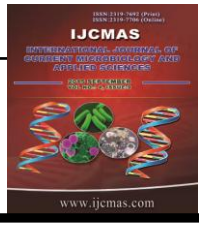
Int.J.Curr.Microbiol.App.Sci (2015) 4(9): 136-145

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 136-145**
http://www.ijcmas.com



Original Research Article

Physico-Chemical and Bacteriological Analysis of Well Water Used for Drinking and Domestic Purposes in Ogbomosho, Nigeria

I. C. Oladipo* and O. A. Adeboye

Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso 210214, Oyo State, Nigeria; 3Department of Microbiology, University of Ibadan, Ibadan 200284, Oyo State, Nigeria
**Corresponding author*

Keywords

pH,
well water,
antibiotic
susceptibility
and
total
viable count

A B S T R A C T

Samples of well water were collected from five different locations within Ogbomosho metropolis in Nigeria and analyzed microbiologically and physico-chemically using standard methods. Total viable count was between 1.0 x 10^3 and 2.2 x 10^3 cfu/ml. The pH ranged from 5.9 to 6.9 while the turbidity of the well water samples ranged from 0.67 to 1.00. The chemical oxygen demand of the samples was within the range of 1.10 to 3.33 while the chloride content of the sample was within the range 7.43–18.47, none of the water sample met the chloride content standard set by EPA. The total bacterial counts for all the water samples were generally high exceeding the limit of 1.0X10^2 CFU/ml which is the standard limit of heterotrophic count for drinking water. The Isolated organisms were identified to be *Bacillus polymxa, Bacillus alvei, Pseudomonas fluorescens, Bacillus megaterium* and *Bacillus licheniformis.* Conclusively, proper well location and construction, control of human activities to prevent sewage from entering water body is the key to the avoiding bacterial contamination of drinking water. Household treatment such as boiling should be encouraged before water from these wells is used for drinking and domestic purposes.

Introduction

Water of good drinking quality is of basic importance to human physiology and man's continued existence depends very much on its availability (Lamikaran, 1999; FAO, 1997). The provision of portable water to the rural and urban population is necessary to prevent health hazards (Nikoladze and Akastal, 1989; Lemo, 2002). Before water can be described as potable, it has to comply with certain physical, chemical and microbiological standards, which are designed to ensure that the water is palatable and safe for drinking (Tebutt, 1983). Potable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health (Ihekoronye and Ngoddy, 1985). Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, spring and wells (Kolade, 1982). Unfortunately, clean, pure and safe water only exists briefly in nature and is

immediately polluted by prevailing environmental factors and human activities. Water from most sources is therefore unfit for immediate consumption without some sort of treatment (Raymond, 1992).

The presence of bacteria is of great importance in the water industry with regards to water-borne diseases. Some of such diseases are dysentery, typhoid fever, paratyphoid fever, cholera, infantile paralysis, poliomyelitis, infectious hepatitis, guinea worm, amoebic dysentery, etc (Szewzyk *et al.,* 2000). Transmission of the causative micro-pathogenic organism is through direct or indirect contamination of water source by human excreta. Since it is extremely difficult to isolate and identify different forms of pathogens, the microorganisms which are of significance to water quality are those of enteric pathogenic origin (Szewzyk *et al.,* 2000).

In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on well, stream and river water for domestic use. The bacterial qualities of groundwater, pipe borne water and other natural water supplies in Nigeria, have been reported to be unsatisfactory, with coliform counts far exceeding the level recommendation by World Health Organization (Dada *et al.,* 1990a, 1990b, Edema *et al.,* 2001). Water quality assessment has become a big issue today because of the potential hazards associated with the use of contaminated water supply. Various researchers have reported on the serious and severe illness like typhoid fever, Cholera, dysentery resulting from the use of contaminated water supply (Mather, 1984). Also water of poor physico-chemical quality may have adverse health effect causing unavoidable economic and human losses (Mather, 1984).

In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system. Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population (Edema *et al.,* 2001). Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water borne diseases to the barest minimum in addition to being pleasant to drink, which implies that it must be wholesome and palatable in all respects (Edema *et al.,* 2001).

The principal objectives of municipal water are the production and the distribution of safe water that is fit for human consumption (Lamikaran, 1999). A good knowledge of the chemical qualities of raw water is necessary so as to guide its suitability for use. Thus, regular physico-chemical analysis of water at source must be carried out to determine or check the effectiveness of treatment process. This work is aimed at evaluating the microbiological quality and physico-chemical parameters of water sources used for drinking and cooking purposes in Ogbomosho, Nigeria.

Materials and Methods

Collection of samples

Five different well water samples were collected from five different locations in Ogbomosho. These samples were transported aseptically to the laboratory for analysis.

Culture media

The media used include Mac Conkey and Nutrient Agar. These were prepared

according to the manufacturer's specification and sterilized at 121^0C for 15 minutes in an autoclave.

Isolation of microorganisms

One milliliter of each sample was serially diluted, one milliliter of an appropriate dilution was inoculated on agar plates and the plates were incubated for 24hours at 37^0C. After 24 hours sterile wire loop was used to pick the pure colonies from the plate and was streaked on a freshly prepared nutrient and MacConkey agar then incubated for 24 hours at 37^0C. Pure cultures were then stored in a refrigerator at 4^0C. The routine laboratory method of Cruickshank *et al.* (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Total colony count

One milliliter of each sample was mixed with sterile de-ionized water and serially diluted. One milliliter of appropriate dilution was seeded on plate count agar using spread plate method, and then incubated at 37^0C for 24 hours. The plate count agar was examined and colonies present were counted and recorded after incubation at 37^0C for 24 hours, to get the total colony count in CFU/mL.

Determination of antibiotic susceptibility profile

The antibiotic susceptibility of the bacterial species isolated was performed on Müller-Hinton agar. Then, 0.1 mL of each bacterial isolates (105–106 cells/mL**)** was seeded into each of the Petri dishes containing sterile Müller-Hinton agar and were allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs (Himedia, India) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 30°C for 24 h. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (Oladipo *et al.*, 2014).

Physico-chemical analysis

The physic-chemical tests included the determination of turbidity, colour, total solid, total dissolved solid, total suspended solid, pH, total hardness and chloride content using the method of FAO (1997)

Determination of Chemical Oxygen Demand (COD)

The COD was determined according to the method reported by Rand and Taras (1975). Ten milliliter of the sample was taken in a100 ml bottle then 5 ml of conc. H_2SO_4 was added and about 1g of copper sulphate ($CuSO_4$) also added. Then 3 ml of prepared N/40 $KMnO_4$ solution was added and the bottle was immersed in boiling water for 30min while keeping the surface of the boiling water at the higher level than the surface of the sample. Then 3 ml prepared N/40 sodium oxalate ($Na_2C_2O_4$) was added and immediately titrated with N/40 potassium permanganate ($KMnO_4$) until violet color appeared then repeated for the blank separately under same condition using 10 ml of distilled water instead of 10 ml of sample. Then,

$$\text{COD as mg } O_2/L = \frac{1/40 \times 8000}{\text{ml of sample}}$$

Where:

A = ml of $KMnO_4$ used for sample.
B = ml of $KMnO_4$ used for blank.

1/40 = molarity of $KMnO_4$.
8000 = milliequivalent weight of oxygen × 1000 ml/L.

Determination of Biochemical Oxygen Demand (BOD)

The BOD-5 was determined using Winkler method as described by (Rand and Taras, 1975). Two 100 ml bottles was obtained with lid and cleaned well. 25 ml sample was taken in each bottle and 75 ml of distilled was added to the two bottles. Then the two bottles closed well. One bottle was kept in the incubator at 20–22°C for 5 days. Then 10 ml of manganese sulphate solution and 2 ml of alkali- iodide solution were added to the other bottle well below the surface of the liquid by using a syringe. Then the bottle closed and mixed by inverting the bottle several times. When the precipitate settles leaving a clear supernatant above the precipitate mixed again slowly by inverting the bottle, and when the setting has produced at least 50 ml supernatant 8 ml of con. HS_2O_4 were added. Then the bottle was closed and mixed by gentle inversion until dissolution was completed. Then 100 ml of the sample was titrated with 0.05 M $Na_2S_2O_3$ solution until a pale yellow solution is reached. Then 2 ml of freshly prepared starch solution was added and titration was continued until a blue color appeared. The procedure was then repeated using 100 ml distilled water (blank). Then, repeated for incubated sample after 5 days. The BOD was calculated as follows:

$$\text{BOD as mgO}_2\text{/L} = 16(V1 - V2)$$

Where:

$V1$ = ml of $Na_2S_2O_3$ used for the sample before incubation.

$V2$ = ml of $Na_2S_2O_3$ used for the sample after incubation.

Determination of Total Solids and Volatile Solids

The total solids and volatile solids of the samples were determined as described by (Punmia and Jan, 1998). Cleaned dish was taken and ignited to constant weight ($W1$). Then 25 ml of well mixed sample transferred to the above dish. Then the sample evaporated to dryness at 103°C for 24 hours, in constant temperature oven. Then cooled the dish in a desicator and weight was determined ($W2$). Then the dish was ignited at 600°C in furnace for 30 min. The dish was cooled in a desicator and its weight was determined ($W3$).

The total solids content was calculated as follows:

$$\text{Total solids (ST)} = \frac{(W2 - W1) \times 100\text{mg/l}}{V}$$

$$\text{Total volatile solids (SV)} = \frac{(W2 - W3) \times 100\text{mg/ml}}{V}$$

Where:

$W1$ = Weight of empty dish.
$W2$ = Weight of the dish after evaporation.
$W3$ = Weight of the dish after ignition.
V = Volume of the sample.

Determination of Suspended Solids (Non-Filterable Solids)

The total suspended solids were determined according to the method described by (Punmia and Jan, 1998). Cleaned crucible with filter paper was ignited to constant weight in an oven ($W1$). Then 25 ml sample was taken and filtered through the crucible. Then the crucible was dried in a constant temperature oven maintained at 103°C for 24 hours, then cooled in a desicator and weigh ($W2$). The suspended solids were then calculated as follows:

$$\text{Suspended solid} = \frac{(W2 - W1) \times 100\text{mg/ml}}{V}$$

Where:

W1 = Weight of empty crucible plus filter paper.
W2 = Weight of the crucible and filter paper after drying.
V = Volume of the sample.

Determination of total dissolved solids

Total dissolved solids were determined by evaporating the water samples to dryness (AOAC, 1990). In this method 50 ml of samples were transferred to a weighed evaporating dish, and evaporated to dryness by heating for 1–2 hours at 180°C to a constant weight. Total dissolved solids were calculated as follows:

$$\text{mg/l of TDS} = \frac{(\text{mg residue}) \times 1000}{\text{ml of sample}}$$

Determination of Color

The color was measured using Lovibond Tintometer (model E). It is a visual colorimeter used widely in the oil industry, but it is used in foods that are in liquid form (Pomeranz and Meloan, 1987). The instrument has a set of permanent glass colors filters in the three primary colors: red, yellow and blue. The colors were calibrated on a decimal scale in units of equal depth throughout each scale. The water samples were placed in glass cell and the filters were introduced into the optical system until color match was obtained.

Determination of Turbidity

The turbidity was determined photo-electrically using photometer 7000. The turbidity of water depends on the quantity of solid matters present in the suspension state. Turbidity is a measure of light-emitting properties of water, and turbidity test is used to indicate the quality of waste discharges with respect to colloidal matter. The turbidity depends up on the strength of the water samples. The results were expressed in term of formazin turbidity units (VTU).

Results and Discussion

Bacterial pathogens were isolated from well water samples collected from five different locations within Ogbomosho metropolis in Nigeria and were subjected to microscopical, macroscopical, physiological and biochemical tests. They were identified to be *Bacillus polymxa* (2), *Bacillus alvei* (1), *Pseudomonas fluorescens* (2), *Bacillus megaterium* (2), *Bacillus licheniformis* (2). The distribution of the microorganisms in the samples is shown in table 1. *Pseudomonas fluorescens* can survive and replicate in moist reservoirs, and as a result, nosocomial outbreaks often lead to the investigation of water sources (Anaissie *et al.*, 2002). It has generally been regarded to be of low virulence and an infrequent cause of human infection (Hsueh *et al.*, 1998).

Bacillus licheniformis is a facultative anaerobe commonly found in dust, soil and water. It is considered now pathogen for humans (Gale *et al.*, 2002). Most infections are associated with the experience of invasive trauma (e.g. catheters, surgery) and/or a debilitate health state, thus it is often encountered as a nosocomial pathogen (Gale *et al.*, 2002). *Bacillus licheniformis* is also a well-known cause of food poisoning, resulting in diarrhea and vomiting. Infections are rarely known to be fatal, although fatal food poisoning has been reported. Ocular infections, bacteremia, sepsis/septicemia, ventriculitis, peritonitis are the reported types of infection and they

are usually treated with antibiotics. Cases of long-term persistence or recurrence or of extended latency have not been found (Fritze and Pukall, 2001). Hence, the presence of these bacterial pathogens in the tested water samples suggests that infection may arise following consumption of these water samples.

The result of the total bacterial count showed that Sample E had the highest colony count of 2.2×10^3 CFU/mL while sample C had the lowest colony count of 1.0×10^3 CFU/mL as shown in table 2. Antibiotics susceptibility profile of the isolated organism was determined using commercially available antibiotics discs. It was observed that all the isolates were sensitive to all commercially available antibiotics used as shown in table 3. The total bacterial counts for all the water samples were generally high exceeding the limit of $1.0X10^2$ CFU/ml which is the standard limit of heterotrophic count for drinking water (EPA, 2002). The primary sources of these bacteria in water are animal and human wastes. These sources of bacterial contamination include surface runoff, pasture, and other land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities and natural soil /plant bacteria (EPA, 2002).

In the United States, several rivers were reported to be reservoirs of antibiotic resistant bacteria (Ash *et al.*, 2002). Antibiotic resistant may occur spontaneously, by selective pressure or because of antibiotic abuse by humans or over use in animals (White *et al.*, 2005). Although antibiotic resistant is common, antibiotics are still indicated in the management of diarrhea. Antibiotics shorten the duration of diarrhea, decrease stool output and may mitigate complications (Black, 1993). The antibiotic susceptible pattern of isolates in this study indicates that 100% of the isolates were sensitive to clinically relevant antibiotics.

The physico-chemical parameters analysis of the well water samples was also carried out and the pH of the well water samples was within the range of 5.9–6.9 and the color was within the range of 0.17–0.67 Hazen unit while the turbidity of the samples was within the range of 0.67–1.00 VTU. The chemical oxygen demand of the samples was within the range of 1.10–3.33 mg/L. The chloride content of the sample was within the range of 7.43–18.47 mg/L as shown in table 4. The physiochemical analysis of the well water samples was compared with World health organization and Environmental protection agency standards. The pH range of samples B, C, D, E was within the WHO and EPA standard except sample A that was below the standard. The turbidity of the samples was within the range of EPA standard but not up to WHO standard. The total solid of the samples were higher than WHO and EPA standard. The acidity of samples A, C, D and E were below WHO and EPA standard while sample B was higher than both WHO and EPA standards.

The pH of most the water samples were in agreement with pH assigned by EPA (as the standard pH of water ranges from 6.5 – 8.5), except pH of water sample B (EPA, 2002). None of the water samples met the color standard limit for drinking water set by EPA. The standard color limit recommended by EPA is 15 Hazen Unit (EPA, 2002) while the color of the water samples in this work ranged from 0.17 to 0.5. The low turbidity observed with the waters is in agreement with EPA standards on turbidity.

Table.1 Distribution of the isolate in the well water samples

Samples	*B. polymyxa*	*B. alvei*	*P. fluorescens*	*B. megaterium*	*B. licheniformis*
A	+	+	-	-	-
B	+	-	+	-	-
C	-	-	+	+	-
D	-	-	-	-	+
E	-	-	-	+	+

+ = present, - = absent,

Table.2 Total viable count of the well water samples

Samples	Total viable count (CFU/mL)
A	1.7×10^3
B	1.7×10^3
C	1.0×10^3
D	1.3×10^3
E	2.2×10^3

Table.3 Antibiotics susceptibility pattern of the isolates

Isolates	PEF	GN	APX	Z	AM	R	CPX	S	SXT	E
Bacillus polymyxa	22.5	22.5	15.0	12.0	16.5	20.0	22.5	22.5	22.5	22.5
Bacillus megaterium	20.0	18.0	19.5	20.5	18.0	18.5	21.0	20.0	17.5	19.5
Bacillus licheniformis	19.0	19.5	18.5	14.5	20.5	17.0	20.5	19.5	19.5	16.0
Bacillus alvei	19.5	19.5	20.0	15.5	15.0	19.5	20.0	19.0	20.0	18.5
Pseudomonas fluorescens	20.0	21.5	19.5	21.0	20.0	21.0	20.0	21.0	17.5	17.6

KEY: PEF (10µg) = Pefloxacin, GN (10µg) = Gentamycin, APX (30 µg) = Ampiclox, Z (20 µg) = Zinnacof, AM (30 µg) = Amoxacillin, R (25 µg) = Rocephin, CPX (10 µg) = Ciprofloxacin, S (30 µg) = Streptomycin, SXT (30 µg) = Septrin, E (10 µg) = Erythromycin

Table.4 Comparison of the physicochemical analysis of water samples from water sources with WHO and EPA standards

PARAMETERS	A	B	C	D	E	WHO STANDARD	EPA STANDARD
pH	6.50	5.90	6.70	6.70	6.90	6.50	6.50-8.50
Conductivity(Ms/cm)	296.00	82.33	160.33	492.67	151.67	-	-
Color(Hazen Unit)	0.17	0.67	0.33	0.17	0.50	6.00	15.00
Turbidity(VTU)	0.80	1.00	1.00	0.67	0.83	6.00	0.00-5.00
Total Solids(mg/100g)	1363.33	1440.00	1506.67	1760.00	1423.33	500.00	500.00
Total Suspended Solids(mg/L)	1250.00	1363.33	1426.67	1580.00	1350.00	-	-
Total Dissolved Solids(mg/L)	113.33	43.33	80.00	180.00	71.67	500.00	500.00
Acidity(mg/L)	0.23	0.47	0.13	0.13	0.17	0.30	0.30
Total Hardness(mg/L)	72.30	67.67	64.70	69.17	58.47	500.00	500.00
Chloride(mg/L)	7.43	8.33	10.40	18.47	8.50	200.00	250.00
Alkalinity(mg/L)	0.23	0.33	0.07	0.10	0.13	-	-
Dissolved oxygen(mg/L)	4.10	3.6	3.30	2.80	3.40	-	-
BOD(mg/L)	0.90	0.83	1.00	2.03	1.40	-	-
COD(mg/L)	1.13	1.30	1.57	3.33	1.10	-	-

High turbidity is often associated with higher levels of disease causing microorganism such as bacteria and other parasites (EPA, 2002; Schwartz *et al.*, 2000). Wells may get contaminated from underground runoff, which thereby increases its turbidity, which is a measure of cloudiness of water (EPA, 2002; Schwartz *et al.*, 2000). Fewer number of disease causing microorganisms may be an indication of lower turbidity value experienced with well samples. At no time can turbidity (Cloudiness of water) go above 5 VTU (EPA, 2002).

All water samples exceed the value set by EPA for standard limit for total dissolved solids, 500mg/L. Total dissolved solid in drinking water has been associated with natural sources, sewage urban runoff, industrial waste water and chemical used in the water treatment process (EPA, 2002), though of aesthetic rather than health hazards (EPA, 2002). The chloride content or limit recommended by EPA is 250mg/l; none of the water sampled is in agreement with the chloride content set by EPA (2002).

Generally, well water is believed to be pure (Gordan and John, 1996; Prescott *et al.*, 2002) because of the purification properties of the soil however, it can also be contaminated. Wells are found to be contaminated due to improper construction, shallowness, animal wastes, and proximity to toilet facilities, sewage, refuse dump sites, and various human activities around the well (Bitton, 1994).

Conclusively, proper well location and construction, control of human activities to prevent sewage from entering water body is

the key to the avoiding bacterial contamination of drinking water (EPA, 2002). Water borne diseases may be due to improper disposal of refuse, contamination of water by sewage, surface runoff, therefore programmes must be organized to educate the general populace on the proper disposal of refuse, treatment of sewage and the need to purify our water to make it fit for drinking because the associable organisms are of public health significance being implicated in one form of infection or the other (Bitton, 1994). In areas lacking in portable water as in rural dwelling, educative programmes must be organized by researchers and government agencies to enlighten the villagers on the proper use of well water. More surveys of water quality analysis should be carried out in the municipality. Household treatment such as boiling should be encouraged before water from these wells is used for drinking and domestic purposes.

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