Evaluation of the Seasonal Variation of Bacteria and Fungi from Rivers and Lake

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ABSTRACT

Aquatic ecosystems, encompassing rivers and lakes, serve as critical habitats supporting diverse microbial communities crucial for ecosystem functioning and human well-being. Seasonal changes in certain environmental factors greatly influence the microbial communities in these aquatic ecosystems. The aim of this research was to evaluate the seasonal variation of bacteria and fungi isolated from rivers and lakes. A total of forty-two water samples were collected in triplicates from seven (7) water bodies randomly selected from each town representing the seven (7) Local Government Area in Anambra Central Senatorial District. Bacteria and fungi were isolated from these samples using standard isolation techniques. The isolates were identified phenotypically through morphological, microscopic and biochemical characteristics. All the generated data were statistically analysed using SPSS software version 22. A total of two hundred and sixty-six (266) isolates were recovered from the samples. Bacteria isolates represent 241(90.60%) of the total number of microbial isolates compared to the fungi isolates 25(9.40%). The characteristics of the studied bacteria suggests the identities of the bacteria to be Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiellapneumoniae and Salmonella Typhi. The characteristics of the fungi colonies represent that of Aspergillusniger, Aspergillusflavus, Candida albicansetc. Our data demonstrated a seasonal variation in the occurrence of bacteria across all sample location with high percentage occurrence recorded for rainy season than dry season although the observed variation was not statistically significant at p-value > 0.05. Various seasonal dependent socioeconomic activities could lead to the depletion of water quality, and thereafter changes the seasonal ecology of the rivers.

KEYWORDS: *Aquatic ecosystems, seasonal variation, rainy season, dry season*

Introduction

Water covers approximately 71% of the Earth's surface, with continents and islands accounting for the remaining 29% (Victor-Aduloju et al., 2023; Agu et al., 2023).Much of the earth's surface is occupied by water, and understanding the seasonal variation of bacteria and fungi populations in rivers and lakes is crucial for assessing water quality, ecosystem health, and potential risks to human and environmental health (Agu*et al.*, 2014; Agu*et al*., 2017;Agu and Odibo, 2021). Bacteria and fungi play significant roles in nutrient cycling, decomposition, and overall ecosystem functioning within aquatic environments. Moreover, changes in their abundance and diversity across seasons can reflect shifts in environmental conditions, such as temperature, precipitation, and nutrient availability.

Seasonal variations in microbial communities have been extensively studied in freshwater ecosystems worldwide. These studies have highlighted the dynamic nature of microbial populations, with fluctuations occurring in response to seasonal changes in environmental parameters. Factors such as temperature, sunlight exposure, water flow, and nutrient inputs influence microbial growth, metabolism, and community composition over time.

Several recent studies have investigated the seasonal variation of bacteria and fungi in freshwater ecosystems, providing valuable insights into microbial ecology and environmental health. For instance, a study by Smith et al. (2021) examined the seasonal dynamics of bacterial communities in a river system, revealing distinct shifts in community composition and diversity between wet and dry seasons. Similarly, Jones et al. (2022) investigated the

seasonal variation of fungal populations in a lake ecosystem, highlighting the influence of temperature and nutrient availability on fungal abundance and diversity over the course of a year.

Understanding the seasonal dynamics of bacteria and fungi in rivers and lakes has important implications for water resource management and conservation efforts. Monitoring changes in microbial communities can serve as an early warning system for detecting pollution events, assessing the impact of human activities on aquatic ecosystems, and guiding management strategies to protect water quality and biodiversity.

MATERIALS AND METHODOLOGY

Study Area

Anambra State of Nigeria is one of the 36 States of Nigeria. Located in the South-Eastern parts of the Country, it is situated between Latitudes 5° 32' and 6°45'N and Longitude 6°43' and 7° 22'E respectively. With an estimated land area of 4,865sqkm or 486,500ha, the State is varied in terms of topography, population distribution and regional development (Ezenwaji*et al.*, 2014). Its State capital, Awka is an emergent urban area which is about 440kms from Abuja, the Federal Capital in central part of Nigeria and about 65kms to Enugu the old Eastern Nigerian regional capital. According to the National Population Commission (2010), the State had a population of 2,796,475 in 1991, but rose to 4,182,032 in 2006 and 4,461,942 in 2011.

Study Design

One (1) river was randomly selected from one (1) town in each of the seven (7) Local Government Areas in Anambra Central Senatorial District. They include: Awka South (Nibo), Awka North (Amansea), Anaocha (Agulu), Dunukofia (Ukpo), Njikoka (Enugwu-ukwu), Idemili North (Eziowelle) and Idemili South (Nnobi).

Sample collection

One hundred and fifty centiliter (150cl) of water was collected from each sample point from about 10cm from the river surface from three sample points of each river sampled in each town representing the Local Government Area (Fig. 3.1.) (Amiteye*et al*., 2019). Same sampling was done at both seasons (Rainy and Dry), giving a total of 42 samples collected. The names of the rivers and their location are: Obizi river (Nibo), Ezu river (Amansea), Agulu Lake (Agulu), Ndibe (Ukpo), OnuNgene River (Enugwu-ukwu), Ukwuakpu River (Eziowelle) and MmiliMgbo (Nnobi). These samples were collected and transported to the Laboratory immediately for microbial examination.

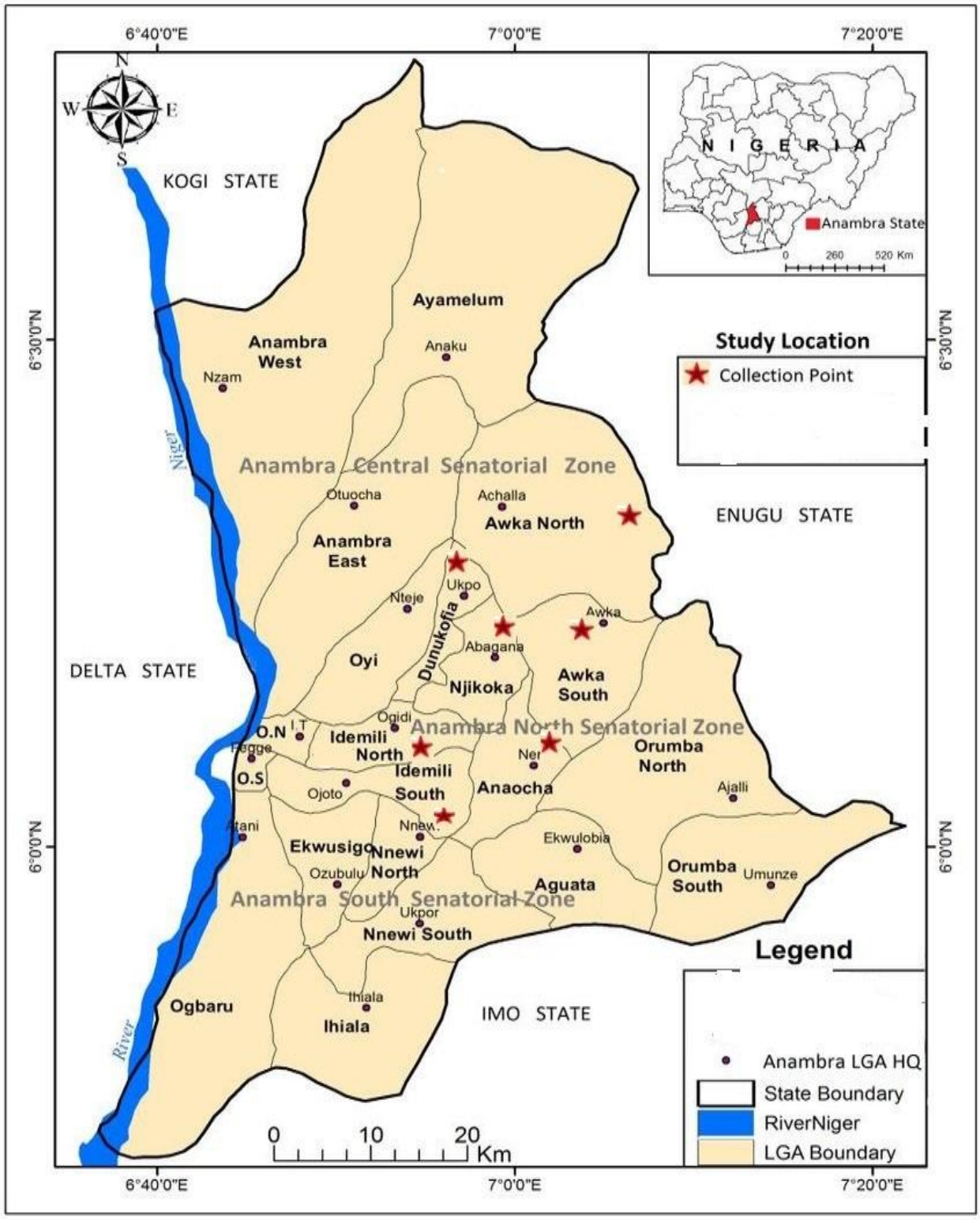

Fig. 3.1: Map showing study location (Source: Okeke and Anukwonke, 2016)

Preparation of media

All the culture media (Nutrient agar, Potato Dextrose agar and Muller Hinton Agar) were prepared according to the manufacturer's instruction and sterilized in an autoclave at 121ºC at 15Psi pressure for 15 minutes. Then, allowed to cool at warm touch before they were dispensed into Petri dishes.

Microbial Isolation

The 50ml of each water samples collected was inoculated into 50ml of sterilized peptone water in conical flask and incubated for 24hours at 37ºC to maintain the viability of the microbes in the sample. After which the water samples were serially diluted with sterile water to a 10- and 100-fold dilutions. Aliquots (0.1ml) from each water sample was spread onto respective agar plates for isolation of bacteria and fungi.

Potato Dextrose Agar (PDA) supplemented with *Chloramphenicol* was used for the isolation of fungi while Nutrient agar (NA) was used to isolate bacteria. The bacteria and fungi culture plates were incubated in an inverted position at 37^0C and 28 ± 2^0C respectively, till visual growth of culture is observed. All isolates were maintained on NA and PDA agar slants at 4 °C until use (Kumar *et al.,* 2012; Thiyagarajan *et al.,* 2014).

Microbial colonies that showed antagonism in the mixed culture plates were selected for further studies on antimicrobial activities. Antagonism was described as the appearance of inhibition zones around each individual colony (Ezekwuechee*t al.,* 2019).

Cultural Characterization

Culture plates of pure colonies of the isolate were examined for colonial appearances such as colony colour, surface, opacity, size, elevation and edges and of colonies.

Slide Culture for Fungal Identification

The microscopic morphology was accessed by the improved slide culture technique of Agu and Chidozie (2021). A sterile glass slide was placed on the bottom of a sterile petri dish. With the aid of a sterile 2 ml syringe, 0.5 ml of the molten Saboraud Dextrose Agar (SDA) maintained at 45 ^{O}C in a water bath was dispensed on the sterile glass slide. The cover of the petri dish was replaced and the molten agar allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus at the centre of the bump. Therefter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The plates were incubated at room temperature for 3 to 5 days depending on the growth rate of the fungus. When desired growth was observed, few drops of Lactophenol cotton blue stain was dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases

Grams Staining

The isolated bacteria were stained using Grams staining technique to differentiate between Gram positive bacteria and Gram-negative bacteria. The technique employed during staining process follows:

i. From pure culture of individual isolate (24hour old), a smear of a bacterial colony was made on a grease free slide,

ii. The slides were allowed to air dry and hit fixed over a Bunsen burner,

iii. Crystal violet stain was used to flood the slide for 1min., and afterwards rinsed with distilled water,

iv. Gram's Iodine, a mordant was used to flood the slide for 1min., and afterwards rinsed with distilled water,

v. Alcohol (75%) was applied onto the stained slides for 10sec, and rinsed with distilled water,

vi. Safranin, a counter stain was used to flood the slide for 1min., and afterwards rinsed with a distilled water.

vii. The stained slides were air dried and viewed under the microscope using x 100 lens with an immersion oil. The stain character and shape of the bacterial shape was recorded.

BIOCHEMICAL TESTS

The staining was followed by biochemical tests and culturing in selective and differential medium for suspected organisms. The tests employed in this study as described in Cheesbrough, 2002, are as follows;

Catalase test

The catalase test is used to differentiate *Staphylococci* (catalase-positive) from S*treptococci* (catalase-negative). The catalase test facilitates the detection of the enzyme catalase in bacteria. The procedure is as follows,

i. Using a sterile inoculating loop, a small amount of organism from a pure culture of 18-24hour old colony was collected and placed onto the microscope slide.

ii. Using a Pasteur pipette, a drop of 3% H_2O_2 was placed onto the organism on the microscope slide.

iii. Observation for immediate bubble formation was made and recorded as positive.

Citrate test

The test was used to differentiate Gram-negative bacteria on the basis of citrate utilization. It is useful in selecting organisms that uses citrate as its main carbon and energy source. Simmons citrate agar was prepared according to manufacturer's instructions. Five milliliter (5ml) of completely dissolved Simmons citrate agar was dispensed into bijou bottles and sterilized at 121^0C for 15mins. The bijou bottles were allowed to cool in a slant mode to form an agar slant. Test bacterial colonies were picked from a pure culture and inoculated into the slope of the agar after which it was incubated at 37^oC for 48hours. A green to blue color change of in the medium after 24hours incubation was recorded as positive.

Coagulase Test

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) which produce the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative) which do not produce coagulase.

Procedure (Slide Test)

A drop of saline was placed on each end of a slide. Then, using a wire loop, a portion of the isolated colony from a fresh pure culture was emulsified in each drop to make two thick suspensions. A drop of human plasma was added to one of the suspensions, and mixed gently. Clumping of the organism was looked out for within 10 seconds. No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping (Cheesbrough, 2002). The presence of clumps was indicative of *Staphylococcus aureus*.

MacConkey Agar

MacConkey agar is a selective and differential plating medium used for the isolation of Gram-negative enteric bacteria. It also aids in the differentiation of lactose fermenting bacteria (E.g: *Escherichia coli*) from non-lactose fermenting bacteria (E.g: *Salmonella*).

Test Procedure:

Appropriate quantity of MacConkey agar was weighed out and dissolved in required volume of water according to manufacturer's instructions. It was sterilized at 121^0C for 15mins after which it was aseptically poured into sterile Petri dishes and allowed to gel. Test organisms were directly inoculated onto the surface of agar plate. It was incubated aerobically at 37°C. Observation for specific cultural characteristics and color changes were made after 18-24 hours of incubation.

Interpretations

• Organisms that utilize the lactose are red/pink. Example: *Escherichia coli* while those that do not ferment lactose are colourless. Example: *Salmonella* species

Sugar Fermentation Test

One percent of glucose, lactose, maltose and sucrose were prepared by weighing out 1g of each sugar and dissolving in 100ml of peptone water separately. Few drops of phenol red were added to the individual liquid medium containing 1% of the sugars to be fermented. Nine mililitre each of the medium was introduced into a test tube. A Durham tube was introduced in an inverted position, sterilized at 121^0C for 10mins and allowed to cool. From a pure culture, colonies of the test organisms were collected with a sterile wire loop and introduced into test tubes labeled according to the sugar it contains. After 24hrs of incubation at 37^0C, gas production by displaying fluid contained in the Durham tube and color changes from pink to yellow were observed and recorded for the individual organisms and sugar.

Mannitol Salt Test

Mannitol fermentation is used as a selective and differential test for the isolation and identification of *Staphylococcus* and *Micrococcus species*.

Procedure: A mannitol salt agar was prepared according to manufacturer's instruction in a conical flask and sterilized. An Approximate volume of 20ml of the sterilized medium was poured into sterile Petri dish under ascetic conditions and allowed to gel. From 18-24hrs old culture of the test organism, a pure colony was picked and streaked on the surface of the mannitol salt agar and incubated at 37^0C for 24hrs. Colour change was observed. A pink to yellow colour change around the colonies were recorded as positive to mannitol salt fermentation.

Motility test

This test was done to determine the organisms that are motile or non-motile. This was done using the hanging drop method. A loopful of the suspension (incubated inoculated nutrient broth) was placed on a cover slip and inverting it over the cavity slide with petroleum jelly applied on it, it was then examined microscopically using 40X objectives (Cheesbrough, 2002).

Oxidase

The oxidase test was used to determine if an organism possesses the cytochrome oxidase enzyme. This test detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in the bacteria and a redox dye- tetramethyl-p-phenylene-diamine. The dye is reduced to deep purple color. This test assists in the identification of *Pseudomonas, Neisseria, Alcaligenes, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella*, all of which produce the enzyme cytochrome oxidase.

Procedure

Oxidase test was performed using Filter Paper Test Method. A small piece of filter paper was soaked in 1% Kovacs oxidase reagent and allowed to dry. A well isolated colony from a fresh (18 - 24hour culture) bacteria plate was picked using a wire loop and rubbed onto the treated filter paper. This was observed for color changes. Microorganisms are oxidase positive when the color changes to dark purple within 5 -10 seconds. Those that are delayed oxidase positive will change to purple within 60-90 seconds. Microorganisms are oxidase negative if the colour does not change or it takes longer than 2 minutes.

Indole test

This was carried out using the method described in the Practical Manual by Kanika, (2011). Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria like (*E. coli, P. vulgaris* and *P. rettgeri*,) that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0ml of deionized water. Ten milliliters of peptone water were dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and incubated at 37˚C for 48 hours. Five drops of KOVAC's reagent were carefully layered onto the top of the freshly incubated culture. The presence of indole was revealed by the formation of red layer colorations on the top of the broth culture.

Identification of Isolate

Identification of the bacteria isolate was done using ABIS (Advanced Bacteria identification software) online software (https://www.tgw1916.net/bacteria_logare_desktop.html).

Pure cultures of common fungi were tentatively identified by comparing the characteristic features of fungi described in Atlas of Fungi accessed online (https://universe84a.com/atlas-of-fungi/). The isolated fungal strains were identified at genus level on the basis of macroscopic characteristics like color, colony, morphology, shape, texture, diameter, and appearance of colony and by microscopic characteristics like mycelium, presence of specific reproductive structures, structure and shape of conidia and presence of mycelium. The microscopic morphology was accessed by viewing the Lactophenol cotton blue stained slides under the microscope using x40 objective lens.

3.10 Data Analysis

Data obtained were organized, analyzed and presented using statistical software; SPSS (Statistical Package for Social Sciences) version 22 and Microsoft Excel version 2016. Seasonal variations were determined using Chi-square (χ^2). Values of $P<0.05$ were considered statistically significant.

RESULTS

Forty – two (42) water samples randomly collected from different water bodies (rivers and lake) in Awka Central Senatorial District revealed the presence of bacteria and fungi colonies. The cultural, microscopic and biochemical characteristics of both the bacteria and fungi isolates are presented in Tables 4.1 and 4.2 respectively. The characteristics suggests the identities of the bacteria isolates to be *E. coli, Enterobacter* spp., *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Shigella* spp., *Serratia* spp., *Proteus* spp. and *Salmonella* spp. The characteristics of the fungi colonies represent that of *Aspergillus niger*, *Aspergillus flavus, Geotrichum* spp., *Candida* spp., *Rhizopus* spp. and *Penicillium* spp.

A total of two hundred and sixty-six isolates were recovered from the samples. The distribution of these isolates with respect to culture type and sample locations are shown in Figures 4.1 and 4.2. Bacteria isolates represent 90.60% of the total number of microbial isolates compared to the fungi isolates (9.40%). *Bacillus* spp and *Proteus* spp. are shown to be the predominantly occurring bacteria across the sample locations, with Nibo and Enugwu-ukwu recording the highest prevalence of this species. *Pseudomonas* spp and *Shigella*spp. are the least occurring bacteria with low prevalence (below 10%) across all sample locations except in Nnobi where *Pseudomonas* spp recorded a prevalence above 10%. The most occurring fungi in this study is *Rhizopus*spp, followed by *Aspergillusflavus* and *Aspergillusniger*. The result showed the absence of fungal growths in samples from Nibo and Eziowelle. Also, no fungi isolate except for Rhizopus was reported for samples from Nnobi.

Table 4.1: Morphological and Cultural Characteristics of Bacteria Isolates from Water Bodies

Isolates	Grams Reaction/shape	Cell arrangement	Appearances	Colony shape/ margin	Texture/ consistency	Pigmentation/odor	Opacity	catalase	Citrate	oxidase	Indole	glucose	Lactose	maltose	sucrose	Suspected Organism
DS1	- rod	singly	Milky on NA. Red on MCA	Round/ entire	Smooth and Shiny	None/odor present	Translucent	+	-	-	+	+G	+	-	+	***E. coli***
DS2	- rod	singly	Milky on NA Pinkish on MCA	Irregular/ lobate	Smooth and shiny	None/pungent smell	Opaque	-	+	-	-	+	+	+	+	***Enterobacter*spp**
DS3	- rod	Single short rods	White on NA Colorless on MCA	Irregular/ lobate	Smooth/ mucoid	green/pungent smell	Translucent	+	+	+	-	-	-	-	-	***Pseudomonas* spp**
DS4	+ rod	singly	White on NA	Irregular/ undulate	Smooth/ Viscid	odorless	Opaque	+	+	-	-	+	-	-	-	***Bacillus* spp.**
DS5	+ cocci	Clustered	Milky on NA Yellow on MSA	circular/ entire	Smooth/ Viscid	odorless	Opaque	+	-	-	-	+	+	+	+	***Staphylococcus* spp**
DS7	- rod	singly	White on NA. Pinkish on MCA	Round/ entire	smooth/ mucoid	None/pungent smell	Opaque	-	+	-	-	+	+	+	+	***Klebsiella*spp**
DS8	- rod	pairs	Colorless on MCA	Round/ entire	Mucoid	None/odor present	Transparent	+	-	-	-	+	-	-	-	***Shigella*spp**
DS9	- rods	Singly	Purple on NA	Round/ entire	Smooth	None	Opaque	+	-	-	-	+	-	-	-	***Serratia* spp.**
DS11	- rods	Short singly rods	Colorless and swarming on NA	Irregular	Smooth	None/ odor present	translucent	+	+	-	-	+G	-	+	+	***Proteus* spp**
DS12	+ rods	Rods in chains	Milky on NA	Round/ lobate	Rough	none	Opaque	+	+	-	-	+	-	-	-	***Bacillus* spp**
DS16	- rod	Short single rods	Colorless	irregular	Smooth/ mucoid	none	Transparent	+	-	-	-	+G	-	-	-	***Salmonella* spp.**
RS3	+ rod	singly	White on NA	Irregular/ undulate	Smooth/ Viscid	odorless	Opaque	+	+	-	-	+	-	-	-	***Bacillus* spp.**
RS5	+ rod	singly	Grayish white on NA	Small circular/ entire	Rough	None/fruity smell	Opaque	+	+	+	-	+	+	+	-	***Bacillus* spp**
RS23	+ rod	singly	Milky on NA	Large circular/ entire	Rough	None	Opaque	-	-	+	-	+	+	+	-	***Bacillus* spp**

Key: + = Positive; - = Negative; G = Gas produced; NA = Nutrient Agar; spp = Specie; MCA = MacConkey Agar

MSA = Mannitol Salt Agar

Table 4.2: Cultural and Microscopic characteristics of fungi isolates from different water bodies

ISOLATE NO	COLOUR (R/F)	TEXTURE	Microscopy	Suspected Organism
1	Black / white	Mycelia (filamentous)	Conidial fungi, Septate, biseriate	*Aspergillus Niger*
2	Yellow to green/ White	Smooth, raised	Septate, biseriate with rough conidiophore	*Aspergillus flavus*
3	Black / white	Filamentous rough edge	Septate mycelium	*Geotrichum*spp
4	Yellow	Mucoid smooth	Blastoconidia singly/clusters, pseudohyphae	*Candida*spp
5	White/Milkfish yellow	Filamentous smooth	Non-septate sporangiophore	*Rhizopus*spp
6	Green / white	Filamentous	Conidiophore like brush, septate	*Penicillium*spp
7	Milky	Smooth	Blastoconidia singly/clusters, pseudohyphae	*Candida*spp

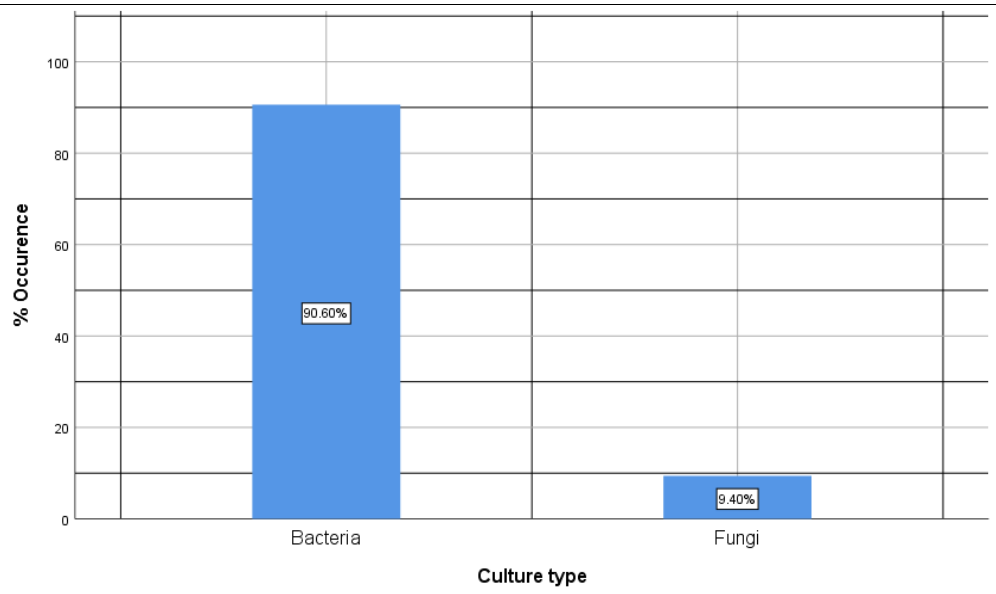

Figure 4.1: Microbial type occurrence

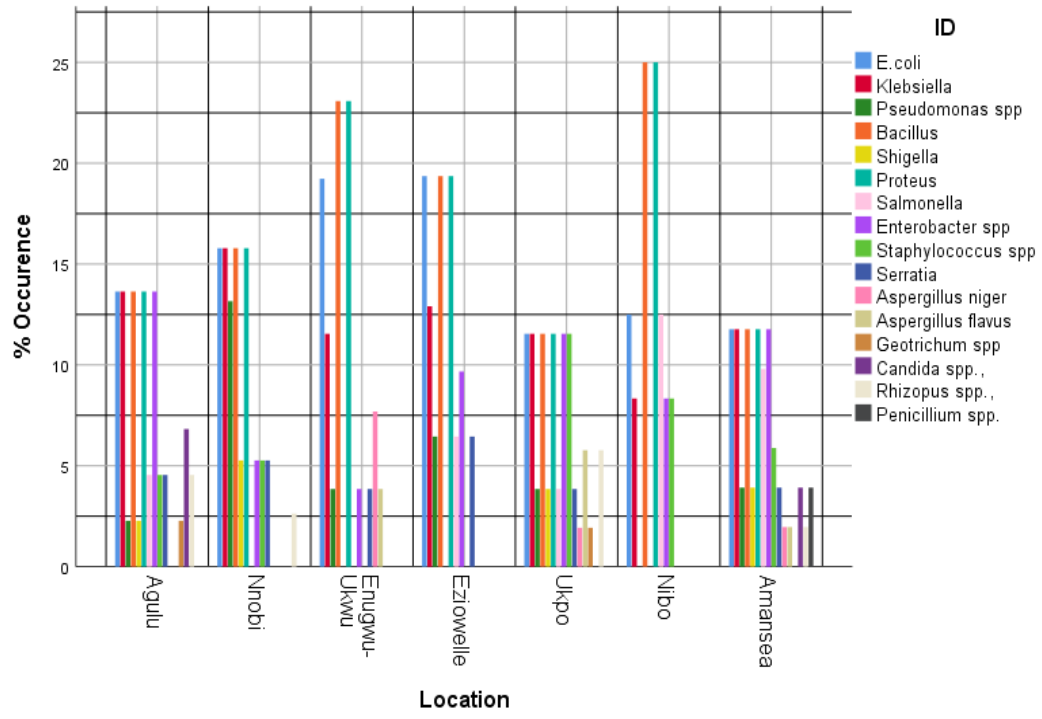

Fig. 4.2: Distribution of isolates across study sample sites

Table 4.3 shows the seasonal variation of isolates from different water bodies. No fungi were isolated during the dry season so no seasonal for fungi comparison could be done.

There was no observable difference in the occurrence of *Bacillus* and *Proteus* species in both seasons across all sample sites.

The occurrence of *E. coli* was higher in rainy season for all sample sites except for samples from Nnobi river with higher occurrence in dry season and Amansea river with no statistical difference in occurrence for both seasons.

There was no observable difference in the occurrence of *Klebsiella* species in both seasons across all sample sites except for Enugwu-ukwu and Nibo rivers with higher occurrence during rainy season.

The occurrence of *Pseudomonas* spp. was higher during rainy season for all sample sites except for samples from Eziowelleriver and Amansea river no statistical difference in occurrence for both seasons. Also, *Pseudomonas* spp. was not isolated in Nibo river.

The presence of *Shigella*sppwas not reported for samples from Enugwu-ukwu, Eziowelle and Nibo rivers. There was no difference in occurrence of *Shigella* for both seasons in samples from Nobi and Ukpo. A 100% occurrence was recorded for *shigella*spp in samples from Agulu and Amansea during dry season but none in rainy season.

The presence of *Salmonella* spp. was not reported for samples from Nnobi, and Enugwu- rivers. There was no difference in occurrence of Shigella for both seasons in samples from Eziowelle and Ukpo rivers. *Salmonella* spp. was isolated in samples from Agulu in dry season but not in rainy season. Samples from Nibo and Amansea had a higher occurrence of *Salmonella* spp. (66.7% and 60% respectively) during rainy season.

There was no observable difference in the occurrence of *Enterobacter* spp. during both seasons for samples from Agulu, Ukpo, Nnobi and Amansea rivers. Higher occurrences were recorded for samples from Enugwu-ukwu (100%), Eziowelle (66.7%), Nibo (100%) during rainy season.

There was no observable difference in the occurrence of *Staphylococcus* spp. during both seasons for samples from Agulu, Ukpo, Nibo rivers. There was no report for the presence of *Staphylococcus*spp in samples from Enugwu-ukwu and Eziowelle rivers. Higher occurrences (100%) were recorded for samples from Nnobi and Amansea rivers during dry season.

*Serratia*spp were more prevalent in rainy season for all the sample sites except in Agulu and Ukpo with no difference in occurrence. Serratia spp. was not isolated from Nibo river.

However, the seasonal variations observed across sample locations were not statistically significant at p-value > 0.05.

Table 4.3: Seasonal Variation of Microbial Isolates with respect to sample location.

		Season	
ID	Location	Dry N (%)	Rainy N (%)
E. coli.	Agulu	2 (33.30)	4 (66.70)
	Nnobi	4 (66.70)	2 (33.30)
	Enugwu-ukwu	2 (40.00)	3 (60.00)
	Eziowelle	3 (50.00)	3 (50.00)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	0 (0.00)	3 (100.00)
	Amansea	3 (50.00)	3 (50.00)
	Total	17 (44.70)	21 (55.30)
	Chi-square (p value)	1.564(0.655)	
Klebsiella spp.	Agulu	3 (50.00)	3 (50.00)
	Nnobi	3 (50.00)	3 (50.00)
	Enugwu-ukwu	1 (33.30)	2 (66.70)
	Eziowelle	2 (50.00)	2 (50.00)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	0 (0.00)	2 (100.00)
	Amansea	3 (50.00)	3 (50.00)
	Total	15 (45.50)	18 (54.50)
	Chi-square (p value)	2.078(0.912)	
Pseudomonas spp.	Agulu	0 (0.00)	1 (100.00)
	Nnobi	2 (40.00)	3 (60.00)
	Enugwu-ukwu	0 (0.00)	1 (100.00)
	Eziowelle	1 (50.00)	1 (50.00)
	Ukpo	0 (0.00)	2 (100.00)
	Nibo	0 (0.00)	0 (0.00)
	Amansea	1 (50.00)	1 (50.00)
	Total	4 (30.80)	9 (69.20)
	Chi-square (p value)	2.672(0.750)	
Bacillus spp.	Agulu	3 (50.00)	3 (50.00)
	Nnobi	3 (50.00)	3 (50.00)
	Enugwu-ukwu	3 (50.00)	3 (50.00)
	Eziowelle	3 (50.00)	3 (50.00)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	3 (50.00)	3 (50.00)

	Amansea	3 (50.00)	3 (50.00)
	Total	21 (50.00)	21 (50.00)
	Chi-square (p value)	0(1)	
Shigella spp.	Agulu	1 (100.00)	0 (0.00)
	Nnobi	1 (50.00)	1 (50.00)
	Enugwu-ukwu	0 (0.00)	0 (0.00)
	Eziowelle	0 (0.00)	0 (0.00)
	Ukpo	1 (50.00)	1 (50.00)
	Nibo	0 (0.00)	0 (0.00)
	Amansea	2 (100.00)	0 (0.00)
	Total	5 (71.40)	2 (28.60)
	Chi-square (p value)	2.100(0.555)	
Proteus spp.	Agulu	3 (50.00)	3 (50.00)
	Nnobi	3 (50.00)	3 (50.00)
	Enugwu-ukwu	3 (50.00)	3 (50.00)
	Eziowelle	3 (50.00)	3 (50.00)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	3 (50.00)	3 (50.00)
	Amansea	3 (50.00)	3 (50.00)
	Total	21 (50.00)	21 (50.00)
	Chi-square (p value)	0(1)	
Salmonella spp.	Agulu	2 (100.00)	0 (0.00)
	Nnobi	0 (0.00)	0 (0.00)
	Enugwu-ukwu	0 (0.00)	0 (0.00)
	Eziowelle	1 (50.00)	1 (50.00)
	Ukpo	1 (50.00)	1 (50.00)
	Nibo	1 (33.30)	2 (66.70)
	Amansea	2 (40.00)	3 (60.00)
	Total	7 (50.00)	7 (50.00)
	Chi-square (p value)	0(1)	
Enterobacter spp.	Agulu	3 (50.00)	3 (50.00)
	Nnobi	1 (50.00)	1 (50.00)
	Enugwu-ukwu	0 (0.00)	1 (100.00)
	Eziowelle	1 (33.30)	2 (66.70)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	0 (0.00)	2 (100.00)
	Amansea	3 (50.00)	3 (50.00)
	Total	11 (42.30)	15 (57.70)
	Chi-square (p value)	2.784(0.835)	
Staphylococcus spp.	Agulu	1 (50.00)	1 (50.00)
	Nnobi	2 (100.00)	0 (0.00)
	Enugwu-ukwu	0 (0.00)	0 (0.00)

	Eziowelle	0 (0.00)	0 (0.00)
	Ukpo	3 (50.00)	3 (50.00)
	Nibo	1 (50.00)	1 (50.00)
	Amansea	3 (100.00)	0 (0.00)
	Total	10 (66.70)	5 (33.30)
	Chi-square (p value)	3.750(0.441)	
Serratia spp.	Agulu	1 (50.00)	1 (50.00)
	Nnobi	0 (0.00)	2 (100.00)
	Enugwu-ukwu	0 (0.00)	1 (100.00)
	Eziowelle	0 (0.00)	2 (100.00)
	Ukpo	1 (50.00)	1 (50.00)
	Nibo	0 (0.00)	0 (0.00)
	Amansea	0 (0.00)	2 (100.00)
	Total	2 (18.20)	9 (81.80)
	Chi-square (p value)	4.278(0.510)	

Key: *E. coli* = *Escherichia coli* spp = specie N = Total number of isolates

DISCUSSION

The aquatic environment especially the rivers and lake within the State of Anambra, Nigeria have been such that serves as avenue for different activities ranging from domestic to religious, agricultural, and flood. Thus, its ability to encourage the proliferation of microorganisms.

In this study, the results established the presence of both bacteria and fungi in varying degrees. The frequency of occurrence of bacteria isolates was significantly higher than the fungi isolate in all the sample locations. This is consistent with the recent report suggesting that bacteria greatly exceeded fungi numerically in all habitat including aquatic habitat (Bahram *et al.,* 2021). Also, Dai and his colleagues reported that bacteria were significantly more abundant than fungi in both sediment and water, in all their sampling areas (Dai *et al.,* 2021). Bacteria, are some of the smallest and oldest organisms on the planet, are prevalent in all water systems and practically every environment, present in the millions per milliliter (mL), and in the hundreds of millions per milliliter in waterways resulting to contamination and threat to great public health (Wang *et al.,* 2019). As part of the biofilm, bacteria find their way to surface water samples, through decaying matter (such as dead wood or leaves), or covering on surface of rocks, stones, and sand grains (the slippery coating on hard surfaces in rivers). The presence of fungi in aquatic environment play a crucial role in the decomposition of plant matter in aquatic systems, as they are among the only creatures capable of decomposing plant structural components such as cellulose and lignin (Osono*et al.,* 2021).

Our investigation revealed the presence of *E. coli*, *Enterobacter* spp., *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Shigella* spp., *Serratia* spp., *Proteus* spp. and *Salmonella* spp. across the water samples from study sites. These organisms have been implicated in samples of aquatic source in Southern Nigeria. Ogbonna and Inana reported the presence of *Escherichia coli*, *Pseudomonas putida*, *Salmonella* spp, *Shigella*spp, *Staphylococcus aureus*, *Enterobacter*sppand *Enterococcus faecalis* from fishes a potential source of contamination in water samples from Port Harcourt Rivers State Nigeria (Ogbonna and Inana 2018). Similarly, Oku and Amakoromo, 2013, reported the presence of these organisms from samples emanating from fresh water. In Anambra State, similar bacteria species have been reported to be present in water samples in Otuochariver (Amuneke*et al.,* 2020).*Bacillus* spp and *Proteus* spp. among others bacteria isolated in this study are shown to be the predominantly occurring bacteria across the sample locations.

The findings of this research showed a low frequency of fungi compared to reports of other researchers suggesting a higher frequency of fungi in surface water samples (Al-gabr*et al.,* 2014). Al-gabr and colleagues reported the presence of *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp., *Mucor* sp., and the most dominant being *Rhizopus* spp. In agreement to the findings of Al-gabr and colleagues, our finding suggested *Rhizopus* spp. as

the most dominant fungi specie, while on the contrary, we did not detect the presence of *Fusarium* spp., *Trichoderma* spp., *Mucor* spp. The presence of fungi in rivers has been associated with increased organic matter concentration (Pietryczuk*et al.,* 2018). We detected the presence of bacteria and species of fungi that are well-known for causing infectious diseases and the production of mycotoxins and therefore may directly cause human health problems.

Our data demonstrated a seasonal variation in the occurrence of bacteria across all sample location with high percentage occurrence recorded for rainy season than dry season. Although the observed variation was not statistically significant, our findings corresponds with the reports of other researchers that showed increased microbial occurrence in rivers during rainy season (Tornevi*et al*., 2014; Waithaka *et al.,* 2020). Many bacteria wash into rivers and streams from the surrounding land, and their numbers can grow substantially after a rainfall. Furthermore, majority of the Rivers under study were used for many worship rituals and various seasonal dependent socioeconomic activities, which could lead to the depletion of water quality, and thereafter changes the seasonal ecology of the rivers.

In conclusion, evaluating the seasonal variation of bacteria and fungi in rivers and lakes is essential for understanding ecosystem dynamics, assessing environmental health, and informing management decisions. Ongoing research using advanced analytical techniques continues to enhance our understanding of microbial ecology in freshwater environments and its significance for ecosystem function and human well-being.

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