Microorganisms and Physico-chemical Profile of Aquatic Ecosystem in Akwa Ibom State

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Authors’ contributions

This work was carried out in collaboration between both authors. Author MAE designed the study and performed the statistical analysis. Author BA carried out the analysis and wrote the first draft of the manuscript under the supervision of author MAE. Both authors read and approved the final manuscript.

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ABSTRACT

Microbiological and physicochemical profiles of water samples collected from three aquatic ecosystems in Akwa Ibom State were determined using standard microbiological and chemical techniques. The antibiotic susceptibility profiles of the isolates were determined using disc diffusion technique. Physicochemical parameters of the water samples were also carried out using standard chemical methods. The total heterotrophic bacterial counts and total coliform counts of the water samples ranged from 2.0x10⁷±0.17 to 2.6x10⁷±0.44 CFU/ml and 1.8x10⁵±0.38 to 3.3x10⁵±0.3 CFU/ml, respectively. The total fungal and total vibrio counts ranged from 1.2x10⁵±0.07 to 1.7x10⁵±0.49 CFU/ml and 5.2x10⁴±2.31 to 1.8x10⁵±0.19 CFU/ml, respectively. The faecal coliform and total salmonella-shigella counts of the water ranged from 6.8x10⁴±0.3 to 2.3x10³±0.38 CFU/ml and 1.2x10³±2.58 to 3.8x10³±0.24 CFU/ml, respectively. Nine bacterial genera comprising Bacillus, Salmonella, Escherichia, Pseudomonas, Shigella, Staphylococcus, Vibrio, Proteus and Streptococcus species were isolated from the samples, while the fungal isolates were Aspergillus, Mucor, Penicillium, Rhizopus and Candida species. The bacterial isolates were highly resistant to Amoxicillin/Clavulanates and Cefuroxime, while high sensitivities to Gentamicin were observed among E. coli, Staphylococcus spp. and Pseudomonas spp. Analysis of variance showed that there was a significant difference (p<0.05) between total viable counts obtained in the three sample locations while the correlation coefficient showed positive relationship between the total viable

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counts and some of the physicochemical parameters studied. The aquatic ecosystem studied, based on the bacteriological and physicochemical parameters revealed that the human, animal and agricultural activities plays significant role in the contamination of the water source. Consequently, the water should be properly processed before use.

Keywords: Aquatic ecosystems; antibiotic susceptibility; faecal coliform.

1. INTRODUCTION

Water is the most important natural resource on earth. It is essential for all known forms of life, and is approximated to cover 70.9% of the earth surface [1]. Despite its abundance, the quality and accessibility of potable water remains a global challenge; more so, in rural and semi-rural communities in the developing countries [2]. Contamination of water is a serious environmental problem as it adversely affects human health and biodiversity in the aquatic ecosystem. The use of indicator bacteria such as faecal coliforms and faecal streptococci for assessment of faecal pollution and possible water quality deterioration in freshwater sources is widely used [3]. Currently coliforms and Escherichia coli are of great importance among bacterial indicators used in water quality definition and health risk [4]. Globally many people do not have access to safe drinking water and as a consequence, there is significant morbidity and mortality due to disease causing organisms in water [5]. Historically water has played a significant role in the transmission of human disease. Typhoid fever, cholera, amoebic dysentery and many other gastrointestinal diseases can be transmitted by water. Contamination of water by sewage and human faecal material present the greatest danger to public health. Though water is abundant, suitable drinking water is limited by geography, demography and affordability [5]. Aquatic environments have varied surface areas and volumes. Microorganisms are found in locations as diverse as the human body, drinks and beverages, and the usual places one would expect rivers, lakes and oceans. They also occur in water-saturated zones in materials we usually describe as soils. These environments can range from alkaline to extremely acidic [6].

In Nigeria, only 58% of inhabitants of the urban and semi-urban areas and 39% of the rural areas have access to potable water supply; the rest of the population depend on ground (well and borehole) and surface water (stream and river) for their domestic water supply [7]. With a growing human population, urbanization, pollution, atmospheric input from fossil fuel burning and environmental degradation, the threats on water supplies from chemical and biological contamination are expected to increase. In the coastal areas where groundwater is used for potable and agricultural purpose, intrusion can be a serious problem resulting in the contamination of waters, necessitating expensive desalination treatment. The southern-state of Akwa Ibom (Nigeria) which contributes more than 30% of Nigerian’s crude oil is presently experiencing an increase in human and industrial activities. This has resulted in the increase in the rate of potable water abstraction. This action, if not checked and properly monitored, will in future leads to encroachment of seawater into the costal aquifers [8]. In Akwa Ibom State, most communities depends solely on natural water bodies as their source of water whereby these water are polluted regularly by faeces deposited by man, animals and surface run-off water from burnt farmland and fertilized farmlands. Unhealthy practices like dumping of waste and use of detergent in clothes washing renders the water unfit for use and also capable of spreading most diseases. The detection of microbial diversity and their variation in water is of great practical and scientific relevance, especially in coastal ecosystems [9].

Because of the associated dangers of contaminated waters, this study was carried out to assess the bacteriological quality and the physicochemical parameters of aquatic ecosystems in Akwa Ibom State, Nigeria.

2. METHODOLOGY

2.1 Study Locations

This research was carried out in three different locations in Akwa Ibom State of Nigeria. The studied locations include; Oron River in Oron Local Government Area, located in the South-eastern part of Akwa-Ibom State. The second location was Itu River through AyaAdeghe, which is located in the North-western part of Akwa-
Ibom State while the third study location was Ibeno Beach through Ukpene kang axis of the Qua Ibo River Estuary in Ibeno Local Government Area which lies in the Southern part of Akwa Ibom State. Akwa Ibom State is located in the Coastal Southern part of Nigeria, lying between latitudes 4°32′N and 5°33′N, and longitudes 5°7′E and 8°25′E. The state is bordered on the east by Cross River State, on the west by Rivers State and Abia State, and on the south by the Atlantic Ocean and the southernmost tip of Cross River State. It is currently the highest oil and gas-producing state in Nigeria.

2.2 Sample Collections

A total of fifteen samples were examined. Five samples were collected from each of the three different study locations. According to the method described by [10], 250 ml sterile sample bottle was dipped into the water in a depth of 30 cm, and placed in the direction of the flow of water. The cork was removed and the sample was taken, leaving space for agitation. The samples were stored in clean well-labelled plastic containers, and transported to the laboratory for analyses in ice cold chest.

2.3 Microbiological Analysis

Standard pour plate technique as described by [3] was used for the microbiological analysis from 10-fold dilutions, nutrient agar medium was used for the enumeration of total heterotrophic bacteria counts (THBC), MacConkey agar was used for total coliform counts (TCC), Salmonella-Shigella agar for total Salmonella-Shigella counts (TSSC), Thiosulphate citrate bile salt sucrose agar for total vibrio counts (TVC) and Sabouraud Dextrose agar for total fungal counts (TFC). The bacterial plates were incubated at 35°C for 24 hours, while fungal plates supplemented with 0.5 mg/l streptomycin were incubated at room temperature (28 ± 2°C) for 3-5 days. Colonies were selected randomly and were characterized using morphological and biochemical test such as gram stain, spore stain, motility, catalase, oxidize, coagulase, indole, MR-VP, Urease and sugar fermentation tests. Bacterial isolates were identified with reference to Cowan and Steel's Manual for the identification of Medical Bacteria [11]. Fungal isolates were identified based on their morphological and cultural characteristics as recommended by [12].

2.4 Antibiotics Susceptibility Testing

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion technique [13], on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute [14]. The antimicrobial drugs used were Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Amoxicillin/Clavulanates (30 µg), Nitrofurantoin (300 µg) and Ampicillin (10 µg).

2.5 Determination of Physico-Chemical Parameters of the Water Samples

The physical parameters which include conductivity, temperature, pH, Dissolved Oxygen and Total Dissolved Solids (TDS) were determined according to the methods described by [3, 15].

2.6 Statistical Analysis

Analysis of variance (ANOVA) and Correlation matrix were used to compare means and values were considered significant at p<0.05. Post-Hoc multiple comparisons for the ANOVA were done using Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

The mean levels of the microbiological loads in the water samples from the three studied locations in Akwa Ibom State are shown in Fig. 1. The total heterotrophic bacteria counts (THBC) of the water samples varied from 2.0x10^5±0.17 to 2.6x10^5±0.44 CFU/ml. The total fungal counts (TFC) ranged from 1.2x10^5±0.07 to 1.7x10^5±0.49 CFU/ml. The total coliform counts (TCC) ranged from 1.8x10^5±2.14 to 3.3x10^5±0.3 CFU/ml. The faecal coliform counts (FCC) varied from 6.8x10^4±0.3 to 2.3x10^5±0.38 CFU/ml. The total vibrio counts (TVC) varied from 5.2x10^4±2.31 to 1.8x10^5±0.19 CFU/ml. While the total salmonella-shigella counts (TSSC) ranged from 1.2x10^4±2.58 to 3.8x10^5±0.24 CFU/ml.

Morphological and biochemical characteristic of the bacteria and fungi isolated from the samples at the different locations are shown in Tables 1 and 2. A total of nine bacteria species belonging to the following genera, Bacillus, Salmonella, Escherichia, Pseudomonas, Shigella, Staphylococcus, Vibrio, Proteus and
Streptococcus and a total of five fungal species were isolated from the samples. These include Aspergillus niger, Mucor, Penicillium notatum, Rhizopus and Candida species.

The results of the antimicrobial sensitivity screening of antibiotics against the test isolates are presented in Table 3. The results revealed that the bacterial species exhibited different sensitivity and resistivity pattern to the antibiotics used. Most of the isolates showed intermediate or resistant pattern towards Amoxycillin/Clavulanates and Cefuroxime. As E. coli, Staphylococcus and Pseudomonas species were sensitive to Gentamicin but resistant to Cefuroxime.

On the bacterial load, pathogenic bacteria were isolated from the three locations. Presence of enteric bacteria like Salmonella spp., Shigella spp., Vibrio spp. and E. coli can be attributed to high level of faecal and municipal waste contamination which may constitute health hazard to the people drinking or using the water for domestic activities or both. A similar conclusion was drawn by [16]. The result of the analysis of variance (ANOVA) on microbial counts showed that there was a high significant difference in total viable counts between the three sample locations (Ibeno, Itu and Oron) with (p<0.05) level of significance.

The total viable counts for the water samples collected from the three sample locations were generally high exceeding the WHO limit of 1.0x10^2CFU/ml which is the standard limit of total bacterial counts for drinking water [17] as total bacterial counts are indicative of the presence of high organic matter in the water. The primary sources of these bacteria in water could be attributed to animal and human activities. 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. The high coliform counts obtained from the samples, is an indication that the water sources have received faecal contamination [18,19]. None of the sampling locations of the water sources complied with WHO standard for coliform in water and this could be supported by evidence advanced by [20] who reported high coliform counts. According to [21], every water sample that contains coliform, should be investigated for the presence of faecal coliforms (E. coli) [17] with a view to ascertaining contamination with human or animal waste and possibly pathogenic bacteria or organisms. Other bacteria isolated from all water samples such as Staphylococcus aureus, Pseudomonas spp., and Proteus spp. are also of public health significance. Staphylococcus aureus is known to produce enterotoxin. Proteus spp. belongs to the intestinal flora but is also widely distributed in soil and water. Bacillus causes a toxin mediated disease rather than infections such as diarrhoea and emetic illness characterized by nausea and vomiting [4].

![Fig. 1. Mean viable microbial counts of water and sediment samples](image)

**Key:** THBC = Total heterotrophic bacteria counts, TFC = Total fungal counts, TCC = Total coliform counts, FCC = Faecal coliforms counts, TVC = Total vibrio counts, TSSC = Total Salmonella-Shigella counts
Table 1. Morphological and biochemical characteristics of bacteria isolates

| Isolates | Cell Shape | Gram's Reaction | Catalase | Coagulase | Motility | Citrate | Urease | Indole | Oxidase | Volesproskaurer | Methyl/Red | Glucose | Lactose | Maltose | Manitol | Dextrose | Sucrose |
|----------|------------|-----------------|----------|-----------|----------|---------|--------|--------|---------|----------------|------------|---------|---------|---------|---------|---------|----------|
| 1.       | Rod        | +               | -        | +         | +        | -       | -      | +      | -       | -              | -          | A       | A       | A       | A       | A       | A        |
| 2.       | Rod        | -               | +        | -         | +        | -       | +      | +      | -       | -              | A          | AG      | A       | AG      | A       | A       | Pseudomonas |
| 3.       | Rod        | -               | +        | -         | +        | -       | +      | -      | -       | -              | A          | AG      | A       | AG      | AG      | A       | Escherichia coli |
| 4.       | Rod        | -               | +        | -         | +        | +      | -      | -      | +       | -              | +          | AG      | A       | AG      | AG      | A       | Shigella sp. |
| 5.       | Rod        | -               | +        | -         | -        | -      | -      | -      | -       | +              | -          | A       | A       | A       | A       | A       | A        |
| 6.       | Cocci      | +               | +        | -        | -        | -      | +      | +      | -       | -              | AG         | A       | A       | AG      | A       | A       | A        |
| 7.       | Cocci      | -               | +        | -        | +        | +      | -      | +      | -       | -              | -          | A       | A       | A       | A       | A       | A        |
| 8.       | Rod        | -               | +        | -        | -        | -      | -      | -      | -       | +              | -          | A       | A       | AG      | A       | A       | A        |
| 9.       | Cocci      | +               | -        | +        | +        | +      | -      | -      | +       | A              | AG         | A       | A       | AG      | A       | A       | A        |

A = Acid production, G = Gas production

Table 2. Morphological characteristics of fungi isolates

| Isolates | Colour hyphae | Colour colony | Nature of hyphae | Type of spore | Special structure | Shape of sporangiophore or conidiophores | Spore character | Probable organism |
|----------|---------------|---------------|-----------------|--------------|------------------|-----------------------------------------|----------------|--------------------|
| 1.       | White         | White to greenish grey with edge | Septate | Globose | Smooth walled erect conidiophores | Dome gradually enlarging | Compact | Aspergillus niger |
| 2.       | White         | White and pale grey | Unseptated | Oval spores | Sporangiophores | Erect sporangiophore | Globose | Mucor sp. |
| 3.       | Blue to green colony | Green | Septate multinucleated, branched | Chain of green round globose | Absent | Erect and branched Conidiophore | Broom like straginata with conidia | Penicillium notatum |
| 4.       | White to grayish brown | White | Coenocytic | Rhizoids Ovoid sporangiophores | Filamentous Stolon | Rhizopus sp. |
| 5.       | Moist milky colony | Unseptated | Blastococidia | Candida sp. |
Table 3. Antibiotic sensitivity pattern of bacterial isolates

| Bacterial isolates     | CAZ 30 µg | CRX 30 µg | GEN 10 µg | CPR 5 µg | OFL 5 µg | AUG 30 µg | NIT 300 µg | AMP 10 µg |
|------------------------|-----------|-----------|-----------|----------|----------|-----------|------------|-----------|
| Streptococcus sp.      | I(20)     | I(20)     | S(25)     | I(20)    | S(30)    | R         | I(19)      | R         |
| Staphylococcus sp.     | S(25)     | R         | S(23)     | S(25)    | S(34)    | R         | I(20)      | R         |
| Vibrio sp.             | R         | I(17)     | I(17)     | R        | S(21)    | I(20)     | R          | R         |
| Shigella sp.           | R         | I(19)     | S(22)     | S(28)    | S(24)    | R         | I(20)      | R         |
| Escherichia coli       | R         | I(17)     | R         | S(28)    | S(28)    | R         | R          | I(16)     |
| Salmonella sp.         | R         | R         | S(21)     | R        | R        | S(30)     | R          | I(20)     |
| Proteus sp.            | R         | R         | S(22)     | S(22)    | S(28)    | R         | S(30)      | R         |
| Bacillus sp.           | I(17)     | I(19)     | R         | R        | R        | R         | R          | I(19)     |
| Pseudomonas sp.        | I(17)     | R         | S(24)     | S(26)    | S(29)    | I(20)     | S(25)      | R         |

Resistant = R(≤15), Intermediate = I(16-20), Sensitive = S(≥21), CAZ = Ceftazidime, CRX = Cefuroxime, GEN = Gentamicin, CPR = Ciprofloxacin, OFL = Ofloxacin, AUG = Amoxycillin/Clavulanates, NIT = Nitrofurantoin, AMP = Ampicillin

Table 4. Mean physiochemical properties of surface water samples by locations

| Parameters                           | Ibeno       | Itu         | Oron        | FEPA        | WHO         |
|--------------------------------------|-------------|-------------|-------------|-------------|-------------|
| pH                                   | 7.23±0.22   | 6.82±0.21   | 7.06±0.22   | 6.0-9.0     | 6.5-8.5     |
| EC (µs/cm)                           | 2,632.50±547.11 | 23.92±2.31 | 1,015.16±163.46 | 200         | 500         |
| TDS (mg/l)                           | 5,883.50±37672.9 | 8.40±2.06 | 2,101.20±119.11 | <2000       | 500         |
| gAcidity (mg/l as CaCO₃)             | 90.25±7.50  | 102.26±25.25 | 99.63±13.74 | -           | -           |
| Alkalinity (mg/l as CaCO₃)           | 76.51±37.64 | 61.11±5.25  | 28.54±2.24  | -           | -           |
| Dissolved Oxygen (mg/l)              | 9.30±0.50   | 6.30±0.19   | 6.68±1.21   | 8.0-10.0    | 8.0-10.0    |
| Biological Oxygen Demand (mg/l)      | 2.63±0.44   | 2.76±0.10   | 2.36±1.11   | <500        | 10          |
| Sulphate (mg/l)                      | 5.13±43.73  | 2.04±0.12   | 2.38±0.42   | <1000       | 250         |
| Phosphate (mg/l)                     | 0.14±0.15   | 0.07±0.02   | 0.24±0.18   | <10         | -           |
| Nitrate (mg/l)                       | 2.64±0.46   | 3.45±0.35   | 3.67±1.00   | <20         | 50          |
| Chloride (mg/l)                      | 3,410.99±891.47 | 6.13±4.59  | 48.28±9.11  | <600        | 250         |
| Basic Cations                        |             |             |             |             |             |
| Ca²⁺ (mg/l)                          | 128.90±15.73 | 119.44±14.18 | 51.52±24.26 | -           | 75          |
| Mg²⁺ (mg/l)                          | 51.66±25.48  | 21.96±4.19  | 15.16±4.55  | -           | 50          |
| Na⁺ (mg/l)                           | 27.18±14.97 | 0.10±0.01   | 2.16±0.19   | -           | 200         |
| K⁺ (mg/l)                            | 12.98±2.28  | 1.26±0.10   | 3.02±0.74   | -           | 10          |
| THC (mg/l)                           | 0.00        | 0.00        | 0.00        | -           | -           |
| Salinity (ppt)                       | 27.50±11.46 | 0.00        | 0.00        | 2000        | -           |

References: [22, 23]
The results of the physicochemical assessment of the water samples are presented in Table 4, showing the descriptive analysis of the physicochemical parameters of the water samples from the three sample locations. The pH values recorded were observed to be within the WHO standard of 6.5 – 8.5, where the pH values ranged from 6.82±0.21 - 7.23±0.22. The results of total dissolved solid (TDS) and electrical conductivity (EC) which ranged from 8.4±2.06 - 5883.50±7672.9 (µs/cm) and 23.92±2.31 - 2,632.50±547.11 (mg/l) respectively, were observed to be higher than the WHO standard, except in Itu study area where it was within the standard limits. The results of the dissolved oxygen (DO) ranged from 5.93±0.50 - 6.68±1.21 (mg/l) and was within the WHO standard permissible limit. The results of some of the physicochemical parameters were shown to be higher in the Ibom sampling location, which have direct effect on human activities [24]. The correlation coefficient showed positive relationship between the total viable counts and some of the physicochemical parameters studied.

4. CONCLUSION

It can be concluded that water from the entire source is not fit for domestic usage without further processing. As increase in industrialization and population causes increase in living standard, this results in decrease in the quality of water. There is, therefore the need for urgent steps to be taken for proper management and sanitation of the aquatic ecosystem in Akwa Ibom State, because input of sewage or other organic rich materials into the water results in the increase in organotropic bacteria, algae and cyanobacteria which not only bring about health implications but further complications for aquatic life. Awareness should be created amongst riverine communities for proper sewage disposal to reduce microbial contamination of aquatic resources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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