity was calculated from the values of conductivity.

Bacteriological analysis

Total and fecal coliform count

The membrane filtration method was used for both total and fecal coliform count. 100ml of the water samples were filtered through a membrane filter (0.45µm pore size) which retains bacteria. In the two step enrichment procedure, the filters containing bacteria were placed on an absorbent pad saturated with lactose broth.
For total coliform count, incubation was done at 35ºC for 2 hours after which the filters were transferred to a Petri dish containing MacConkey agar and incubated for another 24 hours at 35ºC. Visible colonies were counted and expressed as Cfu/100ml of the original sample.
For fecal coliform count, incubation was carried out at 44ºC for 2 hours, after which the filters were transferred to a Petri dish containing Eosin methylene blue agar and incubated for another 24 hours at 44ºC. Visible colonies were also counted and expressed as Cfu/100ml of the original sample.

Bacteria identification

The bacteria isolates were identified and characterized using cultural characteristics, gram reaction and biochemical tests **(Cheesbrough, 2000)**. The biochemical tests carried out include: catalase, citrate, motility, coagulase, oxidase indole, urease, sugar fermentation, methyl red and voges proskauer test.

Statistical analysis

Data were analyzed and presented as mean plus or minus standard error of mean (Mean ±SEM). Pearson's correlation was used to determine the relationship between the parameters at 99% confidence interval ($P<0.01$).

RESULTS

The physicochemical parameters of the water samples A to J are presented in table 1. The temperature ranged from 31.5±0.11 to 33.7±0.11ºC while the pH ranged from 6.20±0.01 to 7.40±0.01. Turbidity and conductivity ranged from 25±0.05 to 150±0.06 NTU and 21±0.58 to 163±0.12µS/cm respectively. Resistivity determined as inverse of conductivity was also shown (Table 1).
Table 2 shows the total and fecal coliform counts recorded in the water samples. Maximum total coliform count of 179±2.31cfu/100ml was observed in well water sample F, while the least count of 46±2.89cfu/100ml was recorded in well water sample E. However, zero coliform count was recorded in well water sample C. Fecal coliform recorded the maximum count of 58±0.58cfu/100ml in well water sample J and the minimum count of 5±0.58cfu/100ml was observed in sample F. Moreover, zero fecal coliform count was recorded in well water samples A and E (Table 2).

Table 1 Physicochemical analysis of the water samples

Samples	Temperature (ºC)	pH	Turbidity (NTU)	Conductivity (µS/cm)	Resistivity (µS/cm)
A	33.7±0.11	7.07±0.01	139±0.23	125±0.17	0.008
B	33.3±0.4	6.32±0.01	150±0.58	71±0.52	0.014
C	33.4±0.17	6.20±0.01	121±0.46	45±0.64	0.022
D	32.7±0.11	6.39±0.01	96±0.58	163±0.12	0.006
E	32.6±0.23	6.50±0.00	106±0.40	35±0.98	0.029
F	33.0±0.11	7.10±0.05	88±0.12	41±1.12	0.024
G	31.5±0.11	7.00±0.03	74±0.29	94±0.40	0.011
H	32.1±0.17	7.40±0.01	25±0.06	21±0.58	0.048
I	32.8±0.11	7.15±0.01	69±0.58	54±0.64	0.019
J	32.4±0.11	7.00±0.00	54±1.15	71±0.87	0.014

Values are mean of triplicates ±SEM

Table 2 Total counts of bacteria present in the water samples

Samples	Total coliform count (Cfu/100ml)	Fecal coliform count (Cfu/100ml)
A	112±1.15	0±0.0
B	85±2.89	10±0.58
C	0±0.0	28±1.73
D	56±1.15	15±1.73
E	46±2.89	0±0.00
F	179±2.3	5±0.58
G	152±1.15	25±0.58
H	56±3.46	33±1.15
I	75±2.31	50±0.00
J	85±1.72	58±0.57

Values are mean of triplicates ±SEM

The cultural and biochemical characteristics of the bacteria isolates are presented in table 3. The result revealed the presence of *Enterobacter aerogenes*, *Salmonella enterica*, *Enterococcus faecalis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia* and *Citrobacter freundii* in the well waters sampled (Table 3).
The correlation analysis (table 4) showed that temperature was highly significant and positively correlated with turbidity ($p = 0.000$; $r = 0.690$). pH was highly significant and inversely correlated with turbidity ($p = 0.000$; $r = -0.697$). pH was also highly significant but positively correlated with total coliform ($p = 0.003$; $r = 0.520$), while turbidity was highly significant and inversely correlated with fecal coliform ($p = 0.000$; $r = -0.655$).

Table 3 Cultural and biochemical characterization of the bacterial isolates

Tests	EA	SE	EF	PV	EC	KP	CF
Morphology	Circular and shiny white colony -rod	Circular, dry and creamy -rod	Circular, smooth and creamy +cocci	Contoured, smooth and creamy -rod	Circular, smooth and whitish -rod	Irregular, glistening and creamy -rod	Circular, moist and grey/shiny -rod
Gram stain	+	+	-	+	+	+	+
Catalase	+	+	-	+	+	-	+
Motility	-	-	-	+	+	-	-
Indole	-	+	-	+	+	+	+
MR	+	-	+	+	-	-	-
VP	+	+	-	-	-	+	+
Citrate	+	-	+	-	+	+	+
Lactose	+	G	+	AG	+	+	+
Glucose	+	-	+	A	Var	+	+
Sucrose	+	+	-	+	-	-	+
Fructose	+	+	+	A	-	+	+
Maltose	-	-	-	-	-	-	-
Oxidase	-	-	-	+	-	+	Var
Urease	EA	SE	EF	PV	EC	KP	CF
Identity							

EA; *Enterobacter aerogenes*, SE; *Salmonella enterica*, EF; *Enterococcus faecalis*, PV; *Proteus vulgaris*, EC; *Escherichia coli*, KP; *Klebsiella pneumonia*, CF; *Citrobacter freundii*, +; positive, -; negative, A; acid, AG; acid and gas, Var; variable.

Table 4 Correlation between the bacteriological and physicochemical parameters

Parameters	P – value	r –value
Temperature/pH	0.056	-0.352
Temperature/turbidity	0.000**	0.690
Temperature/conductivity	0.570	0.108
Temperature/total coliform	0.294	-0.198
Temperature/fecal coliform	0.053	-0.356
pH/turbidity	0.000**	-0.679
pH/conductivity	0.236	-0.223
pH/total coliform	0.003**	0.520
pH/fecal coliform	0.083	0.322
turbidity/conductivity	0.074	0.332
turbidity/total coliform	0.714	-0.070
turbidity/fecal coliform	0.000**	-0.655
conductivity/total coliform	0.487	0.132
conductivity/fecal coliform	0.280	-0.204
total coliform/fecal coliform	0.256	-0.214

**Correlation is significant at $P \leq 0.01$ level

DISCUSSION

The high temperature ranges of 31.5±0.11 to 33.7±0.11ºC observed in this study though fell within the limit of 25 to 36ºC set by WHO **(Osarenmwinda and Idaehor, 2019)**, could be attributed to the climatic conditions at the point of sampling and depth of the well. Similar result was obtained by **Allamin *et al.* (2015)** who reported a temperature range of 29.8 to 33.1ºC from well waters in Kaduna Metropolis. The pH range (Table 1) observed in this study is close to neutrality and fell within the standard range of 6.5 to 8.5 set by WHO and Nigerian Standards for Drinking Water Quality **(NSDWQ, 2015)**, except samples BCD where pH of 6.32, 6.2 and 6.39 were respectively observed to fall slightly below the limits of regulatory agencies. The slightly acidic pH observed in these samples (BCD) could be attributed to the pollution of the water through anthropogenic or industrial as well as natural phenomena. Similar result was obtained by **Eniola *et al.* (2007)** who reported a pH range of 6.54 to 7.80 and 6.54 to 7.90 respectively for borehole waters stored at indoor and outdoor containers. **Bello *et al.* (2013)** reported a pH range of 6.3 to 7.5 for well waters in Ijebu-Ode, Southwestern Nigeria. The pH of groundwater sources could be determined by the nature of the soil as well as the free CO_2 level in the water **(Ogbonna *et al.*, 2010)**.

The turbidity of the well waters sampled was observed to be very high comparable to the standard limits set by regulatory agencies. This increased turbidity may be the reason for the high temperature observed in the well water samples; because of the presence of suspended particles which absorb more heat. Temperature and turbidity was also strongly correlated in this study (p =0.000; r = 0.690, table 4). The high turbidity values may also be attributed to the presence of a number of bacteria isolates from the water samples. **Ali *et al.* (2012)** also observed high turbidity values (above the limits set by WHO and NSDWQ) in well waters located in Zango-Abbatoir, Kaduna-Nigeria.

Conductivity is a measure of the ability of water to pass an electrical current. It is influenced by the presence of inorganic dissolved solids such as chloride, nitrate, sulphate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron and aluminum cations (ions that carry a positive charge) **(EPA, 2012)**. Conductivity is also affected by temperature; the warmer the water, the higher the conductivity and vice versa. The conductivity measurement of the well waters sampled seems high (min 35±0.98 µS/cm; max 125±0.17 µS/cm) but fell within the permissible limits of WHO and NSDWQ **(Osarenmwinda and Idaehor, 2019)** for drinking water supply. The seeming high conductivity could be attributed to the slight increase in the temperature of the well waters as well as the presence of dissolved inorganic compounds. Similar results of high conductivity in well waters were reported by **Allamin *et al.* (2015), Osarenmwinda and Idaehor (2019)**.

The total and fecal coliform counts of the well water samples (Table 2) revealed the presence of total coliforms in all the well water samples except sample C that recorded zero total coliform count. The presence of coliform bacteria in drinking water source indicates contamination of the water with pathogenic bacteria. Also the presence of fecal coliform in all the water samples (except samples A and E) indicated contamination of the well waters with human or animal excreta. Many of the well waters used for domestic activities mostly in rural areas are hand drilled, shallow and water are being drawn with a rope attached to a bucket or other plastic/aluminum materials by rural dwellers. Coupled with the fact that many of these rural dwellers are farmers and cattle rearers, these may have contributed to the high number of total and fecal coliform counts observed in this study. WHO standard recommended zero total coliform per milliliter and zero fecal coliform per 100 ml of water as maximum permissible limit for drinking water. NSDWQ recommended 10cfu/ml for total coliform and 0cfu/100ml for fecal coliform as maximum permissible limit for drinking water sources **(NSDWQ, 2015)**. Thus most of the well waters sampled for this study are not suitable for human consumption because of the high counts of total and fecal coliforms observed, which exceeded that of WHO and NSDWQ guidelines. **Abednego *et al.* (2013)** recorded high coliform counts exceeding WHO permissible limits from water sources in river Ogun. The results of total coliform counts obtained in this study is also similar to that of **Rogbesan *et al.* (2002)** who in their study recorded high number of total coliform above the limits of WHO in more than 60% of water sampled.

The presence of *Enterobacter aerogenes*, *Salmonella enterica*, *Enterococcus faecalis*, *Proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia* and *Citrobacter freundii* in the well waters (Table 3) suggested fecal contamination. Some of these isolates have been implicated in gastrointestinal illnesses such as typhoid fever, dysentery, diarrhea and other forms of infections **(EPA, 2003)** and have been involved in mortality across the globe **(WHO, 2011)**. **Bello *et al.* (2013)** reported the isolation of *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Enterococcus* spp., *Proteus* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* from both borehole and well waters in a Southeastern Nigeria.

CONCLUSION

Most of the well waters analyzed in this study did not meet the chemical and microbiological standard guidelines stipulated by regulatory agencies such as WHO and NSDWQ for drinking water sources. Moreover, some of the bacteria isolates obtained in this study are potential human pathogens and have been implicated in gastrointestinal illnesses such as typhoid fever, dysentery, diarrhea and other forms of infections. Therefore, such water should not be used for human consumption, otherwise, adequate treatment and monitoring should be carried out to remove bacteria and other dissolved organic and inorganic substances found in the water before usage. It is recommended that septic tanks and local latrines should be located away from the wells to avoid leaching of contaminants into the well waters. Moreover, adequate hygiene should be maintained around the wells and the wells should be properly covered to avoid contamination through biotic and abiotic factors. Hand pumps for drawing water should also be installed in the wells rather than using a rope attached to a bucked to avoid introducing contaminants into the well waters.

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