INTRODUCTION

Water is the most vital element among the natural resources; it is the most indispensable need for existence of all living things. Its decreasing availability in terms of quality and quantity has been a major public health concern in Africa, particularly in Nigeria (WHO, 2004; Saraveanan and Peter, 2009). According to a recent UNICEF report, about 80 million people in Asia and Africa are living without access to safe water. Consequently, this has caused many people to suffer from various diseases (Tanwir et al., 2003). In developing countries such as Nigeria, most of the rural community lack access to potable water supply and rely mainly on river and stream sources for their household use and other purposes (Banwo, 2006).

Many water sources in developing countries are unhealthy because they contain harmful physical, chemical and biological agents. Unfortunately, many of the available water sources are not potable without some form of treatment which is seldom or not available in most rural settings which expose the rural populace to waterborne diseases (Oketola et al., 2006). In some rural areas in Nigeria, domestic wastes, sewage and faeces are discharged into streams which also serve as their water sources for daily needs. When the load of organic matter or wastes is too heavy, the self-purification power of the stream are unable to remove these materials added and there will be pollution of these water sources which can be dangerous to human and the environment as a whole (Adetokunbo and Grilles, 2003). The microbiological quality of drinking water is of a great primary importance, and the monitoring of bacterial indicators such as total coliform. Microbial indicators have been used worldwide to indicate if human wastes have contaminated water body. Microbes typically utilized are those that are found in elevated concentrated in human faecal coliform, Escherichia coli and Enterococci (Brooks et al., 2006).

An additional indicator, Clostridium perfringens can be used for monitoring stream water quality (Eggerongbe et al., 2010). The outbreaks of diarrhea or gastroenteritis in rural communities have all been attributed to the consumption of water of poor microbial...
quality (Ashbolt, 2004). It is therefore not an option but an imperative to critically monitor the quality of water supply in rural areas in order to further highlight their despicable water supply situation and to provide the impetus for sustainable government intervention (Gucker et al., 2006). The aim of this study was to evaluate the microbial qualities of Nkwuaku and Ogbaru streams located in Awgu Local Government Area in Enugu State, Nigeria.

MATERIALS AND METHODS

Study Area

The study took place in Awgu Local Government Area of Enugu State, Nigeria. The Local Government is made up of towns such as Agbogugu, Isu-Awa, Ituku, Ihe, Ogbaku, Owelli, Ogugu, Agbudu, Amoli, Mmaku, Ugbo, Obeagu, Mgbidi, Ugwueme, Nkwe, Ezere, Awugu, Nenwenta, Awgunta and Mgbowo. It has clay and stony lands with hilly topography. Subsistence farming, petty trading, livestock rearing, palm wine tapping and stone quarrying are the major occupation of the people. There is no notable industry located in this area and its environs.

Collection of Samples

Water samples for this study were collected from two different streams (Nkwuaku and Ogbaru) within the study area. The samples were collected in sterile containers at different collection points and analyzed within 24 hours for physicochemical and bacteriological qualities.

Sterilization of Materials

All glassware which were used for this study were properly washed and rinsed using distilled water and sterilized in a hot air oven at 250°C for 1 hour. These glassware included glass Petri-dishes, test tubes, pipette, Bijou bottles and McCartney bottles.

Determination of Physicochemical Characteristics of the Sample

The physicochemical properties which were examined included temperature, pH, taste, turbidity, colour, conductivity, odour, total solids, total dissolved solids, total suspended solids, acidity and alkalinity.

Determination of pH, Temperature and Conductivity

The pH was determined using a pH meter (HI96107 HANNA pH Meter), which was standardized with a neutral buffer solution of pH 7.0 in a beaker. A 100ml aliquot of each sample was measured into a beaker and the pH probe was immersed into the sample. This was allowed for some minutes until a stable reading was obtained, and value was recorded. An aliquot of 50ml of each sample was measured into a 100ml beaker and a simple mercury-in-glass thermometer calibrated in degrees centigrade immersed in the water. The reading on the thermometer for each sample was recorded. Conductivity of the two samples was determined using a digital conductivity meter (4520JENWAY, Serial No. 01263). The meter was turned on and allowed to warm up for about 15 minutes. It was then standardized with 0.01M KCl solution where a conductivity value of 1413 microsiemen per-centimetre was obtained. The electrode was thoroughly rinsed with distilled water, wiped and then dipped into 100ml of each water sample in the beaker and left for some minutes to obtain a stable reading. The value for each sample was recorded.

Determination of Colour and Turbidity

Colour was determined immediately each sample arrived the laboratory. This was carried out after the two samples had been allowed to rest on a bench to attain room temperature. Approximately 25 ml of water from each of the samples was measured into the sample cell for colour analysis. Colour instrument (HACH DR/890) was zeroed with 25ml distilled water before samples were determined and the readings were recorded. Approximately 10ml of distilled water was measured into the sample cell of a turbidity meter (HANNA, LP2000), which was used to zero the instrument. Later, 10ml each from samples was measured into the same cell and the readings were recorded.

Determination of Total Solids (TS) and Total Dissolved Solids by Gravimetric Method

Total solids were determined as the residues left after evaporation of the unfiltered samples. Approximately 10ml of each of the two unfiltered samples was taken and evaporated in an evaporating dish in an oven at a temperature of 103-105°C for two and half hours. The evaporating dish was then cooled in desiccators and weighed. Total dissolved solids were determined as the residues left after evaporation of the filtered samples, approximately 10ml of filtered sample from each of the samples was measured into a pre-weighed evaporating dish. The residues collected were then dried in an oven at temperature of 103-105°C for one hour and final weights were taken after cooling the evaporating dish in a desiccator.

Total solids were calculated using the formula given below:

\[
\text{Total solids (mg/L)} = \frac{(W_1 - W_2) \times 1000}{V}
\]

Where:

- \(W_1\) : Initial weight of dried residue + dish in mg
- \(W_2\) : Final weight of the dish in mg
- \(V\) : Volume of sample used in ml

Determination of Total Suspended Solids (TSS)

For total suspended solid (TSS), 10ml each of the water samples were filtered through a pre-weighed filtered
paper. The filtered papers were dried at 103-105°C in oven and TSS was determined by the following formula.

\[
\text{TSS (mg/L) = \frac{\text{Filter post weight} - \text{filter pre weight} \times 1000}{\text{Volume of sample (ml)}}
\]

**Determination of Alkalinity and Acidity**

To 100ml of each of the water samples, 3 drops of phenolphthalein indicator was added. The samples were titrated with 0.02N H₂SO₄ to pH 8.3 and phenolphthalein alkalinity was estimated (phenolphthalein indicator was changed colour from pink to colourless at pH 8.3). Finally, the phenolphthalein alkalinity of water was calculated as follows:

\[
\text{Alkalinity (mg/L) = \frac{A \times N \times 50 \times 1000}{V}}
\]

Where:
- \(A\) : Volume of H₂SO₄ in ml
- \(N\) : Normality of H₂SO₄ used to titrate
- \(V\) : Volume of sample used in ml

**Determination of Acidity**

To 100ml of each of the water samples, 3 drops of methyl orange indicator was added. The samples were titrated with 0.02N standard NaOH to pH 8.3. (Methyl orange indicator was changed colour from orange-red to yellow at pH 8.3). The acidity of water was calculated as follows:

\[
\text{Acidity (mg/L) = \frac{A \times N \times 50 \times 1000}{V}}
\]

Where:
- \(A\) : Volume of NaOH titrant used in ml
- \(N\) : Normality of NaOH used
- \(V\) : Volume of sample used in ml

**Microbiological Analysis**

**Preparation and Inoculation of Samples**

Serial dilution of each sample was made in sterile distilled water by inoculating 1ml of each sample into the first test tube containing 9ml of distilled water. The solution was mixed thoroughly and 1ml of it was transferred into the second test tube containing 9ml of distilled water. The procedure was repeated with all the remaining tubes. 0.1ml was pipetted out from 10⁵ of the diluted factor and was inoculated in the plate using spread plate technique. The plates were incubated for 24 hours at 25°C for bacterial isolation and at 20°C for 3 days for fungal isolate using Potato Dextrose Agar. The number of colonies were counted and recorded for both bacteria and fungi.

**Purification of Isolates**

The resulting colonies from the Nutrient Agar Medium, MacConkey, Potato Dextrose Agar, Eosin Methyl Blue Agar, Manitol salt agar, Blood Agar and Salmonella – Shigel Agar plates were purified by sub-culturing on freshly prepared nutrient agar plates. The plates were incubated at 25°C for 24 hours for bacterial incubation and 20°C for 3 days for fungi. After the appropriate periods of incubation, the resulting discrete colonies were transferred onto agar slants in McCartney bottles and kept in the refrigerator as stock culture for subsequent tests during identification.

**Characterization and Identification of Bacterial Isolates**

Bacterial isolates were analyzed based on morphological features, Gram staining and other biochemical characterization which included citrate, indole, catalase, coagulase, motility, oxidase, urease, glucose, lactose, fructose and mannitol tests. Confirmation of biochemically characterized bacteria was made using the most Probable Number (MPN) technique.

**Identification of Fungal Isolates**

Fungal isolates were identified based on their colonial morphology and cell morphology. A small portion of fungal growth was isolated with a sterile wireloop and placed on a grease free glass slide and teased with a drop of distilled water. A drop of lactophenol cotton blue stain was added and covered with a greased free cover slip. The slide was observed using x10 and x40 objective lenses. Lactophenol cotton blue wet mount preparation is the most widely used method of staining and observing fungi.

**RESULTS**

Table 1 shows the various physicochemical characteristics exhibited by the stream water samples. Ogbaru stream had higher colour value of 10HU than Nkwuaku stream which had a colour value of 6HU. Nkwuaku stream had lower turbidity than Ogbaru stream sample had an objectionable odour while Ogbaru stream sample had a lower pH value of 6.7 while Ogbaru had a pH value of 8.3. Turbidity was higher in Ogbaru stream and lower in Nkwuaku stream sample. Ogbaru stream had a higher temperature of 27°C than Nkwuaku (26°C). Conductivity was higher in Ogbaru stream sample (678 μS/cm) than in Nkwuaku stream sample (468 μS/cm). The value for total solids was lower (430 mg/l) in Nkwuaku stream and higher (530 mg/l) in Ogbaru sample. Nkwuaku sample recorded a lower total dissolved solids (210 mg/l) than Ogbaru (270 mg/l). Total suspended solids was 220 mg/l in Nkwuaku stream and 260 mg/l in Ogbaru stream sample. Ogbaru stream sample’s acidity was 4.0 mg/l and that of Ogbaru stream was 6.0 mg/l. Alkalinity was recorded to be 5.0 mg/l in Ogbaru sample and 6.0 mg/l in Nkwuaku sample.
Table 1. Physicochemical Characteristics of the Stream Water Samples in Awugu Local Government Area, Enugu State.

| Parameters       | Sample A Nkwuaku Stream | Sample B Ogbaru Stream |
|------------------|-------------------------|-------------------------|
| pH               | 6.7                     | 8.3                     |
| Temperature (°C) | 26                      | 27                      |
| Conductivity (µs/cm) | 468                  | 678                     |
| Colour (HU)      | 6.0                     | 10.0                    |
| Turbidity (NTU)  | 8.0                     | 10.0                    |
| Odour            | U                       | O                       |
| Total solids(mg/l) | 430                   | 530                     |
| Total dissolved solids(mg/l) | 210              | 270                     |
| Total suspended solids(mg/l) | 220            | 260                     |
| Alkalinity(mg/l) | 6.0                     | 5.0                     |
| Acidity(mg/l)    | 4.0                     | 6.0                     |

Key: U = Unobjectionable; O = Objectible; HU = Hazen Unit; NTU = Nephelometric Turbidity Unit.

Table 2 shows the colonial characteristics of each isolates on Nutrient agar, Eosin methylene blue agar, MacConkey agar, Manitol salt agar, Salmonella-shigella agar and Blood agar. The isolates which were obtained include *staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella* species, Klebsiella species, *Pseudomonas aeruginosa*, Proteus species and Campylobacter species.

Table 2 shows the total bacterial and fungal plate counts of the stream samples. The total bacteria count revealed that Ogbaru stream had a higher total bacteria count of 5.1 x 10^7 cfu/ml than Nkwuaku stream which had 1.2 x 10^7 cfu/ml. This indicates that Nkwuaku stream sample had the lower bacterial indicators compared with Ogbaru stream sample. Also, the total fungal count revealed that Nkwuaku had a higher total fungi count (4.0 x 10^5 cfu/ml) than Ogbaru stream (1.6 x 10^5 cfu/ml).

Table 2. Colonial Characteristics of Isolates from Ogbaru and Nkwuaku Stream Water on Different Growth Media.

| Isolate Code | Media Plates |
|--------------|--------------|
| NA           | EMB          | MAC          | MSA          | SSA          | BA            |
| OG1          | Smooth, golden yellow, convex colonies | Pink, smooth, convex and opaque colonies | Yellow, smooth, convex and opaque colonies | -            | Light golden yellow, smooth, convex and hemolytic colonies |
| OG2          | Thick, grayish white, moist, smooth & opaque colonies | Green metallic sheen smooth colonies | Moist, smooth, flat & pink colonies | -            | Slight growth pink colonies |
| OG3          | Low convex, smooth, grayish white translucent colonies | - | Low convex, smooth, colourless and transparent colonies | -            | Black colonies with offensive odour |
| OG4          | Irregular, low convex, smooth, mucoid, greenish yellow opaque colonies | Metallic sheen and glass appearance colonies | Low convex, smooth, mucoid, colourless transparent colonies | -            | Irregular slight growth & nearly colourless colonies |
| NK1          | Whitish, round raised, glistering colonies | Light pink raised large colonies | - | Yellow colonies | -            | White non-hemolytic colonies |
| NK2          | Mucoid, grayish white, raised opaque colonies | Convex, mucoid, pink translucent colonies | Convex, mucoid, pink-red opaque colonies | -            | Smooth slight growth, pink colonies |
| NK3          | Smooth, opaque, irregular, glistening grayish white translucent colonies | Glistening, colourless, transparent colonies | Low convex, smooth, colourless transparent colonies | -            | Colorless with black center colonies |
| NK4          | Whitish droplet colonies | - | - | - | -            | Smooth, greyish, convex, glistening & non-hemolytic colonies |

- = No growth; NA = Nutrient Agar; EMB = Eosin Methylene Blue agar; MAC = MacConkey Agar; MSA = Manitol Salt Agar; SSA = *Salmonella – Shigella* Agar; BA = Blood Agar; OG = Ogbaru; NK = Nkwuaku.
Table 3. Total Bacterial and Fungal Plate Counts Obtained from the Stream Water Samples.

| Sample Source | Bacteria (CFU/ml) | Fungi (cfu/ml) |
|---------------|------------------|---------------|
|               | NA               | EMB           | MAC           | SSA           | PDA           |
| Nkwuaku       | 4.2 x 10^7       | 2.8 x 10^7    | 3.9 x 10^7    | 2.2 x 10^7    | 4.0 x 10^7    |
| Ogbaru        | 5.1 x 10^7       | 1.5 x 10^7    | 3.5 x 10^7    | 1.9 x 10^7    | 1.6 x 10^7    |

PDA = Potato Dextrose Agar; NA = Nutrient Agar; EMB = Eosin Methylene Blue agar; MAC = MacConkey agar; SSA = Salmonella – Shigella Agar

Table 4 shows various groups of microorganisms isolated and identified from this study. They include; Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Proteus spp, Pseudomonas aeruginosa, Salmonella spp. and Campylobacter spp.

Table 5. Morphological Characteristics of Fungal Isolates in the Stream Water Samples.

| Macroscopy           | Microscopy                        | Organism(s)          |
|----------------------|-----------------------------------|----------------------|
| Dark-brown mycelium  | Conidiophores smooth walked and non-septate | Aspergillus niger |
| Yellow pink creamy   | Cylindrical to ovoid conidia, curved septate conidiophores | Fusarium oxysporum |
| colonies             |                                   |                      |
| Light green and      | Long, erect septate, conidiophores | Aspergillus flavus   |
| powdery              |                                   |                      |
| Gray-green fluggy    | Long, erect, non-septate conidiophores | Aspergillus fumigatus |
| colonies             |                                   |                      |

Table 5 shows various fungi isolates and identified from the stream samples by their morphological characteristics. They are; Aspergillus niger, Fusarium oxysporum, Aspergillus flavus and Aspergillus fumigatus.

Table 6 shows the result of the MPN tests for occurrence of the presumptive coliforms (E. coli). The result revealed a gross coliform contamination in the two stream samples.

Table 6. MPN Values per 100ml of the Stream Water Samples.

| Water Samples | 5 of 10ml | 5 of 1ml | 5 of 0.1ml | MPN (Per 100ml) |
|---------------|-----------|----------|-----------|-----------------|
| Nkwuaku Stream| 0         | 1        | 0         | 2               |
|               | 1         | 1        | 0         | 4               |
|               | 1         | 0        | 1         | 4               |
|               | 1         | 1        | 1         | 6               |
|               | 1         | 0        | 0         | 2               |
|               | 2         | 0        | 0         | 5               |
|               | 2         | 0        | 1         | 7               |
|               | 3         | 1        | 0         | 11              |
|               | 1         | 1        | 1         | 6               |
|               | 0         | 2        | 0         | 4               |

MPN (Per 100ml) = Most Probable Number per 100ml of water sample.
Table 7 shows the percentage occurrence of bacterial isolates from the stream water samples which includes; *Escherichia coli*, 2(16.66%); *Enterococcus faecalis* 1(8.33%); *Salmonella* spp. 2(16.66%); *Klebsiella* spp. 1(8.33%); *Staphylococcus aureus* 2(16.66%); *Pseudomonas aeruginosa* 1(8.33%); *Proteus* spp. 2(16.66%) and *Campylobacter* spp. 1(8.33%).

| S/N | Isolates                    | Frequency Occurrence | % Occurrence |
|-----|-----------------------------|----------------------|--------------|
| 1.  | *Escherichia coli*          | 2                    | 16.66%       |
| 2.  | *Enterococcus faecalis*     | 1                    | 8.33%        |
| 3.  | *Salmonella* spp.           | 2                    | 16.66%       |
| 4.  | *Klebsiella* spp.           | 1                    | 8.33%        |
| 5.  | *Staphylococcus aureus*     | 2                    | 16.66%       |
| 6.  | *Pseudomonas aeruginosa*    | 1                    | 8.33%        |
| 7.  | *Proteus* spp.              | 2                    | 16.66%       |
| 8.  | *Campylobacter* spp.        | 1                    | 8.33%        |
|     |                             |                      | 12           | 100%         |

Table 8 shows the percentage occurrence of fungal isolates from stream water samples. They are; *Aspergillus niger* 1(20%); *Aspergillus flavus* 2(40%); *Fusarium oxysporum* 1(20%) and *Aspergillus fumigatus* 1(20%).

| S/N | Isolates       | Frequency Occurrence | % Occurrence |
|-----|----------------|----------------------|--------------|
| 1.  | *Aspergillus niger* | 1                  | 20.0%        |
| 2.  | *Aspergillus flavus* | 2                  | 40.0%        |
| 3.  | *Fusarium oxysporum* | 1                  | 20.0%        |
| 4.  | *Aspergillus fumigatus* | 1                  | 20.0%        |
|     |                 |                      | 5             | 100%         |

**DISCUSSION**

Evaluation of microbial contamination of Nkwaku and Ogbaru streams showed that the pH of Nkwaku stream sample was 6.7 while that of Ogbaru sample was 8.3. This is in agreement with pH assigned by the US Environmental Protection Agency (EPA) as the standard pH of water which ranges from 6.5 – 8.5 (EPA, 2002). The higher pH values measured from the stream samples could be attributed to more rain water deposition which dilutes the acidity of water by raising the pH. Thus indicated that the measured pH values of the stream samples were within permissible value which will not cause any harmful effect to the consumers.

Ogbaru stream sample had a higher temperature value (27°C) than Nkwaku sample (26°C) and this could be attributed to the fact the samples were collected early in the morning. This conforms with the findings of Okoye and Umo (2006) who stated that cool water are generally more potable for drinking, because high water temperature enhances the growth of microorganisms and causes taste, odour, colour and corrosion problems.

In the case of turbidity, Ogbaru sample recorded higher value of 10 NTU while Nkwuaku recorded a lower value of 8 NTU. The obtained values are far greater than the value specified and approved (5NTU) by the World Health Organization (WHO) (WHO, 2004). The higher values obtained could have been caused by rainfall and human activities such as; grazing of ruminants and improper management of solid and liquid wastes within the streams. High turbidity is also associated with high levels of disease causing microorganisms such as bacteria, fungi and other parasites. Thus, waters from these streams are not good for drinking purpose.

The total dissolved solid (TDS) of the samples are in agreement with the EPA standard of 500mg/L. Ogbaru recorded higher value of 260mg/L while a lower value of 210mg/L was recorded in Nkwuaku sample. Odour of Nkwaku sample was perceived to be unobjectionable whereas the odour of Ogbaru sample was objectionable. The objectionable odour of Ogbaru sample could be due to the presence of aquatic lives (fungi, bacteria etc) and waste disposal from toilets, industries, household etc. and this makes the water unfit for drinking.

The percentage occurrence of bacterial and fungal isolates from the stream samples were determined and the result revealed that *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Proteus* spp. recorded the highest percentage occurrence (16.66%) while *Enterococcus faecalis*, *Klebsiella* spp., *Pseudomonas aeruginosa* and *Campylobacter* spp. recorded lower percentage occurrence (8.33%).

The high number of *Salmonella*, *Proteus* spp and *Staphylococcus aureus* in the stream samples are not in agreement with EPA water standard for recreational use which states that these pathogenic organisms must not be present in water, because they are of public health significance, having been associated with gastrointestinal infections, diarrhoea, typhoid fever and other form of infection (EPA, 2005).

The total bacteria and fungi counts for the two samples were generally high, exceeding the limit of 1.0 x 10² cfu/ml which is the standard limit of heterotrophic count for drinking water (EPA, 2002). This indicates the presence of high organic matters and nutrient sources which supported the growth of the microbes. The primary sources of microbial contamination include the surface runoff, sewage treatment facilities and improper management activities of the inhabitants like washing, refuse dumping, open defaecation and dropping of different materials into water bodies.

Various groups of microorganisms were isolated and identified during the study. They include *Escherichia*...
coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Proteus sp, Pseudomonas aeruginosa, Salmonella spp. and Campylobacter spp. for bacteria. The fungi isolated include Aspergillus niger, Fusarium oxysporum, Aspergillus flavus and Aspergillus fumigatus. The presence of some of these organisms signifies contamination of water from domestic sources. The Staphylococcus species are known to produce enterotoxins (Okonko et al., 2008). Proteus species are intestinal flora, but also widely distributed in soils and water (Sclegel, 2002). Pseudomonas aeruginosa is an example of non-faecal coliforms, while E. coli is a faecal coliform.

CONCLUSION

From this study it can be observed that from the microbial qualities of these that they are unfit for human consumption though they can be used for other purposes. Many of the inhabitants of Awgu Local Government Area depend on these two streams for drinking water supplied. In view of this, it is recommended that the Government should ban and discourage all indiscriminate disposal of wastes within stream environments.

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