Original Research Article

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

Abstract

Aim: To determine microbiological quality of fresh water in Isiokpo community.
Study Design: This study employs statistical analysis and interpretation.
Place and Duration of the Study: Fresh water samples were collected from Isiokpo community in Ikwere L.G.A. of Rivers State, Nigeria and the study lasted for six months. Sampling was done every two weeks in a month from three stations of freshwater. The samples were transported with ice pack cooler to the Microbiology Laboratory of Rivers State University, Port Harcourt, and immediately analyzed on reaching the Laboratory.
Methodology: Ten- fold Serial dilution technique was adopted in which 1 ml of the stock which was prepared by adding 1ml of water samples into 9 ml of sterile diluent which was pipetted into test tubes containing 9 ml diluents. Spread plate technique was employed for the isolation of microorganisms. The Duncan multiply 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 ± 0.25log_{10}CFU/ml. Total Vibrio counts ranged 1.77 ± 1.97 log_{10}CFU/ml to 4.25 ± 0.09 log_{10}CFU/ml. Total *Pseudomonas* counts ranged from 2.48 ± 1.93log_{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. pH 5.60 to 6.80, Conductivity 35 to 40µs/cm,Total suspended solids (TSS) 48mg/L to 54.00mg/L, Total Dissolved Solids 5.20 to 6.50mg/L, , Nitrate 1.00 to 1.45 mg/L, Sulphate 1.00 to 1.30mg/L, Calcium 6.00 to 9.20mg/L and BOD 5.30 to 6.20 mg/L in all the stations. The microorganisms isolated were the genera of *Bacillus* species, *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Staphylococcus* species, *Sshigella* species, *Vibrio* species, *Aspergillus* species, *Penicillium sp, Mucor* and *Rhizopus* species.
Conclusion: From the study, the presence of *E. coli* and fungi were enough to suspect that the water are contaminated with fecal matters and pathogenic bacteria, hence the water should not be used for human used because the water is of a low quality.

Keywords: Freshwater, Microorganisms, Spread plate, Isolation Analysis of variance, *E. coli*

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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,

agricultural practices such as irrigation, recreational activities (swimming pools), 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 compare to other sources of water including groundwater and rainwater (5). This is because of indiscriminately waste disposal, sewage disposal, pesticides from agricultural activities, domestic waste water may be sources of bacteria and other organisms capable of producing disease in man and animals. Other sources include livestock manure, from municipals, schools, feedlots and swaps (6).

Faecal coliforms are used as indicator of faecal contamination in water a s well as *Aeromonas* and *Pseudomonas* and the presence of these pathogens may cause health risk on consumers and mostly on the immune compromised (7). Although coliform bacteria were not known to cause any illnesses but their presence in water was thought to be a predicator of other disease causing agents. Faecal pollution of water may introduce various forms of intestinal pathogens which may cause mild disease like mild gastroenteritis to severe and sometimes fatal dysentery, diarrhea, cholera, typhoid and hepatitis A (8).

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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, Pseudomonas aeruginosa* which causes gastrointestinal tract infection and when present in large quantities for prolonged period of time can cause health problem.

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, Hence the essence of this study is to determine the microbiological and physic-chemical parameters of Isiokpo fresh water in Rivers State.

2. MATERIALS AND METHODS

2.1 Site Description

The study area was Isiokpo in Ikwerre local government Rivers State, Nigeria. Isiokpo is 4^0 $58^1$35.334" N latitude to $6^0$$52^1$55.122" E longitude.

2.2. Sampling Stations

The study area was zoned into three: Station A, B and C, based on the features observed as well as various activities being carried out on at different points. The Stations were A washing/ bathing, B drinking water site, and C waste dump.

2.3. Collection of water Sample.

Water samples were collected from different stations twice a month at two-week interval for six months (February –July). 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 within 2 hours of collection for analysis (2). During sample collection, standard procedures recommended by the American public Health Association (APHA) were adhere to for data quality and consistency like handling, preservation and analysis

2 Diluents preparation

About eight and half grams of sodium chloride was solubilized in one thousand millilitres of distilled water. Using sterile pipettes, nine millilitres of the prepared diluents were pipetted into test tubes which were autoclaved so as to achieve sterility of the diluents.

3.2.3 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.

2.4. Collection of Samples for physico-chemical Analysis.

Physico-chemical characteristics were determined by collecting water samples and Placed into clean bottles that are rinsed with the sample before collection and closed tightly. They are placed in an ice pack box and taken to the laboratory for analysis.

2.5. Cultivation and enumeration of bacterial and Fungal Isolates.

One milliliter of each water samples were aseptically transferred into 9 ml of normal saline and diluted serially up to 10^{-5}. An aliquots of 10^{-2} dilution were 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 heterotrophic fungal counts respectively. The inoculated plates were incubated at 37^{o}C for 24- 48 hours for bacteria while for fungi 1.0g of tetracycline antibiotics solution was added and aseptically pour on sterile petri dish plate and were incubated at 35^{0}C for 7 days . All inoculation were made in duplicate and the spread plate method using a sterile bent glass rod. Discrete colonies that developed were counted and recorded as colony forming unit (CFU) and were subculture to obtain pure bacteria isolate for further investigations (4).

2.6 Characterization and Identification of Bacterial Isolate.

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 (12). The pure cultures were stored on 10% v/v frozen glycerol suspension. It served as a means of long term storage for further used.

2.7 Characterization and Identification of fungal Isolates

Fungal colonies were subculture on sabouraud dextrose agar and examined macroscopically and microscopically using the needle mouth technique. Their identification was performed according to (13, 14).

2.8 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 microbial count, occurrence of the organisms and physico-chemical parameters of the fresh water are shown in Table 1 -3. Table 1, shows the microbial counts of microorganisms isolated from freshwater water samples. The mean counts 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 counts of coliform bacteria ranged from 4.28± 0.25 log_{10} CFU/ml to 4.60 ± 0.25 log_{10} CFU/ml. The mean counts of *Vibrio* ranged from 1.77 ± 1.97 log_{10} CFU/ml to 4.25 ± 0.09log_{10} CFU/ml while the mean counts of *Pseudomonas* ranged from 2.48 ± 1.93 log_{10} CFU/ml to 4.0217 ± 0.34 log_{10} CFU/ml and the mean fungal count ranged from 2.31 ± 1.81 log_{10} CFU/ml to 4.01±0.42 log_{10} CFU/ml in all the stations of freshwater. The ANOVA, $p \leq 0.05$ showed that there was a significant different in the mean counts for total *Vibrio* counts, total *Pseudomonas* counts and fungal counts along the stations while the ANOVA, $p \geq 0.05$ showed no significant difference in the mean counts for aerobic bacteria count and coliform count along the stations.

The occurrences of isolated organisms are shown in Table 2. For the bacterial isolates, *E. coli* occurred highest in station A (waste dump site) while *Staphylococcus* sp. occurred highest in station B (washing/ bathing site) and *Klebsiella* sp. and Staphylococcus sp occurred highest on station C (drinking site). The fungal isolates: *Penicillium* sp. has the highest occurrence both on station A and B and fungi were not isolated on station C.

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The percentages of the microorganisms isolated are shown in Figure 1& 2. For bacterial isolates, *Bacillus* species range from (8.3% to 10.8%). *Escherichia coli* 16.7% to 27.0%, *Klebsiella* species 25.0% to 15.4% , *Pseudomonas* species 8.1% to 11.5%, *Staphylococcus* species 8.1% to 25.0%, *Shigella* species 15.4% to 15.4% and *Vibrio* species 0.0% to 13.5%. For the fungal isolates, *Aspergillus* species 0% to 37.5%, *penicillium* species 0% to 50.0% and *Mucor* species 0% to 28.6% respectively.

Table 3, shows the values of the organic pollutants of the freshwater samples. They ranged as follows: pH 5.60 to 6.80, cConductivity 35 to 40µs/cm, tTurbidity <1.00NTU, tTotal hHardness 17.20-19.40mg/L, tTotal suspended solids (TSS) 48mg/L to 54.00mg/L, Total Dissolved Solids 5.20 to 6.50mg/L, nNitrate 1.00 to 1.45 mg/L, sSulphate 1.00 to 1.30mg/L, cCalcium 6.00 to 9.20mg/L and BOD 5.30 to 6.20 mg/L.

Table 1: Microbial counts (Log_{10} CFU/ml) of Isolates from freshwater Station (Isiokpo)

Isiokpo freshwater	THBC	TCC	TVC	TPC	THFC
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 counts. TCC=Total coliform counts, TVC=total vibrio counts TPC=Total Pseudomonas counts THF=Total heterotrophic fungal. T he columns with the same alphabets showed no significant different at ($p \geq 0.05$). The columns with different alphabets showed significant at ($p \leq 0.05$).

Table 2: Microorganisms Isolated and their Occurrence

Organisms	Station A	Station B	Station C
Bacteria			
Bacillus sp.	4	2	1
E. coli	10	5	2
Klebsiella sp.	6	4	3
Pseudomonas sp.	3	3	1
Vibrio sp.	5	2	0
Shigella sp.	6	4	2
Staphylococcus sp.	3	6	3
Fungi			
Aspergillus sp.	3	2	0
Penicillium sp.	4	3	0
Mucor sp.	1	2	0

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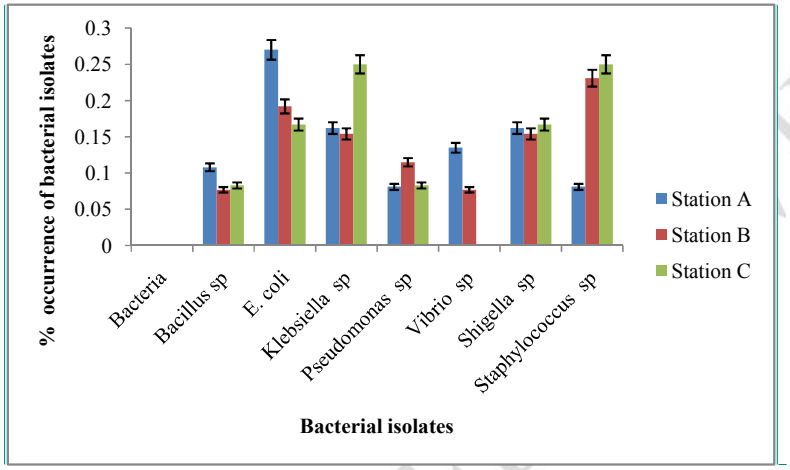

Fig.1: Bacterial isolates Isolated and their Percentage Occurrence

Comment [GP1]: Bacteria ?

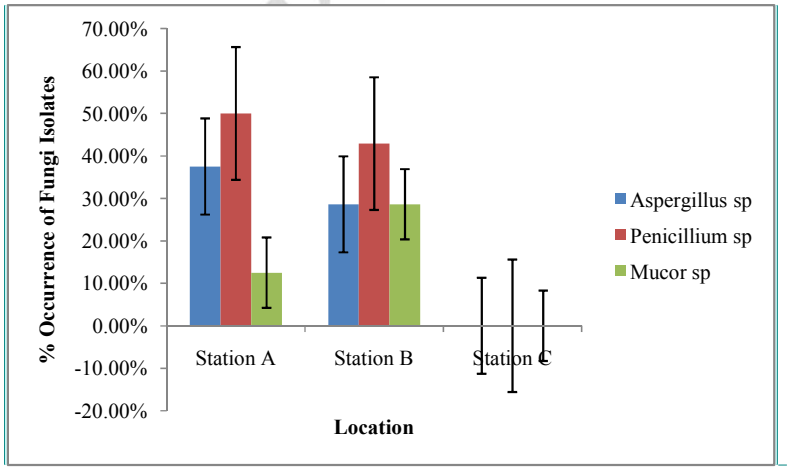

Fig.2: Fungal isolates Isolated and their Percentage Occurrence

Comment [GP2]: Station C? don't put bars

Table 3: Mean values of the organic pollutant of freshwater stations

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	1000
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	30
Total Dissolved Solids (mg/L)	6.00	6.50	5.20	500
Nitrate (mg/L)	1.45	1.20	1.00	10
Sulphate (mg/L)	1.30	1.30	1.00	250
Calcium (mg/L)	9.20	7.50	6.00	70
BOD_5 (mg/L)	6.20	6.10	5.30	15

4. DISCUSSION

The study on microbiological and physico-chemical quality of freshwater in River state revealed high value of coliform counts, presence of *E. coli*, fungal and pollutant which indicates that the water source is contaminated with faecal matter and pathogenic bacteria

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The high microbial counts observed may be as a result of human activities and indiscriminate waste disposal into water source which had negative effect on the quality of the water body. This is in line with the finding (15). Therefore the direct use of Isiokpo stream such as drinking, domestic activities and agricultural purposes indicates danger to public health.

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. These organisms isolated from this study causes gastrointestinal disorders such as diarrhea, upper respiratory infection and other associated symptoms. Other investigators (16, 17) have reported similar result.

The presence of indicator bacteria in the Isiokpo freshwater such as *Escherichia coli* signifies that the water source is contaminated with faecal matters and presence of other enteric pathogens. This is a public health concern and attention should be on such water source. It implied that, the human activities along the stream had adverse effect on its water quality supported by (15). *Escherichia coli* is known to cause diseases like traveler`s diarrhea and other forms of diarrhea. The investigation indicated that bacterial isolated from the freshwater environment includes species that are involved in the degradation of organic matters such as *Bacillus* species, *Escherichia coli Pseudomonas* species and *Staphylococcus aureus* which may have entered the water through leaching and waste dump run-off and at bathing and washing site.

Diseases that are caused by bacteria isolated from the water samples are *Escherichia coli* causes gastroenteritis, *Klebsiella* species causes Klebsiella 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 suppoted by (18). The presence of *Pseudomomas aeruginosa* in water may be as result of discharges from immuno-compromised individual that bath in the water. *Pseudomonas aeruginosa* causes urinary tract infection in the youth and elderly people.

Fungal isolated from the freshwater environment are causative agents of asthma, hypersensitivity pneumonitis and pulmonary mycosis. Other disease cause by fungi isolated from freshwater is aspergillosis by *Aspergillus* species. This concurs with the findings of (19).The fungi isolated from freshwater such as Aspergillus spp and Penicillium spp have been reported by several researchers as petroleum hydrocarbon degraders (20, 21). The presence of these organisms indicates the need to constant monitor the water quality of Isiokpo freshwater.

The physico-chemical parameters determined were within the WHO limit except pH and Total Suspended Solid. The hydrogen ion concentration (pH) of Isiokpo fresh water ranged from 5.60-6.80 which were tending from slightly acidic towards neutrality.

The slightly acidic could be due to influx of biodegradable material and further biodegradation process which releases acidic gases in the surface water as by products. The pH found in this study disagreed with the findings by (22) , which said that pH of most freshwater ranged from 8.0 to 8.3.

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.

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 decreases water clarity. Good and useful water may be turbid sometimes but polluted waters are generally turbid

From the study, all the stations recorded low BOD. This result found may be due to the flow of water which flushes suspended materials away from the drinking site, also due to treated water discharged into the stream, which may decrease the bacterial load in the water which was in agreement with (9,23).

CONCLUSION

The investigation revealed certain physico-chemical parameters that exceeded the WHO limit and the microbiological quality of the water indicated high heterotrophic bacterial counts, high coliform bacterial counts and the presence of *Escherichia coli* indicated that the water bodies are also contaminated with faecal matter from animals, human and presence of pathogenic bacteria. This may be due to human activities and indiscriminate waste disposal which contribute to the contamination of water sources. Therefore the water bodies are not fit for drinking, swimming, agricultural purposes and other domestic activities and should be treated adequately before consumption.

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