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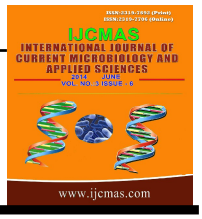


**Original Research Article**

# Physico-chemical and microbiological properties of water samples used for domestic purposes in Okada town, Edo state, Nigeria

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**A B S T R A C T**

In this study, the physicochemical and microbiological examination of drinking water and water used for domestic purposes in Okada town was carried out to ascertain their suitability for consumption, and presence of microorganisms such as typhoid, cholera, hepatitis, giardiasis which are water borne. A total of ten (10) water samples were collected from the various sources of water used for domestic purposes in Okada town. Total viable count was by pour plate technique while most probable number (MPN) counts were by the multiple tube fermentation technique. The pH ranges from 4.35 to 5.39 for the water samples while temperature ranges from 26 to 32$^{o}$C, the turbidity of the water and waste water samples ranges from 2 to 18. All the water samples were found to harbor coliform organisms in numbers greater than the WHO/FAO standards for water. The total viable counts for all the water samples were generally high, exceeding the limit of $1.0 \times 10^2$ cfu/ml for water. The MPN count ranges from 7 to 14 MPN/100ml. the fecal coliform counts on EMB agar plate ranges between 8 and 20 cells, also exceeding the standard limit for water. The isolated organisms were identified to be *Staphylococcus aureus, Salmonella species, Escherichia coli, Pseudomonas aerugionosa, Bacillus species* and *Flavobacterium.* The pathogenic, organic and indicator organisms present in all the water samples studied, as well as their physicochemical implications, render them unfit for human consumption, though they can be used for other purposes.

**Keywords**

Microbiological analysis; standard methods; water, physicochemical properties; morphological characteristics; biochemical characteristics.

## Introduction

The provision of portable water to the rural and urban population is necessary to prevent health hazards. Water houses the largest number of living organisms when compared with other habitats and one of the essential chemicals of life upon which all life forms depend. Physiologically, water is the medium for all biochemical reaction in man. 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 diseases 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, springs and wells. Unfortunately, clean, pure and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human activities.

The transmission of diseases through drinking water is one of the primary concerns for a safe drinking water. Fecal pollution of drinking water may introduce a variety of intestinal pathogens which may cause diseases from mild gastroenteritis to severe and sometimes fatal dysentery, diarrhea, cholera, typhoid, hepatitis, giardiasis etc (Crown, 1986; Wanda, 2006). Therefore, potable water is tested for an indicator of human or animal waste known as coliform bacteria which may include *Escherichia coli type 1, Streptococcus faecalis*, etc (NRDC, 2006). Coliform bacteria are bacteria which are always present in the digestive system of humans and animals and can be found in their waste. They are also present in the soil and plant material (Kathleen, 1998), and are usually gram negative. Ideally, drinking water should not contain any microorganism known to be pathogenic and should be free from bacteria indicative of faecal pollution (FEPA, 1999).

Although chemical composition of water may affect the safety, taste and appearance, bacterial contamination cannot be detected by appearance, taste or smell. This can only be detected by testing the water for the presence of indicator organisms and determining the *E. coli* and total coliform organisms present (Gomes and Martinis, 2004). Water samples that contain any coliform bacteria are generally reported as "total coliform positive". Federal regulations now require that public drinking water found to be "total coliform positive" must be analysed with a faecal coliform or *E. coli* test. These faecal bacteria when present in any concentration in water supply are unaccepted (Kathleen, 1998).

There are two types of water pollution, namely; point source pollution- which occurs as a result of release of harmful substances directly into the body of water, and non-point source pollution- which occurs as a result of indirect introduction of pollution into water bodies/sources from the environment (Kerker, 2003). Water pollution in Nigeria occurs both in rural and urban areas. Estimate suggests that nearly 1.5 million people lack safe drinking water. Raw sewage, garbage and oil spill all serve as sources for contamination (Ladeji, 2002). World Health Organization (WHO, 1995) informed that contaminated water, inadequate sanitation, and poor hygiene cause over 80% of diseases in developing countries. Water borne diseases such as cholera, typhoid fever, bacillary and amoebic dysentery kill at least 3.4 million people every year. Most cases of cholera and abdominal infections being reported in hospitals in recent times were traceable to the consumption of harmful particles and microorganisms, in water and beverages (Agha, 2006).

The principal objectives of municipal water are the production and distribution of safe water that is fit for human

consumption (Lamikanra, 1999). A good knowledge of the chemical qualities of raw water is necessary so as to guide its suitability for use. Thus, regular physicochemical analysis of water at source must be carried out to determine or check the effectiveness of treatment process. Thus, this research was conducted to evaluate the different sources of drinking water and water used for domestic purposes in Okada town compared with standard table water, for conformity to microbiological and physicochemical standards for treated water samples. Also, to identify possible pathogens which could be responsible for such contamination and to stress the public health implication of consumption of such contaminated water.

## Materials and Methods

### Sample Collection

Ten (10) water samples were collected from different locations in Okada town, Edo State, Nigeria. The locations (sites) were labeled A to J and covered all domestic water sources in Okada town. The samples were collected in sterile plastic containers, taken to the laboratory and analyzed immediately. Eva table water (produced by Nigeria Bottling Company, Coca-Cola), approved by NAFDAC, was used as control or standard.

### Physicochemical Properties

The water samples were analyzed for physicochemical properties by the methods of FAO (1997b) and Ademoroti (1996). The turbidity of the samples was determined by measuring the absorbance at 540 nm wavelength using spectrophotometer (HACH DR 2010). Temperature was determined at the point of collection using a digitron thermometer (model 275-K) as described by the methods of FAO (1997) and standardized mercury in glass centigrade thermometer as described by Edema *et al* (2001) and Ademoroti (1996).

### Microbiological Properties

All the media used for bacteriological analysis of the water samples; Plate Count Agar (PCA), Eosin Methylene Blue Agar (EMB), Nutrient Agar (NA) and Lactose Broth (LB), were weighed and prepared according to the manufacturer's specifications. A serial dilution method was used for total viable count and the presumptive test for coliforms. The sterility of each batch of test medium was confirmed by incubating one or two uninoculated tubes or plates along with the inoculated tests. The uninoculated tubes or plates were always examined to show no evidence of bacterial growth. Any uninoculated plate or tube that showed evidence of bacterial growth was discarded. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbon, 1974; Okonko *et al*, 2008).

### Statistical Analysis

All analyses were performed in triplicates. The data were recorded as means ± standard deviation and analyzed using Microsoft excel. Student t-test was used to analyze the significant differences between their means. Differences between means at 5% significant level (P-value < 0.05) were considered.

## Results and Discussion

From the table above (Table 4), the physicochemical properties and Microbiological analysis of water samples obtained from boreholes and taps in various locations in Okada community are not significantly different ($p > 0.05$).

The physicochemical properties of the freshly collected water samples are shown in Table 1. Some of the potable water samples did not comply with the standard limits for drinking water and domestic purposes. The tap water sample E is brownish in colour and most of the water samples had both particulate and suspended particles present in them. The pH range of 4.50 to 5.39 recorded for the untreated water samples and the pH 4.59 for tap water is considered as being unacceptable for natural waters. Borehole water (sample J) had the highest pH value of 5.39. The pH of most natural water sources ranges from 6.5 - 8.5 while deviations from the neutral point (7.0) may be as a result of fluctuations in the $CO_2$/bicarbonate/carbonate equilibrium. For example, the pH of brackish water bodies ranged from 6.5 – 7.4. The generally low pH values obtained in the water might be due to the high levels of free $CO_2$ in the water samples, which may consequently affect the bacterial counts. This was also reported by Edema *et al*, 2001. The pH of water is extremely important. The fluctuations in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water bodies. A temperature range of 26 – $32^0$C of the water samples was recorded. These may have been influenced by the intensity of the sunlight as temperature rose from 26 – $32^0$C on relatively hot days (Mulusky, 1974). This was also reported by Banwo, 2006. A temperature range of 26 and $30^0$C was attributed, in a similar study, to the insulating effect of increased nutrient load resulting from industrial discharge. The turbidity (NTU) of the water samples ranges from 2 – 18. Turbidity was observed to increase as the colour of the water changes from white to light-yellow or brownish (Table 1).

The microbiological analysis of the water and waste water samples is shown in Table 4. The total viable count (TVC) indicates that Tap water sample (D) has the highest microbial load after 24hrs and 48hrs of incubation, with the values $1.8 \times 10^5$ cfu/ml and $2.6 \times 10^5$ cfu/ml respectively, which is higher than the recommended value. The TVCs for all the water samples were generally high, exceeding the limit of $1.0 \times 10^2$ cfu/ml for water (Table 2).

Illegal dumping of domestic wastes, livestock management, fecal deposit and waste dumps affects bacterial concentration in water bodies. The most probable number (MPN) for the presumptive total coliform count of the water samples ranges from 7 to 14 MPN/100ml (Table 3 & 4). It indicates that tap water from location E and location I had the highest total coliform counts of 14 MPN/100ml followed by borehole water from location F, having 12 MPN/100ml, showing that these water samples were contaminated. The coliform count on EMB agar plate showed that tap water from location E had the highest coliform counts of 20 cells/ml, followed by borehole water from location G (18 cells/ml) and tap water from location I (17 cells/ml). Recommended standard for water is less than 2 MPN/100ml (FAO, 1997). The fecal coliform counts per 100ml of the water samples on EMB agar plate ranged between 8 and 20 cells (Table 3 & 4), which also exceeds the standard

limit for water. The presence of coliforms in these water samples generally suggests that a certain selection of water may have been contaminated with feces, either of human or of animal origin. Other more dangerous microorganisms could be present (Richman, 1997).

The morphological characteristics of the isolates obtained from the water samples on Nutrient agar (NA) and Eosin methylene blue (EMB) agar is shown in Table 5. The complete coliform tests showed a positive confirmation for all the water samples. The gram's reaction and endospores staining reaction for the characterization of the isolates obtained are also shown in Table 5.

The biochemical characteristics of the isolates obtained from these water samples are shown in Table 6. The positive results (which are the normal metabolic reactions inherent in most life forms or cells) for the various tests carried out indicates that the isolated bacteria species identified are same with those commonly encountered in water and aquatic environments as was also reported in a study on streams surface water in Wyoming in USA (Banwo, 2006). These identified isolates include *Staphylococcus aureus, Salmonella* species, *Escherchia coli, Pseudomonas aerugionosa, Bacillus* species and *Flavobacterium* species (Table 5).

Water-borne disease could be contracted and spread through drinking and use of contaminated water. Water quality indicates that pollution of the water is increasingly alarming and that it has created serious threat to human health and environment. Bacteriological pollution of drinking water supplies may be either due to the failure of the disinfections of the raw water at the treatment plant, or the infiltration of contaminated water (sewage) through cross connection, leakage points and back siphon-age. In piped supplies, discontinuity increases the likelihood of contamination as the risk of back siphon-age into the distribution network is increased when pipes are at lower pressure than the surrounding soil, which often contains leaked out effluents from leaking sewers. Thus, the quality of the water consumed is critical in controlling infectious diseases and other health problems. According to the WHO, the lack of safe water supply and of adequate means of sanitation is blamed for as much as 80% of all diseases in developing countries. In Okada, morbidity rates from water borne disease are considered high particularly among children below the age of five (5). A regular monitoring of the water quality for improvement not only prevents disease and hazards, but also checks the water resources from going further polluted. The conservation of water sources is very important to provide safe water. As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, protozoan and helminthes parasites. The control of drinking water quality in distribution networks remains a major challenge in urban and rural areas. The protection of sources, treatment and distribution management are all critical strategies in maintaining and improving piped water supplies.

The pathogenic, organic and indicator organisms present in all the water samples studied render them unfit for human consumption, though they can be used for other purposes. Water should meet different quality specifications depending

**Table.1** Physicochemical properties of the water samples

| Sample | Colour | Odour | Taste | Presence of particles | Temp ($^0$C) | pH (25$^0$C) | Turbidity (NTU) |
|---|---|---|---|---|---|---|---|
| A (Spring) | Colourless | Odourless | Tasteless | Suspended | 26 | 4.50 | 3 |
| B (Tap) | Colourless | Odourless | Tasteless | None | 30 | 5.11 | 2 |
| C (BoreHole) | Colourless | Odourless | Tasteless | Particulate | 28 | 4.68 | 9 |
| D (Tap) | Colourless | Odourless | Tasteless | Particulate | 30 | 5.28 | 5 |
| E (Tap) | Brownish | Odourless | Tasteless | Suspended | 31 | 5.05 | 5 |
| F (BoreHole) | Colourless | Odourless | Tasteless | None | 30 | 4.75 | 18 |
| G (BoreHole) | Colourless | Odourless | Tasteless | None | 32 | 4.35 | 4 |
| H (BoreHole) | Colourless | Odourless | Tasteless | Suspended | 30 | 4.86 | 3 |
| I (Tap) | Colourless | Odourless | Tasteless | None | 28 | 4.59 | 5 |
| J (BoreHole) | Colourless | Odourless | Tasteless | Particulate | 31 | 5.39 | 2 |
| Standard limit | Colourless | Odourless | Tasteless | None | Varies | 6.8 - 8.5 | 20 |

Data are presented as results for three (3) independent determinations (i.e. n = 3).

**Table.2** Total Viable Count (TVC) of water samples

| SAMPLES | 24 HR OF INCUBATION (CFU/ML) | 48 HR OF INCUBATION (CFU/ML) |
|---|---|---|
| A (Spring) | $1.4 \times 10^{4a}$ | $1.7 \times 10^{4a}$ |
| B (Tap) | $1.0 \times 10^{4b}$ | $1.2 \times 10^{4b}$ |
| C (Bore Hole) | $1.6 \times 10^{4}$ | $1.8 \times 10^{5}$ |
| D (Tap) | $1.8 \times 10^{5c}$ | $2.6 \times 10^{5c}$ |
| E (Tap) | $1.4 \times 10^{3d}$ | $1.7 \times 10^{3d}$ |
| F (Bore Hole) | $1.6 \times 10^{6e}$ | $1.8 \times 10^{7e}$ |
| G (Bore Hole) | $1.0 \times 10^{4f}$ | $1.2 \times 10^{4f}$ |
| H (Bore Hole) | $1.3 \times 10^{4g}$ | $1.5 \times 10^{4g}$ |
| I (Tap) | $1.5 \times 10^{4h}$ | $1.9 \times 10^{4h}$ |
| J (Bore Hole) | $1.0 \times 10^{3i}$ | $1.2 \times 10^{3i}$ |
| **Standard Limit** | $2.0 \times 10^{2j}$ | $2.0 \times 10^{2j}$ |

Means with the same letter superscripts, across rows, are not significantly different ($p > 0.05$).

**Table.3** Most Probable Number (MPN) of water samples

| Samples | MPN/100ml | Coliform Count on EMB (Cfu/ml) |
|---|---|---|
| A (Spring) | 10 | $10 \times 10^2$ |
| B (Tap) | 08 | $08 \times 10^2$ |
| C (Bore Hole) | 11 | $12 \times 10^2$ |
| D (Tap) | 10 | $14 \times 10^2$ |
| E (Tap) | 14 | $20 \times 10^2$ |
| F (Bore Hole) | 12 | $16 \times 10^2$ |
| G (Bore Hole) | 10 | $18 \times 10^2$ |
| H (Bore Hole) | 09 | $11 \times 10^2$ |
| I (Tap) | 14 | $17 \times 10^2$ |
| J (Bore Hole) | 07 | $15 \times 10^2$ |

**Table.4** Descriptive statistics of physicochemical properties and Microbiological analysis of water samples obtained from bore holes and tap outlets in various locations in Okada community.

| Properties | Water Source | Number of Samples | Mean ± S.E.M |
|---|---|---|---|
| Temperature ($^0$C) | Bore hole | 6 | $29.50 \pm 0.89^{a}$ |
| | Tap | 4 | $29.75 \pm 0.63^{a}$ |
| pH value | Bore hole | 6 | $4.76 \pm 0.15^{b}$ |
| | Tap | 4 | $5.01 \pm 0.15^{b}$ |
| Chlorine (PPM) | Bore hole | 6 | $113.33 \pm 15.63^{c}$ |
| | Tap | 4 | $82.50 \pm 8.54^{c}$ |
| Total dissolved solid (PPM) | Bore hole | 6 | $50.00 \pm 4.47^{d}$ |
| | Tap | 4 | $40.00 \pm 0.00^{d}$ |
| Total hardness (PPM) | Bore hole | 6 | $140.00 \pm 21.60^{e}$ |
| | Tap | 4 | $115.00 \pm 31.22^{e}$ |
| Turbidity (NTU) | Bore hole | 6 | $6.50 \pm 2.51^{f}$ |
| | Tap | 4 | $4.25 \pm 0.75^{f}$ |
| 24HRS Incubation (CFU/ML) | Bore hole | 6 | $275666.66 \pm 264875.40^{g}$ |
| | Tap | 4 | $51600.00 \pm 42892.04^{g}$ |
| 48HRS Incubation (CFU/ML) | Bore hole | 6 | $3037533.33 \pm 2992620.96^{h}$ |
| | Tap | 4 | $73175.00 \pm 62376.26^{h}$ |
| Most probable (NUM/100ML) | Bore hole | 6 | $9.83 \pm 0.70^{i}$ |
| | Tap | 4 | $11.50 \pm 1.50^{i}$ |
| Coliform count on EMB (CFU/ML) | Bore hole | 6 | $1366.67 \pm 128.24^{j}$ |
| | Tap | 4 | $1475.00 \pm 256.17^{j}$ |

Data represents Mean ± S.E.M (n = 3). Means with the same letter superscripts, across columns, are not significantly different ($p > 0.05$).

**Table.5** Morphological characteristics of Isolates

| ISOLATE | MORPHOLOGICAL CHARACTERISTICS | ORGANISM |
|---|---|---|
| B1 | Dark centered, gram negative, non endospores forming colony on Salmonella Shigella Agar | *Salmonella sp* |
| B2 | Gram negative, circular, low convex, with entire margin, mucoid, opaque, small non endospores forming rod shaped, pinkish glistering with metallic sheen colony on Eosin Methylene Blue (EMB) Agar. | *E. coli* |
| B3 | Non-spore forming, gram negative short rods, colourless colony on Nutrient Agar. | *P. aerugionosa* |
| B4 | Spore forming, gram positive rods, creamy white colony on Nutrient Agar with entire margin. | *Bacillus sp* |
| B5 | Gram negative rods that appeared yellowish with entire margin on Nutrient Agar. | *Flavobacterium* |
| B6 | Non-spore forming and non-motile, gram positive cocci, circular, low convex with entire margin, smooth, medium, opaque, golden yellow colony on Nutrient Agar. | *S. aureus* |

**Table.6** Biochemical characteristics of isolates

| TEST | B1 | B2 | B3 | B4 | B5 | B6 |
|---|---|---|---|---|---|---|
| Catalase | + | + | + | + | + | + |
| Oxidase | - | - | - | - | - | - |
| Motility | - | - | + | + | + | - |
| Indole | + | + | - | - | - | - |
| Methyl red | - | + | - | - | + | - |
| Voge-prokauer | + | - | + | + | + | + |
| Citrate utilization | + | - | + | - | - | - |
| Urease | - | - | - | - | - | + |
| Hydrogen sulphide | + | ND | + | + | - | ND |
| Starch hydrolysis | - | - | - | - | - | - |
| Gelatin hydrolysis | + | - | + | + | + | - |
| Nitrate utilization | - | + | - | + | + | - |
| Coagulase | ND | ND | ND | ND | ND | + |
| 10% NaCl | - | + | ND | - | - | - |
| Glucose fermentation | AG | A | AG | AG | AG | A |
| Xylose fermentation | - | A | ND | - | ND | - |
| Lactose fermentation | AG | A | AG | A | - | - |
| Sucrose fermentation | AG | A | AG | AG | AG | A |
| Maltose fermentation | AG | A | - | A | A | A |
| Mannitol fermentation | A | A | AG | AG | A | A |
| Galactose fermentation | AG | ND | ND | AG | A | ND |
| Fructose fermentation | ND | A | A | A | A | A |
| Sorbitol fermentation | ND | A | - | A | A | A |
| Arabinose fermentation | ND | A | A | AG | AG | AG |

**Key:** B1 – Salmonella sp.; B2 – Escherichia coli; B3 – Pseudomonas aerugionosa; B4 – Bacillus sp.; B5 – Flavobacterum sp.; B6 – Staphylococcus aureus
A – Acid production only; ND – Not determined; AG – Acid and gas production

on the particular uses. Thus, potable and domestic water should be harmless for the health of man, have proper organoleptic properties, and suitable for domestic use. Water quality should be controlled in order to minimize acute problem of water related diseases, which are endemic to the health of man.

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