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Effect of seasons on the bacteriological quality of Iyifeyi stream in Ugwobi Abbi, Enugu State, Nigeria

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Abstract

Good quality water is essential to human metabolic activities. The effect of seasons on the bacteriological quality of Iyifeyi stream in Ugwobi Abbi in Uzo-Uwani Local Government Area of Enugu State, Nigeria was studied using the spread plate and multiple tubes methods. Total bacterial load of the stream water examined during the dry season was $32x10^2$cfu/ml to $56x10^2$ cfu/ml; total coliforms, 14MPN/100ml to 21MPN/100ml and the faecal coliforms 0MPN/100ml. The total bacterial load of the stream during the wet season was 102×10^2cfu/ml to 160×10^2cfu/ml; total coliforms, 20MPN/100ml to 43MPN/100ml and the faecal coliforms, 6MPN/100ml to 14MPN/100ml. Shigellae load was 0cfu/ml to 2cfu/ml while Salmonellae, Vibrio cholerae, Enterococcus faecalis and Clostridium perfringens were not detected during both seasons. These results indicated that Iyifeyi stream is unsafe for drinking due to the high bacterial load including the presence of coliforms. Micrococcus varians, Bacillus cereus, Enterobacter aerogenes, Escherichia coli, Shigella dysenteriae and Pseudomonas aeruginosa were detected in the stream water during both seasons. The antibiotics sensitivity test revealed that all the identified bacteria were resistant to Amikacin and Tetracycline. The water from the stream is therefore recommended for treatment such as boiling, sand filtration, chlorination and ozonation before drinking.

Keywords: Bacteriological, Water, Iyifeyi Stream, Seasons, Ugwobi Abbi.

Introduction

Water is essential to the growth and development of all living organisms. It is used for drinking, cooking, recreation, irrigation, bathing and industrial purposes. Water meant for drinking must comply with the physical, chemical and biological parameters established by regulating bodies. The various sources of water include rainfall, hail, snow, rivers, seas, oceans, ponds, streams, springs, lakes and wells.

In Nigeria, the public water supply has ceased to exist in most of the urban and rural communities and as a result of this, many of the citizens rely on surface water such as stream as source of potable water. Many of these streams are often dumping sites for untreated human and animal faeces, industrial, domestic, municipal and agricultural wastes and sewage while some are used for activities such as fishing and swimming even by people of questionable health status. These activities lead to the pollution of such streams thereby exposing the users to great risks of water-borne diseases such as cholera, diarrhea, typhoid fever and diphtheria. Eyankware and Obasi[1] observed that most surface water resources accessible to households in rural areas are subject to chemical and biological contaminations which may come from animals, septic tanks and storm water runoff.

Millions of people die yearly from diarrheal disease and a larger proportion are children aged below five years[2]. Besides causing death, water related diseases also prevent people from working and living active lives[3]. Microbial indicators which have been used worldwide to indicate the contamination of water bodies by human faeces include Escherichia coli, Enterococci and Clostridium perfringens[4]. Surveillance of water quality to guarantee bacteriological safety is crucial in the prevention of water- borne diseases[5], thereby ensuring improved health, socio economic development and poverty reduction.

Iyifeyi stream which is located at Owereze village in Ugwuobi Abbi community is one of the sources of potable water in the community. Information on the bacteriological quality of the stream is not available and there are reported cases of gastroenteritis, typhoid fever, dysentery and diarrhea among the people of Ugwobi Abbi community following the consumption of the water from the stream during both seasons, therefore this research work was carried out to determine the effect of seasons on the bacteriological quality of Iyifeyi stream in Ugwobi Abbi community in Enugu State of Nigeria.

Materials and methods

Study area: This work was carried out on Iyifeyi stream in Ugwobi Abbi, Uzo-Uwani Local Government Area of Enugu State. The stream is one of the sources of drinking water supply in the community which is located on a high plain with a small flat top. Farming is the major occupation of the community. Its geographical coordinates are 6°50'0" North, 7°14'0" East. Uzo-Uwani L.G.A. has distinct wet and dry seasons and shares its borders with Kogi and Anambra States, Nigeria.

Study design: Two sampling seasons were employed (wet and dry seasons). The dry season samples were collected between November, 2017 and January, 2018 while the wet season samples were collected between May and July, 2018. The research was restricted to Iyifeyi stream.

Samples collection: Water samples were collected from three different points using sterile glass bottles (250ml). The sampling points were upstream, midstream and downstream. Three samples were collected from each of the three sampling points. Prior to sample collection, the containers were thoroughly rinsed with the water to be sampled. Laboratory coat, hand gloves and nose masks were worn to avoid contamination during sample collection. The samples were introduced in ice-packed containers and transported to the Microbiology Laboratory of Nnamdi Azikiwe University, Awka, Nigeria where they were analyzed within twelve hours of collection.

Bacteriological Analyses: Determination of total bacterial load: The total bacterial load of the stream water was determined using the spread plate method described by Cheesbrough[6]. Serial dilutions of the samples were prepared using sterile distilled water and 0.1ml aliquot of the serially-diluted sample (10^2) was spread on the surface of sterile nutrient agar medium contained in Petri dishes and incubated at 37°C for 24 hours after which the colonies that developed were counted and the result expressed as colony forming unit per milliliter (cfu/ml).

Coliform test: Presumptive coliform test: Total coliforms load was determined using the Most Probable Number (MPN) technique described by Cheesbrough[6]. MacConkey broth was prepared and five drops of bromocresol purple were added to it. The first five tubes contained 10ml double strength MacConkey broth and the second and third five tubes contained 10ml single strength MacConkey broth. Inverted Durham tubes were introduced into the tubes which were thereafter sterilized by autoclaving at 121°C for 15 minutes and allowed to cool to 45^{o}C. The first, second and third five tubes received 5ml, 1ml and 0.1ml of the stream water with the aid of sterile syringes and were thereafter incubated at 35°C for 48 hours. The tubes that produced acid and gas were recorded as positive and the MPN of total coliforms was obtained using McCrady's Probability Table.

Confirmed E. coli test: Confirmed test was carried out as described by Cheesbrough[6] by transferring a loopful of culture from positive tubes from presumptive coliform test into other tubes of freshly prepared MacConkey broth with bromocresol purple as the indicator and Durham tubes. The tubes were incubated at 44.5°C for 48 hours. Those that showed gas production were recorded as positive. The Most Probable Number of *E. coli* was read from McCrady's Probability Table. A loopful of culture from the positive presumptive coliform test was also plated on Eosin methylene blue agar (EMB) to obtain discrete colonies. The plates were incubated at 35°C for 24 hours and examined for metallic sheen colonies of *E. coli.*

Completed coliform test: The method used by Cheesbrough[6] was adopted. Several colonies from EMB agar plates were subcultured into lactose broth fermentation tubes and on nutrient agar slope and incubated at 35°C for 24 hours. The broth was examined for gas production while the nutrient agar slope that contained the inoculum was used for Gram reaction for the detection of Gram negative organisms. Indole, methyl red, voges proskauer and citrate tests were carried out thereafter to confirm the coliform bacteria.

Isolation of *Salmonellae and Shigellae species:* Salmonella Shigella agar (SSA) was used to detect the Salmonella and Shigella species as described by Cheesbrough[6]. 0.1ml aliquot of each water sample (undiluted) was spread on the surface of the dried and sterilized SSA plates and incubated at 37°C for 48 hours. Pure cultures were obtained by repeated sub-culturing and the colonies were identified using their morphological and biochemical characteristics.

Screening for *Vibrio cholera:* The test was carried out as described by Cheesbrough[6]. Thiosulphate citrate bile salt sucrose (TCBS) agar was used to screen for the presence of *Vibrio cholerae*. 0.1ml of each water sample (undiluted) was transferred on the dried TCBS agar plates using sterile syringes and spread evenly with sterile wire loops. The plates were incubated in an incubator at 35°C for 48 hours and examined for colonies of *Vibrio cholerae.*

Examination for *Enterococcus faecalis:* *Enterococcus faecalis* was examined using the Most Probable Number method described by Cheesbrough[6] and the medium used was glucose azide broth. 10ml of the stream water were introduced into each of five tubes containing 10ml of double strength glucose azide broth while 1ml of stream water was added to each of five tubes of 5ml of single strength glucose azide broth. 50ml of samples were added to 50ml of double strength medium and the inoculated tubes were incubated at 37°C for 72 hours after which they were observed for a change in colour.

Examination for *Clostridium perfringens:* The method described by Cheesbrough[6] was used. The water samples were heated at 70°C for 10 minutes to destroy non-sporing organisms. Multiple portions of the heated water were inoculated into Differential Reinforced Clostridial Medium in screw-capped bottles, leaving no air space. The bottles were incubated at 37°C for 48 hours and observed for the production of black colour.

Characterization and identification of the bacterial isolates: The bacterial isolates were characterized using the cultural, morphological and biochemical characteristics. The tests carried out to identify the isolates were Gram staining, catalase, spore, indole, methyl red, voges proskaeur, citrate, coagulase, motility, oxidase and sugar fermentation tests.

Gram staining: This was carried out as performed by Cheesbrough[6]. A smear from a 24-hour culture was prepared on a glass slide. The smear was allowed to dry in air and fixed by gentle heating using a Bunsen burner. Crystal violet solution was applied to the smear and left to stand for one minute after which the solution was washed with water. Lugol's iodine solution was next applied and the slide was left allowed to stand for one minute and thereafter rinsed with running water. The slide was thereafter decolourized with alcohol and left for 10 seconds after which it was washed with water and counter - stained with safranin and left to stand for one minute. It was thereafter washed with water, air-dried and viewed under the microscope.

Catalase test: The method used by Cheesbrough[6] was adopted. 3% hydrogen peroxide was dropped on a clean slide and a loopful of the bacterium was mixed with it on the slide. The slide was thereafter observed for the presence of gas bubbles which indicated a positive reaction.

Spore test: The test was performed as done by Cheesbrough[6]. A smear of the test culture was prepared and flooded with 5% malachite green. A wire loop with cotton wool at the tip was dipped into ethanol and used in heating the slide to steaming for five minutes. The slide was washed with water and counter-stained with 5% safranin for 30 seconds and allowed to dry and examined under the oil immersion lens of the microscope.

Indole test: The test was carried out as performed by Cheesbrough[6]. A loopful of culture was inoculated into 5ml peptone water and incubated at 44°C for 24 hours, after which 0.5ml of Kovac's reagent was added. The production of red colour indicated a positive reaction.

Methyl red test: The method of Cheesbrough[6] was used for the test. The suspected organism was inoculated into sterile glucose phosphate peptone water and incubated at 37°C for 48 hours. Two drops of methyl red indicator were added and shaken with the mixture after the incubation. A bright red colour indicated a positive result.

Voges proskauer test: The method described by Cheesbrough[6] was adopted. The suspected organism was inoculated into a test tube containing glucose phosphate peptone water and incubated at 37°C for 48 hours. 0.6ml of 50% alcoholic naphtol and 0.2ml of 40% potassium hydroxide were added to the inoculated medium after the incubation. The mixture was shaken and left to stand. A red colour indicated a positive result while the development of a yellow colour indicated a negative result.

Citrate test: The test was performed as described by Cheesbrough[6]. Simmon's citrate medium was inoculated with a loopful of peptone water culture and incubated at 37°C for 5 days. A colour change from green to blue indicated a positive result while the absence of any colour change indicated a negative result.

Coagulase test: The method used by Cheesbrough[6] was used for the test. A drop of physiological saline was placed on each end of a slide. A portion of the isolated colony was emulsified in each drop to make two thick suspensions using wire loops. A drop of human plasma was added to one of the suspensions and mixed gently. The clumping of the organisms was observed within 10 seconds. No plasma was added to the second suspension (control) to differentiate any granular appearance of the organism from true coagulase clumping.

Motility test: The test was carried out using the method described by Cheesbrough[6]. A semi-solid agar medium was prepared in a test tube and inoculated with a straight wire. A single stab was made down the center of the tube to half the depth of the medium which was thereafter incubated at 37°C for 24 hours. The tube was held up to the light and the stab- line was examined for motility. Non-motile bacteria gave growths that were confined to the stab-line, had sharply defined margins and left the surrounding medium clearly transparent. Motile bacteria gave diffused, hazy growths that spread throughout the medium rendering it slightly opaque.

Oxidase test: The method described by Cheesbrough[6] was adopted. Filter paper was moistened with the oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) and the colony was mixed with the reagent on the filter paper using a wire loop. Positive result was indicated by development of dark purple color within 10 seconds while negative result was indicated by the absence of color change.

Sugar fermentation test: The test was performed as done by Cheesbrough[6]. The isolates were inoculated into peptone water each containing 1% of the following sugars; glucose, lactose, fructose, galactose and mannitol and bromocresol purple indicator. Durham tubes were inserted, followed by sterilization, inoculation and incubation at 37°C for 48 hours. Gas development in the Durham tubes as well as a change in the colour of the indicator from purple to yellow denoted a positive reaction.

Antibiotics sensitivity test of the isolates: This was determined as described by Jorgensen and Turnidge[7]. Pure culture of the bacterium to be tested was aseptically emulsified from the plate in a sterile saline solution. It was mixed thoroughly to ensure that no solid material from the colony was visible in the saline solution. 0.5ml of a suspension of the test organism was used to streak a nutrient agar plate for growth and the plate was allowed to dry for 5 minutes. Antibiotic discs; amoxicillin (10μg), amikacin (30μg), chloramphenicol (10μg), ciprofloxacin (1μg) and tetracycline (10μg) were placed on the surface of the agar using sterilized forceps. The discs were gently pressed onto the surface of the agar using flame-sterilized inoculation loops.

The inoculated plates were inverted and incubated for 24 hours at 37°C. A metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used after incubation.

The measurement obtained from each antibiotic was compared to the standard table to determine whether the tested bacterial species was sensitive or resistant to the tested antibiotic.

Statistical analysis of results: The results of the study were subjected to analysis of variance and descriptive statistics.

Results and discussion

The bacterial load of the stream water during the dry season is shown in Table-1. The total bacterial load ranged between 32 and $56x10^2$cfu/ml, total coliforms between 14 and 21 MPN/100ml while faecal coliforms, *Salmonellae, Shigellae, Vibrio cholerae*, *Enterococcus faecalis* and *Clostridium perfringens* were not detected.

The bacterial load of the stream water during the wet season is presented in Table-2. The total bacterial load was $102\text{-}160x10^2$ cfu/ml; total coliform, 20-43 MPN/100ml; faecal coliforms, 6 – 14 MPN/100ml; *Shigellae*, 0-2cfu/ml while *Salmonellae, Vibrio cholerae*, *Enterococus faecalis* and *Clostridium perfringens* were not isolated from the samples.

The average bacterial load of the stream water during the dry and wet seasons is shown in Table-3. The total bacterial counts ranged between 67 and $103x10^2$cfu/ml; total coliforms, 18–32 MPN/100ml; faecal coliforms, 3-7 MPN/100ml; *Shigellae*, 0-1 cfu/ml while *Salmonellae, Vibrio cholerae, Enterococcus faecalis* and *Clostridium perfringens* were not present in the samples.

The morphological and biochemical characteristics of the bacterial isolates from the stream water during the dry and wet seasons are shown in Table-4. The bacteria were *Micrococcus varians, Bacillus cereus, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli* and *Shigella dysenteriae.*

Table-5 showed the distribution of the bacterial isolates in the stream water during the dry and wet seasons. *Micrococcus varians*, Bacillus cereus and *Enterobacter aerogenes* only occurred in the samples during the dry season only while all the isolates were isolated from the samples during the wet season.

Table-6 showed the antibiotics sensitivity results of the bacterial isolates from the stream water during the dry and wet seasons. Micrococcus varians was sensitive to Chloramphenicol, Bacillus cereus was sensitive to Amoxicillin and Ciprofloxacin, Enterobacter aerogenes was sensitive to Chloramphenicol, Pseudomonas aeruginosa was resistant to all the antibiotics, E.coli was sensitive to Chloramphenicol and Ciprofloxacin while Shigella dysenteriae was also sensitive to Chloramphenicol and Ciprofloxacin.

The frequency of occurrence of the bacterial isolates in the stream water during the dry season is shown in Figure-1. *Micrococcus varians* (25%), *Bacillus cereus* (60%) and *Enterobacter aerogenes* (15%) were detected in the samples.

The frequency of occurrence of the bacterial isolates in the stream water during the wet season is presented in Figure-2. *Micrococcus varians* (15%), *Bacillus cereus* (24%), *Enterobacter aerogenes* (8%), *Pseudomonas aeruginosa* (43%), *Escherichia coli* (6%) and *Shigella dysenteriae* (4%) were detected in the water samples.

Figure-3 showed the frequency of occurrence of the bacterial isolates in the stream water during both seasons. *Micrococcus varians* (20%), *Bacillus cereus* (42%), *Enterobacter aerogenes* (11.5%), *Pseudomonas aeruginosa* (21.5%), *Escherichia coli* (3%) and *Shigella dysenteriae* (2%) were detected from the samples during both seasons.

Table-1: Bacterial load of the stream water during the dry season.

Samples	Total bacterial load ($x10^2$cfu/ml)	Total coliform load (MPN/100ml)	Faecal coliform load (MPN/100ml)	*Salmonellae* load (cfu/ml)	*Shigellae* load (cfu/ml)	*Vibrio cholera* load (cfu/ml)	*Enterococcus faecalis load* (cfu/ml)	*Clostridium perfringens* load (cfu/ml)
A	46	14	0	0	0	0	0	0
B	44	16	0	0	0	0	0	0
C	32	20	0	0	0	0	0	0
D	40	21	0	0	0	0	0	0
E	56	20	0	0	0	0	0	0
F	50	20	0	0	0	0	0	0
WHO Standard	100cfu/ml	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table-2: Bacterial load of the stream water during the wet season.

Samples	Total bacterial load (10^2cfu/ml)	Total coliform load (MPN/100ml)	Faecal coliform load (MPN/100ml)	*Salmonellae load* (cfu/ml)	*Shigellae* load (cfu/ml)	Vibrio cholerae load (cfu/ml)	*Enterococcus faecalis* load (cfu/ml)	*Clostridium perfringens load* (cfu/ml)
A	160	28	14	0	2	0	0	0
B	136	20	10	0	0	0	0	0
C	102	28	6	0	0	0	0	0
D	158	43	12	0	0	0	0	0
E	106	42	8	0	0	0	0	0
F WHO Standard	130 100cfu/ml	28 Nil	6 Nil	0 Nil	0 Nil	0 Nil	0 Nil	0 Nil

Table-3: Average bacterial load of the stream water during the dry and wet seasons.

Samples	Total bacterial load (x10^2cfu/ml)	Total coliform load (MPN/100ml)	Faecal coliform load (MPN/100ml)	*Salmonellae* load (cfu/ml)	*Shigellae* load (cfu/ml)	*Vibrio cholera load* (cfu/ml)	*Enterococcus faecalis* load(cfu/ml)	*Clostridium perfringens load* (cfu/ml)
A	103	21	7	0	1	0	0	0
B	90	18	5	0	0	0	0	0
C	67	24	3	0	0	0	0	0
D	99	32	6	0	0	0	0	0
E	81	21	4	0	0	0	0	0
F	90	24	3	0	0	0	0	0
WHO standard	100cfu/ml	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table-4: Morphological and biochemical characteristics of the bacterial isolates from the stream water during the dry and wet seasons.

Isolates	Morphological characteristics on solid media	Cell morphology	Morphological test										Sugar fermentation test					
			Gram staining	Catalase test	Coagulase test	Oxidase test	Motility test	Spore test	Indole test	MR test	VP test	Citrate test	Glucose	Lactose	Fructose	Galactose	Mannitol	Identity
1.	Yellow small convex colonies with smooth edge.	Cocci in clusters	+	+	-	+		-	-	-	-	-	+	-	-	-	-	*Micrococcus varians*
2.	Whitish, glistering and spreading colonies.	Rods	+	+	-	+	+	+	-	-	-		+	-	+	-	+	*Bacillus cereus*
3.	Circular, creamy colonies those are convex and smooth.	Rods	-	+	-	-	+	-	-	-	+	+	+	+	-	+	+	*Enterobacter aerogenes*
4.	Bluish/green colonies those are smooth, large, translucent and convex.	Rods	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	*Pseudomonas aeruginosa*
5.	Circular pink colonies	Rods	-	+	-	-	+	-	+	+	-	-	+	+	+	-	+	*Escherichia coli*
6.	Pale coloured colonies	Rods	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	*Shigella dysenteriae*

MR = methyl red, VP = voges proskauer, + = positive, - = negative.

Table-5: Distribution of the bacterial isolates in the stream water during the dry and wet seasons.

Isolates	Dry Season	Wet Season
Micrococcus varians	+	+
Bacillus cereus	+	+
Enterobacter aerogenes	+	+
Pseudomonas aeruginosa	–	+
Escherichia coli	–	+
Shigella dysenteriae	–	+

Key: + = present, - = absent.

Table 6: Antibiotics sensitivity results of the bacterial isolates from the stream water during the dry and wet seasons.

Antibiotics	Disc content (μg)	Standard values R≤IS≥			Sensitivity pattern (zone of inhibition in mm) of E. coli	Sensitivity pattern (zone of inhibition in mm) of *Pseudomonas aeruginosa*	Sensitivity pattern (zone of inhibition in mm) of *Bacillus cereus*	Sensitivity pattern (zone of inhibition in mm) of *Enterobacter aerogenes*	Sensitivity pattern (zone of inhibition in mm) of *Micrococcus varians*	Sentivity pattern (zone of inhibition in mm) of *Shigella dysenteriae*
Amoxicillin	10	14	16	15	R(5.6)	R(5.0)	S(16.1)	R(3.9)	R(3.5)	R(5)
Amikacin	30	15	17	16	R(6.2)	R(5.7)	R(8.1)	R(7.2)	R(5.2)	R(6.1)
Chloramphenicol	10	14	16	15	S(16.1)	R(15.0)	R(15.1)	S(17.0)	S(17.3)	S(17)
Ciprofloxacin	1	16	18	17	S(18.3)	R(4.4)	S(18.0)	R(7.1)	R(5.5)	S(18.1)
Tetracycline	10	19	21	20	R(9.4)	R(8.8)	R(6)	R(6.1)	R(8.1)	R(7.1)

Key: R = resistance, S = sensitive, I = intermediate.

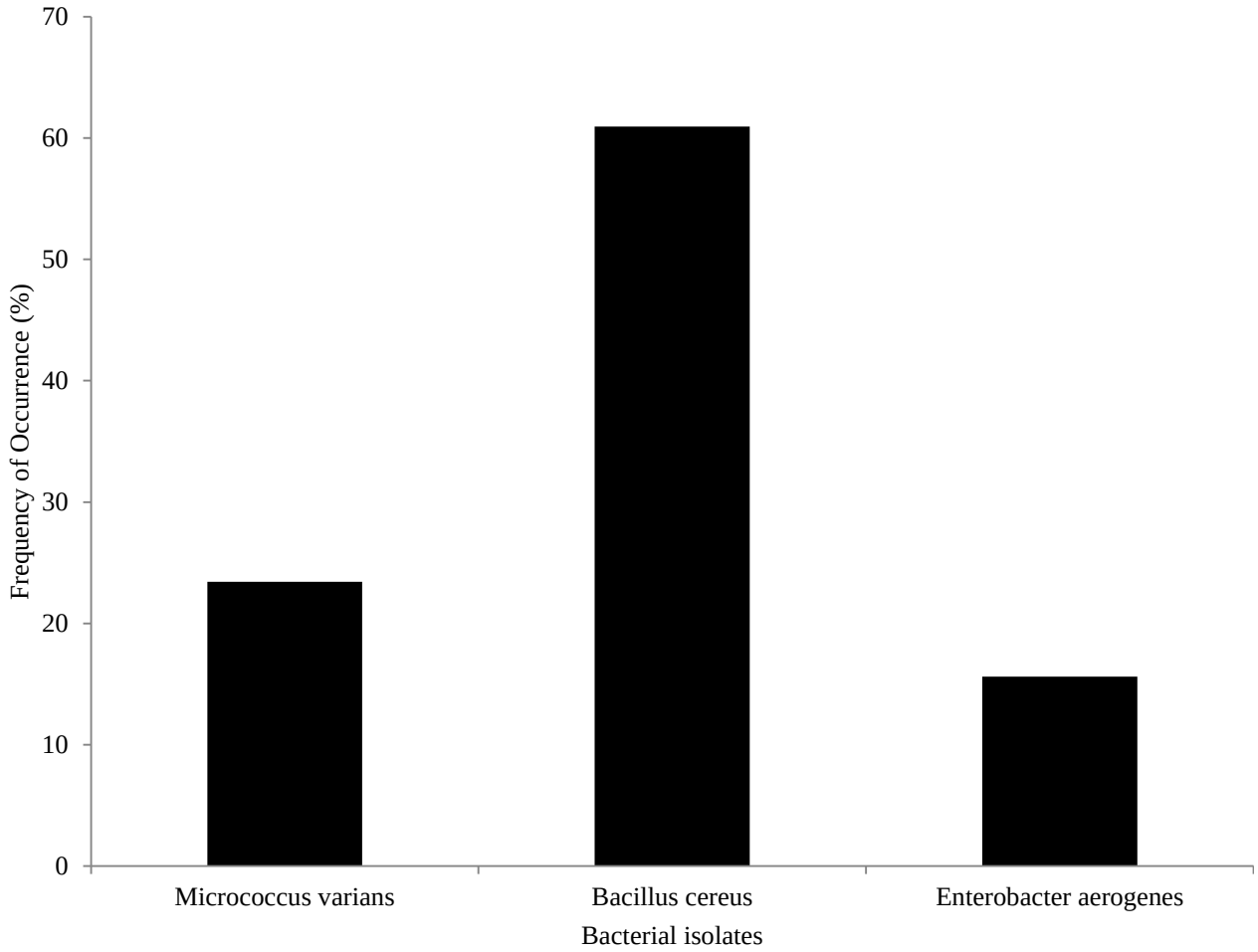

Figure-1: Frequency of occurrence of the bacterial isolates in the stream water during the dry season.

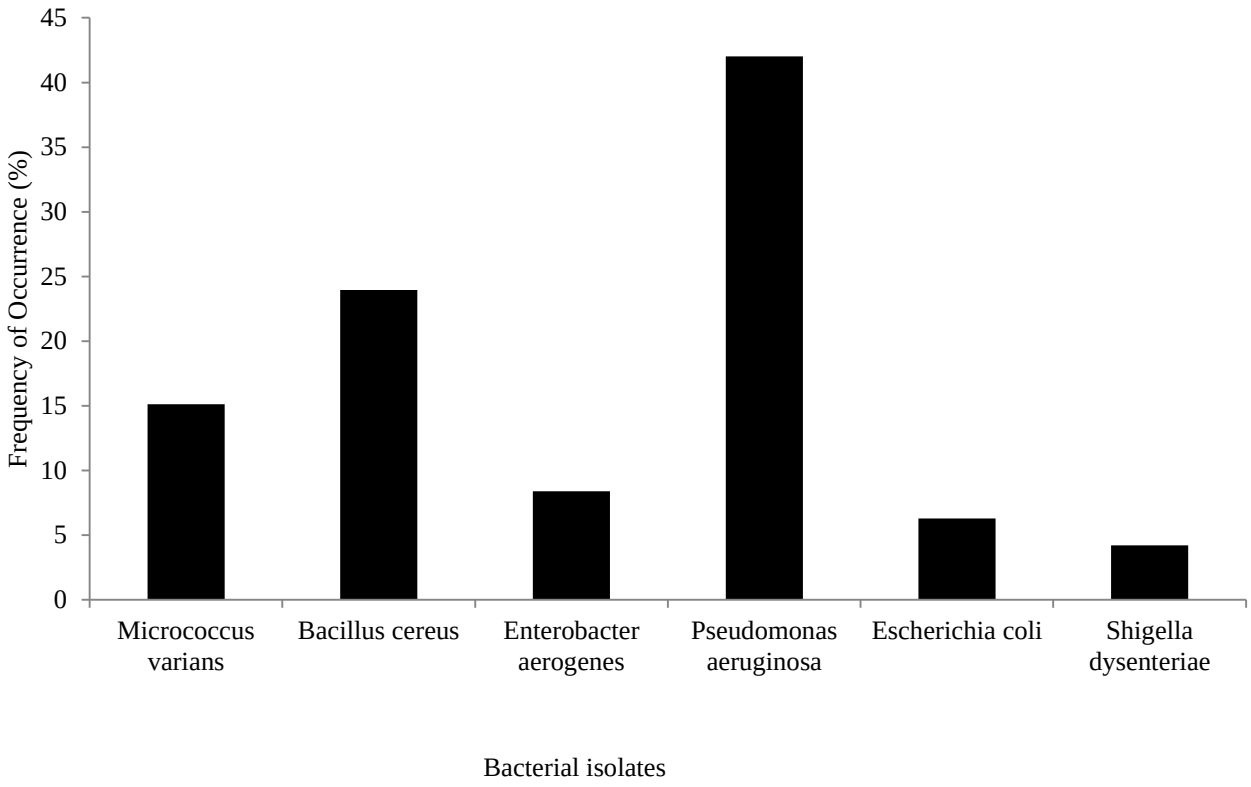

Figure-2: Frequency of occurrence of the bacterial isolates in the stream water during the wet season.

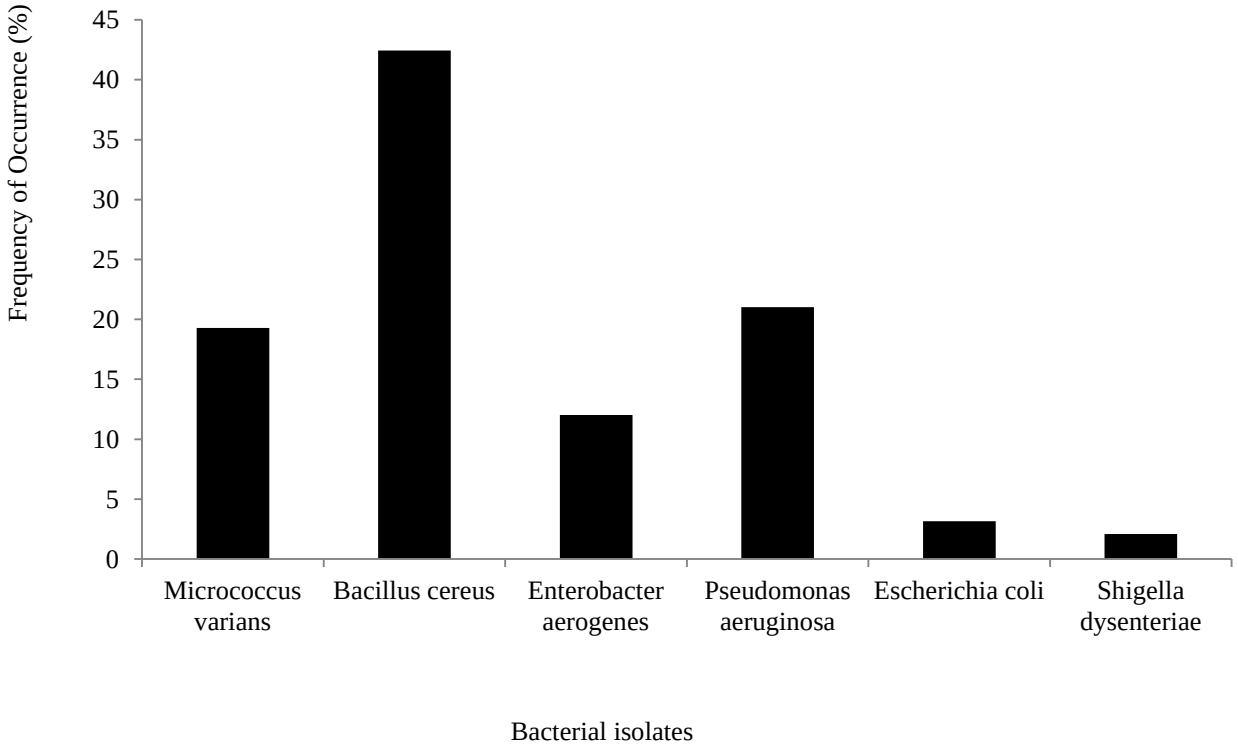

Figure 3: Frequency of occurrence of the bacterial isolates in the stream water during both seasons.

Discussion: The result of the bacteriological analysis during the dry season showed that the values for the total bacterial load ranged from $32x10^2$cfu/ml to $56x10^2$cfu/ml while the total coliform load ranged from 14MPN/100ml to 21 MPN/100ml. Faecal coliforms, Salmonellae, Shigellae, *Vibrio cholerae, Enterococcus faecalis and Clostridium perfringens* were not detected (Table-1). The total bacterial and coliform loads were above the WHO standard for drinking water. The result agreed with Ukpong and Peter[8] who reported that the total bacterial counts for drinking water in Ibeno Local Government Area of Akwa Ibom State were above the WHO standard and there were seasonal variations in the counts.

The total bacterial load during the wet season ranged from 102×10^2cfu/ml to $160x10^2$cfu/ml while the total coliform load and faecal coliforms load ranged from 20MPN/100ml to 43MPN/100ml and from 6MPN/100ml to 14MPN/100ml respectively. *Shigella*e load ranged from 0cfu/ml to 2cfu/ml (Table-2). The values for the wet season samples were also above the WHO limits for drinking water and agreed with the report of Krishna *et al*[9] who observed seasonal variations in the bacteriological characteristics of Kaveri River, India. Statistically, there were seasonal variations in the total bacterial, total coliforms and faecal coliforms loads during the dry and wet seasons.

The total bacterial load was more during the wet than the dry season. This may be attributed to rainwater that may have emptied its contents including organic materials and microorganisms into the stream. This result agreed with Omoigberale et al[10] and Akrong et al[11] that reported higher viable counts during the rainy season in the Ebutte river water samples they examined in Ehor community in Edo State, Nigeria and drinking water sources in communities surrounding Lake Bosomtwe in the Ashanti region of Ghana respectively. However, the result was at variance with Edokpayi et al[12] that reported higher levels of E.coli and Enterococci in the Nzhelele River, South Africa during the dry than the rainy season.

The average total bacterial load of most of the samples studied during both seasons was more than the WHO[13] standard for drinking water. There were counts for total coliforms, faecal coliforms and Shigellae (Table-3) as against WHO standard which stated that such organisms should be absent in any potable water, indicating that Iyifeyi stream is unsafe for drinking. These results agreed with Shitt*u et al*[14] who observed that the total coliform count of the drinking and swimming water samples in Abeokuta, Nigeria exceeded the EPA maximum contamination level of zero total coliform per 100ml of water. The high coliform load particularly *E.coli* obtained in the samples might be an indication that the water source was faecally contaminated. These results also conformed to Akubenyi *et al*[15] who in their assessment of major sources of water for domestic uses in Calabar metropolis, observed that most of the samples recorded total coliforms and faecal coliforms above the WHO limits.

The bacteriological analysis of the water samples during the dry and wet seasons revealed the presence of *Micrococcus varians, Bacillus cereus, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli* and *Shigella dysenteriae* (Table-4). The results showed that the dry season samples were free from *Pseudomonas aeruginosa, Escherichia coli* and *Shigella dysenteriae* (Table-5) while all the isolates were present in the samples during the wet season. These results agreed with Krishna *et al*[9] who also detected some of the bacteria isolated in this study; Akrong et al[11] that isolated *E. coli, Enterococcus, Pseudomonas, Salmonella and Aeromonas* from the drinking water sources they examined in Ghana; Edokpayi et al[12] that isolated *E. coli* and *Enterococci* in water samples from Nzehelele River, South Africa; Omoigberale et al[10] that isolated *E. coli, Klebsiella, Pseudomonas, Bacillus, Enterobacter, Streptococcus, Salmonella, Staphylococcus, Proteus, Clostridium* and *Shigella* in Ebute river in Ehor community, Edo State, Nigeria and Daramola et al[16] that isolated *Bacillus subtilis, Klebsiella pneumoniae* and *Proteus vulgaris* in Landzun stream, Bida, Nigeria; Obioma et al[17] who isolated *Bacillus spp, Shigella spp, Staphylococcus aureus, E.coli, Proteus spp, Pseudomonas spp, Streptococcus spp, Salmonella spp* and *Enterobacter spp* from water samples in Khana Local Government Area of Rivers State, Niger Delta, Nigeria; Onyango et al[18] who detected total coliforms, E.coli, Staphylococcus aureus, Clostridium perfringens and cysts in the water sources in Isiolo County in Kenya and Egberongbe et al[19] that isolated *Proteus, Pseudomonas aeruginosa, E.coli, Salmonella spp, Klebsiella pneumoniae, Campylobacter sp, Staphylococcus aureus* and *Enterococcus faecalis* from stream water in the rural areas of Ijebu North Local Government Area of Ogun State, Nigeria.

These bacteria are important human pathogens associated with a variety of diseases such as gastroenteritis, dysentery, cholera and urinary tract infections[20,21]. Their presence indicated that the water source was not potable and raises serious public health issues. Their entry into the water could be through deliberate and indiscriminate deposition of animal waste and human faeces into the stream. *Enterobacter aerogenes* isolated from the water samples are examples of non-faecal coliforms that could be found on vegetation and in the soil, which could serve as sources through which the pathogens can enter the water. The presence of *E. coli* in water gives an indication of faecal contamination by warm blooded animals. Their occurrence may indicate the presence of other pathogens responsible for infectious diseases such as cholera, gastroenteristis, dysentery and typhoid fever after ingestion of contaminated water[15,22.]

The isolates occurred less frequently in the dry season (Figure-2). This may be as a result of inadequate nutrients required for microbial growth. The isolates however occurred more frequently during the wet season and this may be as a result of availability of organic nutrients due to inflow of such substances into the body of water (Figure-3). *Bacillus cereus* (42%) occurred more frequently during both seasons.

This might be as a result of the availability of nutrients required for its growth in both dry and wet seasons (Figure-4), its ability to produce spores that can withstand adverse environmental conditions and its ability to produce antibiotics that may be inhibitory to other bacteria. The results agreed with Ukpong and Peter[8] who studied the bacteriological characteristics of drinking water in Ibeno Local Government Area of Akwa Ibom State and reported *Bacillus sp* as the predominant bacterium during both the dry and wet seasons. However, Ouma et al[23] studied the seasonal variation of the physicochemical and bacteriological quality of water from five rural catchment Areas of Lake Victoria Basin in Kenya and reported the presence of *E. coli* (69.6%), *Salmonella spp* (18.5%), *Shigella spp* (6.5%) and *Vibrio cholerae* (5.4%) in the samples examined. The antibiotics sensitivity results revealed that all the isolates were resistant to Amikacin and Tetracycline while Pseudomonas aeruginosa only was resistant to all the antibiotics used (Table 6). E.coli. and Shigella dysenteriae were sensitive to Chloramphenicol and Ciprofloxacin while Micrococcus varians and Enterobacter aerogenes were sensitive to Chloramphenicol only. In addition, Bacillus cereus was sensitive to Amoxicillin and Ciprofloxacin.

Conclusion

There were seasonal variations in total bacterial, total coliform and faecal coliform loads. The bacteriological quality of the stream water during both seasons is unacceptable since the samples yielded significant growth of total bacteria and coliforms including faecal coliforms. The water is therefore not potable. Treatment methods such as passage through sand bed filters, boiling, exposure to ultraviolet radiation, sedimentation, chlorination and ozonation as well as frequent bacteriological analysis of the water from the stream is also recommended.

Recommendations: Control of human activities to prevent sewage from entering the stream is the key to avoiding bacterial contamination of the drinking water source. Government should organize a program to educate the populace on the proper disposal of refuse and treatment of sewage before discharge into a water body. Government and non-governmental organizations should also render assistance by providing potable water supply to Ugwobi Abbi community.

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