Analysis of the Physicochemical Parameters and Microbiological Quality of Water Samples Obtained from Mbiaso River and Enang Stream in Ikot Ekpene Metropolis

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Authors’ contributions

This work was carried out in collaboration among all authors. Author EJO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ANI and Author ZGM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The physicochemical, microbiological and heavy metal studies of water samples obtained from Mbiaso River and Enang Stream were investigated. Water samples were collected from two locations with two sampling points from each location along the course of the water body based on their use by the community and analysed using standard procedures. The results obtained were compared with WHO standards for drinking and recreational water. Aside titratable acidity, titratable alkalinity and BOD, all other physicochemical parameters were within the permissible standards. All the heavy metals concentrations were above the permissible limits except for Lead (Pb) that ranged between 0.01mg/l to 0.05 mg/l for all the sampling points. All the water samples were found to harbor coliforms organisms in numbers greater than the required WHO standards for water except for Enang river that lacked the presence of Vibrio spp. The total viable counts for all the water samples were generally high exceeding the standard limit of 1.0 x 10² CFU/ml for water. The total heterotrophic bacteria count, total coliform count, faecal coliform count and total fungal...
1. INTRODUCTION

Water is an important natural resource on earth. It is necessary for all living organisms, ecological system, human health, food production and economic development [1].

Water is the most abundant substance found on the surface of the earth, covering roughly three quarters of the earth’s surface and it plays a very vital role in existence as well as maintenance of life of organisms. Water related diseases continue to be one of the major health problems globally due to consumption of contaminated water. The high prevalence of diarrhea among children and infants can be traced to the consumption of unsafe water [2].

The most dangerous form of water pollution occurs when faecal contaminant like *Escherichia coli* enters the water supply. Also, the faecal-oral routes of transmission, in which pathogens are shed in human or animal faces put contaminants into water supply which may perpetuate many diseases examples of such pathogen are *Salmonella spp*, *Shigella spp*, *Vibrio choierae*, *Escherichia coli* [3].

Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs, and wells [4].

The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life. Natural water contains different types of impurities that are introduced in to aquatic system by different ways such as weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities, including mining, processing and the use of metal based materials [5]. The ensuring of good quality drinking water is a basic factor in guaranteeing public health, the protection of environment and sustainable development [6]. Good quality drinking water is of basic importance to human physiology and man’s continued existence depends very much on its availability [7,8].

The increased use of metal-based fertilizer in agricultural revolution of the government could result in continued rise in concentration of metal pollutions in fresh water reservoir due to the water run-off. Also faecal pollution of drinking water causes water borne disease which has led to the death of millions of people [9].

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 [4]. Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population. 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 [10].

Owing to the dangers associated with contaminated waters, this study attempt to assess the microbiological quality and the physicochemical parameters of two different water sources used for drinking and other domestic activities in Ikot Ekpene.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out in two different locations in Ikot Ekpene Local Government Area of Akwa Ibom State. The studied locations include; Mbiaso River and Enang Stream all in Ikot Ekpene metropolis. Ikot Ekpene, known as the raffia city is a historic town in South-Southern State of Akwa Ibom. It is the political and cultural capital of the Annang ethnic group in Nigeria.
The town is located on the A342 highway that parallels the coast between Calabar to the South-East and Aba to the West, with the capital, Uyo. It lies between latitudes 5°10’N and 54°98’N, and longitudes 7°42’E and 53°32’E (Fig. 1).

Mbiaso River is located within Ibiakpan Ikot Inam and Ikot Nkpo communities and bounded close with Obot Akara Local Government Area. It is a beehive of activities including fishing, sand mining, swimming, bathing etc. Two sampling points were chosen along the course of the Mbiaso River. On the other hand, Enang Stream is located in Nkap community which is within the Ikot Ekpene GRA. A lot of activities take place there including washing, bathing and fishing.

All the sampling points were strategically chosen because of the influx of wastewater and/or human activities/influence around the course of the river and stream. The description of the sampling points is shown in Slides A-D Fig. 2, while the GPS readings were also noted and recorded (Table 1).
Table 1. Location of the study sites with descriptions of the sampling points along mbiaso river and enang stream

| Sampling points | Latitude       | Longitude      | Description                                                                 |
|-----------------|----------------|----------------|-----------------------------------------------------------------------------|
| Point M1        | 5°213336N      | 7°658703E      | The point in the river course where mined sands berth                       |
| Point M2        | 5°212311N      | 7°657029E      | point at which fishing and swimming is prevalent                           |
| Point E1        | 5°175160N      | 7°712012E      | The point at which anthropogenic activities like washing takes place        |
| Point E2        | 5°173459N      | 7°710378E      | point where there is influx of waste water                                  |

Where M1 and M2 represents sampling points 1 and 2 from Mbiaso river; E1 and E2 represents sampling points 1 and 2 from Enang stream

2.2 Sample Collection

Water samples were collected for both physiochemical and microbiological analysis aseptically in sterile bottles during morning hours between 8:00 to 8:45 am. Two sampling points each were strategically chosen along the course of the water bodies. The sterile plastic bottles were opened at the time of sample collection and brought to the laboratory for analysis within 1 hour. The samples were processed immediately. Samples for the heavy metal analysis were acidified with HNO₃ to preserve the metal composition.

2.3 Physicochemical Analysis of the Water Samples

The physicochemical parameters which include Temperature, pH, Electrical Conductivity, Total Dissolved Solids, Total Suspended Solids, Total Alkalinity, Total Acidity, Sulphate, Chloride, Nitrate, Total Hardness, BOD, COD, Lead, Nickel, Cadmium, Chromium, Arsenic were determined by the methods of APHA [11-13] (2012) standards, EPA [14] and FAO [8].

2.4 Media used in the Experiment

The media used for the microbiological analysis of water include MacConkey agar (MCA), Nutrient agar (NA), Alkaline peptone water (APW), Sabourand dextrose agar (SDA), Salmonella-Shigella agar (SSA), Mannitol salt agar (MSA), Thiosulfate citrate bile salts sucrose agar (TCBS) and Eosin methylene blue agar (EMB). All the media used were weighed out and prepared according to the manufacturer’s specification, with respect to the given instructions and directions.

2.5 Enumeration and Total Viable Bacterial and Fungal Counts

A serial dilution method was used for total viable counts. Dilutions of 10⁻¹ to 10⁻⁵ were carried out for all samples by transferring 1ml of the sample to 9mls of sterile diluents to make a 10-fold dilution. For direct counting, spread plate technique was performed as described thus; 0.1 ml of the water sample was transferred by a micro pipette and spread on agar plate in duplicates with a sterile bent glass rod. All the
plates were inoculated at 37°C for 24 hours and 25°C for 5 days for bacteria and fungi respectively. Only 30–300 colonies on a plate were considered for enumeration from the various dilutions. Total counts were expressed as colony forming unit per ml (cfu/ml) [15].

### 2.6 Isolation of Pathogenic Bacteria and Fungi

The water samples were analyzed on different culture media using the standard spread plate technique. Nutrient agar (NA) was used for the heterotrophic bacteria count while Sabouraud dextrose agar (SDA) was used for fungal counts. The bacterial cultures were incubated at 37°C for 24 hours and the fungal cultures at 25°C for 5 days. The samples were also cultured using five differential/selective culture media namely Eosin Methylene Blue (EMB), for the isolation of Escherichia coli, Mannitol salt agar (MSA), for the isolation of Staphylococcus aureus, MacConkey agar for Enterobacter and other coliforms; Salmonella shigella agar (SSA) for isolation of salmonella and some shigella species, while Thiosulfate citrate bile salts sucrose agar (TCBS) for isolation of Vibrio species. This method was adopted from Harrigan, [16] and Adeleke et al. [17].

### 3. RESULTS AND DISCUSSION

The physicochemical analysis of the sample water is as presented in Table 2. The Temperature ranged between 26.7-27.5°C which lie within the range of < 32°C for safe drinking water [18]. The temperature range observed in this work will discourage the rate of chemical and biochemical reactions, solubility of gases in the water which could impact negatively on the taste and odour of the water at higher temperatures [19]. The observation is in contrast to the result obtained by Okonko et al. [4] and Mgbemena and Okwunodulu, [20] who reported that the average temperature was between 28-30°C and 28-29°C respectively in similar study and predicted the increase to be influenced by sunlight intensity. The pH ranged between 7.38–7.51 and 7.81-7.86 respectively for the stream and river water respectively. The pH of most natural waters ranges from 6.5 - 8.5 while deviation from the neutral 7.0 is as a result of the CO₂/bicarbonate/carbonate equilibrium [21]. The pH recorded is similar to that obtained by other researchers [22,4]. The Electrical conductivity (EC) ranged between 13.80 – 14.55μs/cm and 23.06-23.52 μs/cm for Enang Stream and Mbiaso River respectively. The values recorded for EC were within WHO standard permissible limits. The titratable alkalinity ranged between 20.00 - 23.44 mg/l and 16.20-17.65 mg/l while titratable acidity ranged between14.86-14.25 mg/l and 18.27-18.33 mg/l for both Enang stream and Mbiaso river respectively. The values were within the WHO permissible limits of 200-600 mg/l [23] and were in consonance with similar report in a study done in India [22]. The results of the total hardness (TH) varied between 11.00-11.73 mg/l and 14.56-18.22 mg/l in Enang stream and Mbiaso river respectively and indicated that the water was soft. The standard in TH is 0.5-50.0mg/L is considered soft while 100-150mg/L is moderately soft water. The results were within WHO permissible limits and agree with other with the results [24,22]. For aesthetic reasons, a limit of 500mg/L is typically recommended for potable water supplies [25]. The Total suspended solid (TSS) as well as Total dissolved solids (TDS) were within permissible limits set by WHO for drinking water [18]. The values were; 34.31 mg/l, 29.26 mg/l, 11.84 mg/l and 12.32 mg/l for TDS and 56.00 mg/l, 41.45 mg/l, 18.93 and 20.66 mg/l for TDS respectively. The BOD recorded were higher than the WHO permissible standard limits of less than 4.0 mg/l which imply that the water samples were not clean [6]. On the other hand, the COD values ranged between 3.31 mg/l to 6.60 mg/l for all water samples and were within WHO standard for good water quality which is set at less than 10 mg/l. The values recorded for the BOD and COD makes the water suitable for domestic purposes other than potable use [24]. The Chloride, Nitrate and Sulphate concentrations as shown in Table 2 reveals that the values were all within WHO permissible standards [18]. The results recorded is similar to those obtained by Shally et al. [22] and Okereke et al. [26] and poses no risk to consumers either for drinking or domestic usage.

The level of Lead from Table 3, Figs. 3 and 4 shows that Enang stream has concentration of 0.01mg/l to 0.04 mg/l while Mbiaso river has 0.02 mg/l to 0.05 mg/l which is within the maximum permissible limits of WHO. Higher levels of Lead may cause health problems like cancer, anaemia etc. The results obtained in this study indicated that the concentrations of Cadmium as well as that of Chromium at all sites were higher than the WHO maximum permissible limits for fresh water [27].
Table 2. Physicochemical analysis of water samples

| Parameters                  | E1   | E2   | M1   | M2   |
|-----------------------------|------|------|------|------|
| Temperature (°C)            | 27.4 | 27.5 | 27.2 | 26.7 |
| pH                          | 7.51 | 7.38 | 7.81 | 7.86 |
| Electrical conductivity (µs/cm) | 13.80 | 14.55 | 23.06 | 23.52 |
| Titratable acidity (mg/l)   | 20.00 | 23.44 | 16.20 | 17.65 |
| Titratable alkalinity (mg/l) | 14.86 | 14.25 | 18.33 | 18.27 |
| BOD (mg/l)                  | 5.30  | 5.74  | 4.85  | 4.78  |
| COD (mg/l)                  | 3.31  | 2.10  | 6.60  | 6.34  |
| Total suspended solids (mg/l) | 34.31 | 29.26 | 11.84 | 12.32 |
| Total dissolved solids (mg/l) | 56.00 | 41.45 | 18.93 | 20.66 |
| Sulphate (mg/l)             | 15.96 | 14.87 | 18.61 | 19.81 |
| Nitrate (mg/l)              | 2.45  | 2.11  | 3.26  | 3.52  |
| Chloride (mg/l)             | 12.18 | 10.64 | 8.10  | 12.50 |
| Hardness (mg/l)             | 17.73 | 11.00 | 14.56 | 18.22 |

Table 3. Heavy metals concentration of the water samples

| Parameters                  | E1   | E2   | M1   | M2   |
|-----------------------------|------|------|------|------|
| Nickel, Ni (mg/l)           | 0.02 | 0.41 | 1.42 | 1.56 |
| Cadmium, Cd (mg/l)          | 0.10 | 0.25 | 0.05 | 0.08 |
| Chromium, Cr (mg/l)         | 0.12 | 0.05 | 0.41 | 0.56 |
| Arsenic, As (mg/l)          | 0.56 | 0.49 | 0.80 | 0.51 |
| Lead, Pb (mg/l)             | 0.01 | 0.04 | 0.02 | 0.05 |

Heavy Metal Concentration of Enang Stream

![Heavy Metal Concentration of Enang Stream](image)

Fig. 3. Heavy metal concentration of Enang Stream

The level of Nickel in Enang stream varies between 0.02 mg/l to 0.41 mg/l (Fig. 3) and 1.42 mg/l to 1.56 mg/l for Mbiaso river (Fig. 4). This reflects high concentration for Nickel and above the WHO [18] guideline value of 0.07 mg/L. Obasi and Akudinobi, [28] reported concentrations of Nickel ranging from 0 to 1.26 mg/L in water samples. Nickel is noted in special cases of release from natural or industrial nickel deposits in the ground and has an extensive range of carcinogenic mechanisms which include regulation of transcription factors, controlled expression of certain genes and generation of free radicals [28]. It occurs naturally in water, with concentrations normally less than 0.02 mg/L WHO [18].

Arsenic concentration was 0.49 to 0.56 mg/l and 0.51 to 0.80 mg/l in Enang stream and Mbiaso river respectively. These values are above the WHO [18] guideline of 0.01 mg/L for drinking water and have high potential risk. Arsenic contaminations have occurred as a result of both natural geologic processes and the activities of man. Anthropogenic sources of arsenic include human activities such as mining and processing of ores. In water, it is mostly present as arsenate, but in anaerobic conditions, it is likely to be
Lower levels of arsenic exposure can cause nausea and vomiting, reduced production of erythrocytes and leukocytes, abnormal heart beat, pricking sensation in hands and legs, and damage to blood vessels. Long-term exposure can lead to the formation of skin lesions, internal cancers, neurological problems, pulmonary disease, hypertension and cardiovascular disease and diabetes mellitus.

Microbiological analysis of the water bodies is shown in Table 4 and Fig. 5. Total heterotrophic bacteria counts (THBC) were very high in these samples. Enang stream had counts of $4.7 \times 10^4$ and $5.2 \times 10^4$ CFU/ml while Mbiaso river had counts between $1.9 \times 10^4$ and $2.4 \times 10^4$ CFU/ml for THBC respectively. Other researchers has reported similar results when analyzing water bodies [24,4]. On the contrary, Atoyebi and Ekpo, [31] reported Total heterotrophic bacteria counts of $2.0 \times 10^7 \pm 0.17$ to $2.6 \times 10^7 \pm 0.44$ CFU/ml.

### Table 4. Microbial counts from the water samples

| Count type                                      | Enang stream | Mbiaso river |
|------------------------------------------------|--------------|--------------|
|                                                | point A      | point B      | point A      | point B      |
| Total Heterotrophic Bacteria Counts (THBC)     | $5.2 \times 10^4$ | $4.7 \times 10^4$ | $2.4 \times 10^4$ | $1.9 \times 10^4$ |
| Total Fungal Counts (TFC)                      | $2.8 \times 10^3$ | $2.5 \times 10^3$ | $5.3 \times 10^2$ | $3.5 \times 10^2$ |
| Total Coliform Counts (TCC)                    | $3.8 \times 10^4$ | $3.2 \times 10^4$ | $2.3 \times 10^4$ | $2.5 \times 10^4$ |
| Total Staphylococcus Bacteria Counts (TSBC)    | $0.3 \times 10^2$ | $0.4 \times 10^2$ | $0.6 \times 10^2$ | $0.3 \times 10^2$ |
| Faecal Coliforms Counts (FCC)                  | $2.6 \times 10^4$ | $3.0 \times 10^4$ | $1.8 \times 10^4$ | $1.7 \times 10^4$ |
| Total Salmonella-Shigella Counts (TSSC)        | $0.4 \times 10^2$ | $0.26 \times 10^2$ | NIL           | $0.2 \times 10^2$ |
| Total Vibrio Counts (TVC)                      | NIL          | NIL          | $0.8 \times 10^2$ | $1.6 \times 10^2$ |
The total fungal counts (TFC) ranged from $3.5 \times 10^2$ to $2.8 \times 10^3$ CFU/ml, the total coliform counts (TCC) ranged from $2.3 \times 10^4$ to $38 \times 10^4$ CFU/ml, the faecal coliform counts (FCC) varied from $1.7 \times 10^4$ to $30 \times 10^4$ CFU/ml, the total vibrio counts (TVC) varied from 0 to $1.6 \times 10^5$ CFU/ml, total staphylococcus bacterial count ranged from $0.3 \times 10^2$ to $0.6 \times 10^2$ CFU/ml while the total salmonella-shigella counts (TSSC) ranged from 0 to $0.4 \times 10^2$ CFU/ml.

Pathogenic bacteria were isolated from the two study sites. The presence of enteric bacteria like *E. coli*, *Salmonella* spp., and *Vibrio* spp. is indicative high level of faecal and municipal waste contamination which may pose health hazard to those using the water for potable and/or domestic activities. This conclusion is also confirmed by many researchers with regards to high coliform bacteria counts [32,22,31]. The total viable counts for the water samples collected from the two sampling sites were in excess of the WHO permissible limit of $1.0 \times 10^2$ CFU/ml which is the standard limit of bacterial counts for drinking water. The bacterial contamination could be attributed to animal and anthropogenic activities from sources like surface runoff, pastures where animal wastes are deposited, washing, bathing, fishing, dredging etc. Apart from the enteric organisms, there was also the presence of staphylococcus spp., which was within the WHO standard limits for drinking water. There was also higher fungal count in the water samples which can also be attributed to anthropogenic activities and infiltrations into the water bodies during the rainy seasons. These findings are in line with those of Ana et al. [33] who isolated fungi in water bodies.

**4. CONCLUSION AND RECOMMENDATION**

This study concluded that the Mbiaso River and Enang stream is not fit for potable use without further processing. Also, conscious effort should be geared towards limiting the numbers of microorganisms released into the body. The high microbial load in the waters renders it unfit for human consumption though they can be used for other purposes. Water should meet different quality specifications depending on the particular uses.

The water body needs urgent measures to control pollution by controlling anthropogenic activities to prevent sewage from entering the water body which is the key to avoid bacterial contamination of the water, and thus provide means of safe water for use, thereby protecting the water body. Education Programmes must be organized to educate the general populace on the proper disposal of refuse, treatment of sewage and need to purify the water to make it fit for use because the associable organisms are of public health significance being implicated in one form or the other. Regulations should be made and enforced by the various environmental regulatory agencies with regards to proper waste disposal as waste should be treated before disposal.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

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