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# Microbiological and Physiochemical Quality of Freshwater in Isiokpo Community, Rivers State, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author ECC managed the literature searches, wrote the protocol, conducted the experiments and wrote the first draft of the manuscript. Author OO designed the study, also managed the literature searches, managed the analyses of the study and wrote the final draft of the manuscript and author ANP performed the statistical analysis. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To determine microbiological quality of fresh water in Isiokpo community.
**Study Design:** This study employs standard scientific methods, statistical analysis and interpretation.
**Place and duration of the Study:** Fresh water samples were collected from Isiokpo community in Ikwerre L.G.A. of Rivers State, Nigeria. Sampling was done every two weeks from three stations of Isiokpo river for duration of six months (February–July, 2018). Samples were transported in ice packed coolers to the Microbiology Laboratory of Rivers State University, Port Harcourt, and immediately processed for analysis.
**Methodology:** Ten- fold serial dilution technique was adopted after which 0.1 ml of appropriately diluted stock was inoculated onto sterile Microbiological media. Spread plate technique was employed for the isolation, enumeration and identification of microorganisms. The APHA standards were adopted in the determination of physico-chemical parameters which include turbidity, colour,

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odour, pH, conductivity, total suspended solids, total dissolved solids, nitrate, sulphate, calcium and $BOD_5$. The Duncan multiple range test was employed for analysis of variance (ANOVA) of the data obtained.

**Results:** The mean counts for Total heterotrophic bacterial counts ranged from 4.77±0.20 $log_{10}$ CFU/ml to 4.92±0.11 $log_{10}$ CFU/ml. Total coliform bacteria ranged from 4.28±0.25 to 4.60±0.25$log_{10}$ CFU/ml. Total Vibrio counts ranged from 1.77±1.97 $log_{10}$ CFU/ml to 4.25±0.09 $log_{10}$ CFU/ml. Total Pseudomonas counts ranged from 2.48±1.93 $log_{10}$ CFU/ml to 4.0217±0.34 $log_{10}$ CFU/ml. Total heterotrophic fungal counts ranged from 2.31±1.81 $log_{10}$ CFU/ml to 4.21± 0.22 $log_{10}$ CFU/ml in all the stations. The microorganisms isolated belong to the genera of *Bacillus, Escherichia, Klebsiella, Pseudomonas, Staphylococcus, Shigella, Vibrio, Aspergillus, Penicillium, Mucor* and *Rhizopus*. The pH values ranged from 5.60 to 6.80, Conductivity 35 to 40 µs/cm, Total suspended solids (TSS) 48 mg/L to 54.00 mg/L, Total Dissolved Solids 5.20 to 6.50 mg/L, Nitrate 1.00 to 1.45 mg/L, Sulphate 1.00 to 1.30 mg/L, Calcium 6.00 to 9.20 mg/L and BOD 5.30 to 6.20 mg/L in all the stations.

**Conclusion:** The presence of *E. coli* which is an indicator of fecal contamination is enough to suspect that the water body is contaminated with fecal matter and pathogenic bacteria. The water should therefore not be put to human use without proper treatment because the water is of low quality and constitute a danger to public health.

*Keywords: Freshwater, microorganisms, analysis of variance, E. coli, Shigella, fecal contamination*.

## 1. INTRODUCTION

Microbiological quality of water used for human consumption and other domestic activities is crucial as it influences human health and has proved to be useful method of biological assessment of water contamination, which may have be missed in a chemical sampling surveillance programme [1].

Water is very much abundant and it is the most widely distributed substance found in nature. It is invaluable to the existence of life and about 80% of the Earth's surface is totally covered by water [2]. The various sources of water include rain, underground, borehole, surface water and springs. Water is used in various ways such as drinking, cooking, cleaning, personal hygienic, environmental sanitation, industrial, fishing activities (ponds and aquacultures), navigation and movement of submarines and military aircrafts. There are two kinds of water which include the marine and the fresh water. The marine ecosystems are among the largest of Earth's aquatic ecosystems. Examples include salt marshes, intertidal zones, estuaries, lagoons, mangroves coral reefs, the deep sea, and the sea floor. They can be contrasted with fresh water ecosystems, which have a lower salt content. Freshwater exists naturally on Earth's surface in ice sheets, ice caps, glaciers, icebergs, bogs, ponds, lakes, rivers, streams, and underground as groundwater in aquifers and underground streams [3].

Water sources may appear clean, free from characteristics of odor and taste, yet be contaminated [4]. The contamination of water has been associated with sewage effluent. Surface water contains more harmful microorganisms compared to other sources of water including groundwater and rainwater [5]. This is because of indiscriminate wastewater disposal from agricultural, industrial and domestic activities which may be sources of bacteria and other organisms capable of producing disease in man and animals [6].

Fecal coliforms are used as indicators of faecal contamination in water as well as *Aeromonas* and *Pseudomonas* and the presence of these pathogens may cause health risk to unsuspecting consumers [7]. Although coliform bacteria were not known to cause any illnesses, their presence in water is thought to be a predicator of other disease causing agents. Fecal contamination of water may introduce various forms of intestinal pathogens which may cause disease which range from mild gastroenteritis to severe and sometimes fatal dysentery, diarrhea, cholera, typhoid and hepatitis A [8].

Microorganisms found in contaminated water as reported by [9]. were known to be *Salmonella typhi, Salmonella paratyphi, Shigella* species*, Vibrio* species*, Staphylococcus aureus, Campylobacter* species*, E. coli,* and *Pseudomonas aeruginosa* which causes gastrointestinal tract infections and when

present in large quantities for prolonged period of time can cause severe health problems.

Water-borne diseases can cause many health hazards. According to [10,11] water quality requires basic monitoring to check for the level of pollution. Therefore, there is need for physical, chemical and microbiological assessment of water quality and provide information on the quality and safety of the water to ensure continued safety of water supply to the communities. This justifies the present study to determine the microbiological and physico-chemical quality of fresh water in Isiokpo.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Freshwater Samples

The fresh water was collected from Isiokpo river $58^{1}35.334^{"}$N latitude $52^{1}55.122^{"}$E longitude. The river was designated into three stations based on the various activities being carried out at different points or stations along the fresh water. Such activities include washing and bathing, collection of water for drinking and waste dumping. Water samples were collected from different stations twice a month at two-week interval for six months (February–July, 2018). The samples were collected in a sterilized glass containers (200 ml volume) aseptically, sealed after collection to avoid reaction with the atmosphere and labeled properly. The water samples were taken to the laboratory in ice packed coolers within 2 hours of collection for analysis [2,12]. During sample collection, standard procedures recommended by [12] were adhere to for data quality and consistency like handling, preservation and analysis.

### 2.2 Microbiological Analysis

#### 2.2.1 Diluents preparation

Sodium chloride (8½ gm) was solubilized in one thousand millilitres of distilled water. Using sterile pipettes, nine millilitres of the prepared diluent was transferred into test tubes which were autoclaved so as to achieve sterility of the diluents.

#### 2.2.2 Serial dilution of water samples

The dilution method adopted was the Ten-fold technique in which 1 ml of the stock which was prepared by adding 1m of water samples into 9 ml of sterile diluent was pipetted into test tubes containing 9 ml diluent. This was done consecutively until an appropriate dilution was reached.

#### 2.2.3 Cultivation and enumeration of bacterial and fungal isolates

One milliliter of each water samples were aseptically transferred into 9millilitres of normal saline and diluted serially up to $10^{-5}$. An aliquot (0.1ml) of $10^{-2}$ dilution was separately inoculated onto appropriate growth medium (nutrient agar, MacConkey agar, Thiosulphate-citrate bile salt sucrose (TCBS), Cetrimide agar and Sabouraud Dextrose agar) for isolation of heterotrophic bacteria, total coliform bacteria, total *Vibrio*, total *Pseudomonas* and total fungal counts respectively. The inoculated plates were incubated at 37°C for 24 - 48 hours for bacteria while for fungi 1.0 g of tetracycline antibiotics solution was added and aseptically pour on sterile Petri dish plate and were incubated at 35°C for 7 days. All inoculation were made in duplicate using the spread plate method with the aid of a sterile bent glass rod. Discrete colonies that developed after the incubation were counted, recorded and calculated as colony forming unit (CFU) of bacteria or of fungi. Discrete colonies were sub-cultured onto freshly prepared appropriate medium to obtain pure bacterial or fungal isolate for further investigations [4].

#### 2.2.4 Characterization and identification of bacterial isolates

The discrete colonies of bacterial isolate were picked and sub cultured on a sterile dried fresh nutrient agar to obtain pure cultures. The pure isolates were identified by morphological characteristics and routine microbiological tests including gram staining and biochemical tests like catalase test, indole, methyl red, motility, citrate and sugar fermentation [13]. The pure cultures were stored on 10% v/v frozen glycerol suspension. It served as a means of long term storage for further used.

#### 2.2.5 Characterization and identification of fungal isolates

Fungal colonies were subculture on sabouraud dextrose agar and examined macroscopically and microscopically using the needle mount technique. Their identification was performed according to [14,15].

### 2.3 Collection of Samples for Physico-Chemical Analysis

Fresh water samples used for the determination of the physico-chemical characteristics of the fresh water were collected into sterile bottles that are rinsed with the sample before collection and closed tightly. Samples were placed in an ice packed box and taken to the laboratory for analysis. Standard procedures recommended by the [12] were adopted for the collection of samples and for the analysis and determination of the Physico-chemical parameters of the freshwater samples. The physico-chemical parameters determined include turbidity, colour, odour, pH, conductivity, total suspended solids, total dissolved solids, nitrate, sulphate, calcium and $BOD_5$.

### 2.3 Statistical Analysis

One way analysis of variance (ANOVA) was used to ascertain whether there was significant difference in bacterial and fungal counts across the column.

## 3. RESULTS

The result of the microbial count of freshwater samples obtained from the various stations is shown in Table 1. The mean count of total heterotrophic bacteria ranged from 4.77±0.20 $log_{10}$ CFU/ml to 4.95±0.21 $log_{10}$ CFU/ml while the mean count of coliform bacteria ranged from 4.28± 0.25 $log_{10}$ CFU/ml to 4.60±0.25 $log_{10}$ CFU/ml. The mean count of *Vibrio* ranged from 1.77±1.97 $log_{10}$ CFU/ml to 4.25±0.09 $log_{10}$ CFU/ml while the mean count of *Pseudomonas* ranged from 2.48±1.93 $log_{10}$ CFU/ml to 4.0217±0.34 $log_{10}$ CFU/ml. on the other hand, the mean count of fungi ranged from 2.31±1.81 $log_{10}$ CFU/ml to 4.01±0.42 $log_{10}$ CFU/ml for all the stations of freshwater. The ANOVA, $p \leq 0.05$ showed that there was a significant difference in the mean counts for total *Vibrio*, total *Pseudomonas* and of fungi along the stations while the ANOVA, $p \geq 0.05$ showed no significant difference in the mean count of aerobic bacteria and coliform along the stations.

The percentage occurrence of the bacteria isolated from the various stations is shown in Fig. 1. All the bacteria isolated except *Vibrio* which was absent in the drinking water station occurred in all the stations. The waste dump station recorded the highest occurrence (27.0%) of *Bacillus* species, *Escherichia coli*, and *Vibrio* species. The percentage occurrence of fungi isolated from the various stations is shown in Fig. 2. The waste dump station also recorded the highest occurrence *Aspergillus* and *Penicillium* spp. However, none of the fungi isolated during this study occurred in the drinking water station.

The mean values of the physico-chemical constituents of samples from of the different stations of the Isiokpo freshwater and the WHO allowable limit for drinking water are shown in Table 2. The values of pH ranged from 5.60 to 6.80, total suspended solids (TSS) 48 mg/L to 54.00 mg/L, total dissolved solids ranged from 5.20 to 6.50mg/L, and $BOD_5$ ranged from 5.30 to 6.20 mg/L. Generally, all the stations recorded high $BOD_5$ values.

## 4. DISCUSSION

This present study has revealed the microbiology and physico-chemical constituents of the various stations of Isiokpo freshwater in River State. The high counts of total heterotrophs, coliforms, *E. coli*, fungi and high $BOD_5$ indicates that the water body is heavily polluted. This is in line with the finding of [16].

**Table 1. Microbial counts ($Log_{10}$ CFU/ml) of isolates from Isiokpo freshwater stations**

| Station | THBC | TCC | TVC | TPC | TFC |
|---|---|---|---|---|---|
| Bathing/washing | 4.92±0.11$^{a}$ | 4.47± 0.25$^{a}$ | 3.96±0.20$^{b}$ | 3.74±0.45$^{ab}$ | 3.80 ±0.34$^{b}$ |
| Drinking | 4.77±0.20$^{a}$ | 4.28± 0.25$^{a}$ | 1.77±1.97$^{a}$ | 2.48±1.93$^{a}$ | 2.31 ±1.81$^{a}$ |
| Waste dump site | 4.95±0.21$^{a}$ | 4.60 ±0.25$^{a}$ | 4.25±0.09$^{b}$ | 4.0217±0.34$^{b}$ | 4.01±0.42$^{b}$ |

*Legend: THBC=Total heterotrophic bacterial count. TCC=Total coliform count, TVC=total vibrio count TPC=Total Pseudomonas count and TFC=Total fungal count. The means with the same alphabets along the column showed no significant different at ($p \geq 0.05$) while means with different alphabets along the column showed significant at ($p \leq 0.05$)*

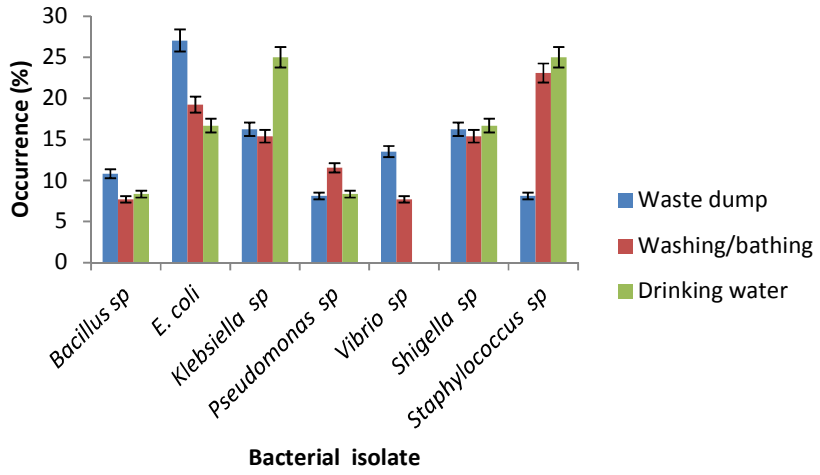


**Fig. 1. Occurrence (%) of Bacterial Isolates in Stations of Isiokpo River**

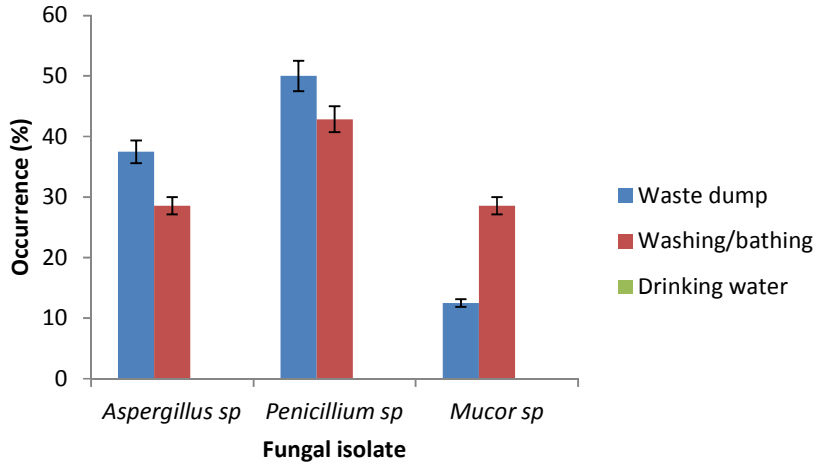


**Fig. 2. Occurrence (%) of fungal isolates in stations of Isiokpo River**

**Table 2. Mean values of the physico-chemical constituents of stations of Isiokpo River**

| Parameter | Station A | Station B | Station C | WHO limit |
|---|---|---|---|---|
| Odour | Unobjectionable | Unobjectionable | Unobjectionable | Unobjectionable |
| Colour (Hazen units) | 1.00 | 1.00 | 1.00 | 15 |
| pH | 5.60 | 5.60 | 6.80 | 6.5-8.5 |
| Conductivity (µs/cm) | 40.00 | 38 | 35 | 400 |
| Turbidity (NTU) | <1.00 | <1.00 | <1.00 | 5 |
| Total Hardness (mg/L) | 19.40 | 19.10 | 17.20 | 100 |
| Total Suspended Solids(mg/L) | 54.00 | 62.00 | 48.0 | ≤ 1 |
| Total Dissolved Solids (mg/L) | 6.00 | 6.50 | 5.20 | 500 |
| Nitrate (mg/L) | 1.45 | 1.20 | 1.00 | |
| Sulphate (mg/L) | 1.30 | 1.30 | 1.00 | |
| Calcium (mg/L) | 9.20 | 7.50 | 6.00 | |
| $BOD_5$ (mg/L) | 6.20 | 6.10 | 5.30 | 0.05 |

The bacteria and fungi identified were *Bacillus* species, *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Shigella* species, *Staphylococcus* species *Vibrio* species, *Aspergillus* species, *Penicillium* species and *Mucor species*. *Bacillus*, *E. coli*, and *Vibrio* species recorded the highest occurrence in the waste dump station, while *Klebsiella*, *Shigella* and *Staphylococcus* species recorded the highest occurrence in the drinking water station. Other investigators [17,18] have reported similar findings.

The presence of indicator bacteria such as *E. coli* and enteric pathogens in the Isiokpo freshwater signifies that the water is contaminated with fecal matter. It implied that, the human activities along the stream had adverse effect on its water quality as reported by [16]. This is a public health concern and attention of the appropriate authorities should be directed to such drinking water source. *Escherichia coli* is known to cause diseases like traveler`s diarrhea and other forms of diarrhea. This investigation has also revealed that some of the bacteria isolated such as *Bacillus* species, *Escherichia coli*, *Pseudomonas* species and *Staphylococcus aureus* are species that have been reported to be involved in the degradation of organic matter which may have entered the water through leaching and waste dump run-off and at bathing and washing site [4,8,19]. These organisms are also known to cause gastrointestinal disorders such as diarrhea, upper respiratory infections and other associated symptoms [20].

Gastroenteritis is caused by *Escherichia coli*. *Klebsiella* species causes pneumonia, blood stream infections, wound infections, urinary tract infections and meningitis. *Shigella* species causes shigellosis (bacterial dysentery). *Staphylococcus aureus* causes staphylococcal food poisoning, characterized with diarrhea and vomiting and is known to produce enterotoxin [19]. The presence of *Pseudomomas aeruginosa* in water may be as result of discharges from immuno-compromised individuals that bath in the water. *Pseudomonas aeruginosa* causes urinary tract infection in the youth and elderly.

Record of the fungi isolated from the freshwater showed that *Aspergillus*, and *Penicillium* species had the highest occurrence in the waste dump station, while *Mucor* species recorded the highest occurrence in the washing and bathing station. These fungi have been reported to be causative agents of asthma, hypersensitivity pneumonitis and pulmonary mycosis. Other disease caused by fungi isolated from freshwater is aspergillosis by *Aspergillus* species [20]. The fungi isolated from freshwater such as *Aspergillus* spp and *Penicillium* spp have been reported by several researchers as petroleum hydrocarbon degraders [21,22]. The presence of these organisms indicates the need to regularly monitor the water quality of Isiokpo freshwater.

The physico-chemical parameters determined were within the WHO limit except pH and Total Suspended Solid. The pH of Isiokpo fresh water ranged from 5.60 to 6.80 which were tending from slightly acidic towards neutrality. This is due to influx of biodegradable material and further biodegradation process which releases acidic gases as by-products into the water. The pH values reported in this study disagreed with the findings of [23], which reported that pH of most freshwater ranged from 8.0 to 8.3.

The total suspended solids (TSS) of Isiokpo fresh water exceeded the WHO limit for drinking water, this could be as a result of silt and disposal of waste materials such as decaying plants, which affect the oxygen in water thereby increasing temperature and decreasing water clarity. Good and useful water may be turbid sometimes but polluted waters are generally turbid.

The $BOD_5$ values reported in this study were highest in the waste dump station and lowest in the drinking water station. Generally, the $BOD_5$ values reported in this study are quite high and this implies that the organic matter load is also high which in turn will surely deplete the amount of dissolved oxygen in the water body. In aquatic environments, total dissolved oxygen is a very important factor to aquatic organisms, because, it causes oxidation of the organic matter in water and respiration of animals meaning it affects their biological process. Therefore, the depletion of dissolved oxygen will adversely affect the ecological community of the water body [9,24].

## 5. CONCLUSION

The present study revealed that, the high counts of bacteria and fungi, and high values of $BOD_5$ shows that the Isiokpo fresh water is contaminated with high load of organic matter. This is as a result of human activities and indiscriminate disposal of waste into the water body. The presence of *Escherichia coli* and

presence of pathogenic bacteria indicated that the water body is also contaminated with fecal matter from humans and other warm blooded animals which constitutes a serious danger to public health. Therefore the fresh water at Isiokpo is not fit for direct human consumption and for other domestic activities without proper and adequate treatment before use.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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