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SEASONAL VARIATIONS ON THE MICROBIAL FLORA AND PHYSICOCHEMICAL PARAMETERS OF EZU RIVER, AWKA, ANAMBRA STATE, NIGERIA.

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Abstract

Water is necessary for life, but many people lack access to clean, safe drinking or safe recreational water and end up becoming victims of water pollution which might lead to the epidemics of water borne diseases. Investigation of the seasonal fluctuations in the microbial flora and a few physicochemical parameters of a nearby river in Awka, Anambra State, was the goal of this study. Standard bacteriological procedures including colony morphology, biochemical tests, and microscopy were used to actualize the aim and objectives of this study. The mean heterotrophic bacterial count during the dry season (October to March) was 6.5944 x 105cfu/ml, which was considerably lower (P-value 0.001) than the mean heterotrophic bacterial count during the rainy season of 12.7833 x 105cfu/ml (April – September). The bacteria species isolated included *Vibrio, Flavobacterium, Bacillus, Micrococcus, Yersinia, Pseudomonas, Escherichia, and Salmonella spp.* In the dry season, the mean heterotrophic fungal count was 28.00 x 105cfu/ml, which was considerably lower with P-√value 0.003 than the mean value of 57.166771 x 105cfu/ml in the wet season. *Fusarium sp., Mucor sp., Aspergillus sp., and Penicillium sp.* were among the fungi species isolated. In the

dry season, the yeast population had a mean of 12.6837 x 103 cfu/ml, and a mean of 24.5335 x 103 cfu/ml in the wet season with a P- value of 0.008(sig). *Saccharomyces and Candida spp*. were the yeasts isolated. However, both hydrocarbon utilizing bacteria and fungi were also isolated with highest numbers obtained at the midstream. These included *Aspergillus, Penicillium, Fusarium and Mucor spp.* while the hydrocarbon-utilizing bacteria were *Pseudomonas, Bacillus, Micrococcus, Klebsiella, Corynebacterium, and Flavobacterium* spp. However, the physicochemical parameters of the river investigated were its P^H, conductivity, temperature, and biochemical oxygen demand (BOD) in both dry and wet seasons. During the rainy season, there were changes in both the flora and the physicochemical factors with a notable spike at the midstream with reduced values during the dry season which may be the result of increased human activities, such as use of chemical fertilizers typical of modern farming and effluent from abattoir sited close to the river which may have an impact on the aquatic microbial load due to eutrophication through run-offs.

Keywords: Seasonal variations, microbial flora, physicochemical parameters aquatic.

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Introduction

The microorganisms associated with water include bacteria genera such as *Pseudomonas, Yersinia, Staphylococcus, Streptococcus, Salmonella, Corynebacterium, Lactobacillus, Agrobacterium, Alcaligenes* and *Acinetobacter species* etc. Some endospore formers include *Bacillus, Clostridium* and*Helibacterium species.* Bacteria present a wide variety of metabolic activities depending on the kind of energy which they use for growth, the source of carbon and the electron donors used for the growth (Lin *et al*, 2014). For instance, some heterotrophic microbes with the capability to process considerable amounts of organic matter can colonize microplastic particles (MP) or other solid hydrocarbon particles in aquatic ecosystems. Weather colonization of microorganisms on these particles will alter the ecological niche. The ecological role of particle-associated microbial communities is context-dependent. This implies that different

environments lead to substantial changes in biomass build-up with associated heterotrophic activities of particle biofilms (Arias-Andes *et al,* 2018).

When contaminants are introduced into an environment such as water, they bring about such problems as instability, disorder, harm, or discomfort in the ecosystem (Ferreira *et al,* 2015). Most water pollutions are caused by the discharge of sewage, industrial effluents or garbage into the rivers, streams, lakes, oceans, and groundwater. The existence of fecal coliform bacteria in the water body specifies that the water has been polluted with the fecal substance of warm-blooded animals. Therefore, the enumeration of fecal bacteria is important for basic as well as applied research in aquatic microbial ecology and the development of parameter-based technologies for drinking water quality assessment. The presence of fecal streptococci and *Klebsiella* also indicates the fecal pollution of water most importantly the presence of *Salmonella, Shigella* spp., and *Proteus* spp. are predominant bacterial flora associated with sewage according to Khan *et al*.(2021).This study was aimed at evaluating the effect of season on the microbial flora and the physicochemical properties of Ezu River in Awka, Anambra State, Nigeria.

METHODOLOGY

Study Area

The research was conducted in Amansea section of Ezu River in Awka, the Capital of Anambra State. Awka is located midway between two major cities, Enugu, and Onitsha in Northern Igboland. For this reason, it was chosen as the administrative center for the colonial authorities and now a base for the Anambra State Government. The river is located at longitude 6˚36˚.0'E and latitude 5˚42˚.0'N within the tropical rain forest belt along the Enugu Onitsha Expressway by Amansea town in Awka South Local Government Area of Anambra State. It covers a reasonable area in both East and North sides of both Enugu and Anambra States. It geographically serves as a boundary between the two States and serves a population of not less than 2.5 million. Ezu River is a semi-enclosed basin subject to pollution and receives a considerable quantity of wastes and effluents from abattoirs, agricultural practices, and wash off from women and children.

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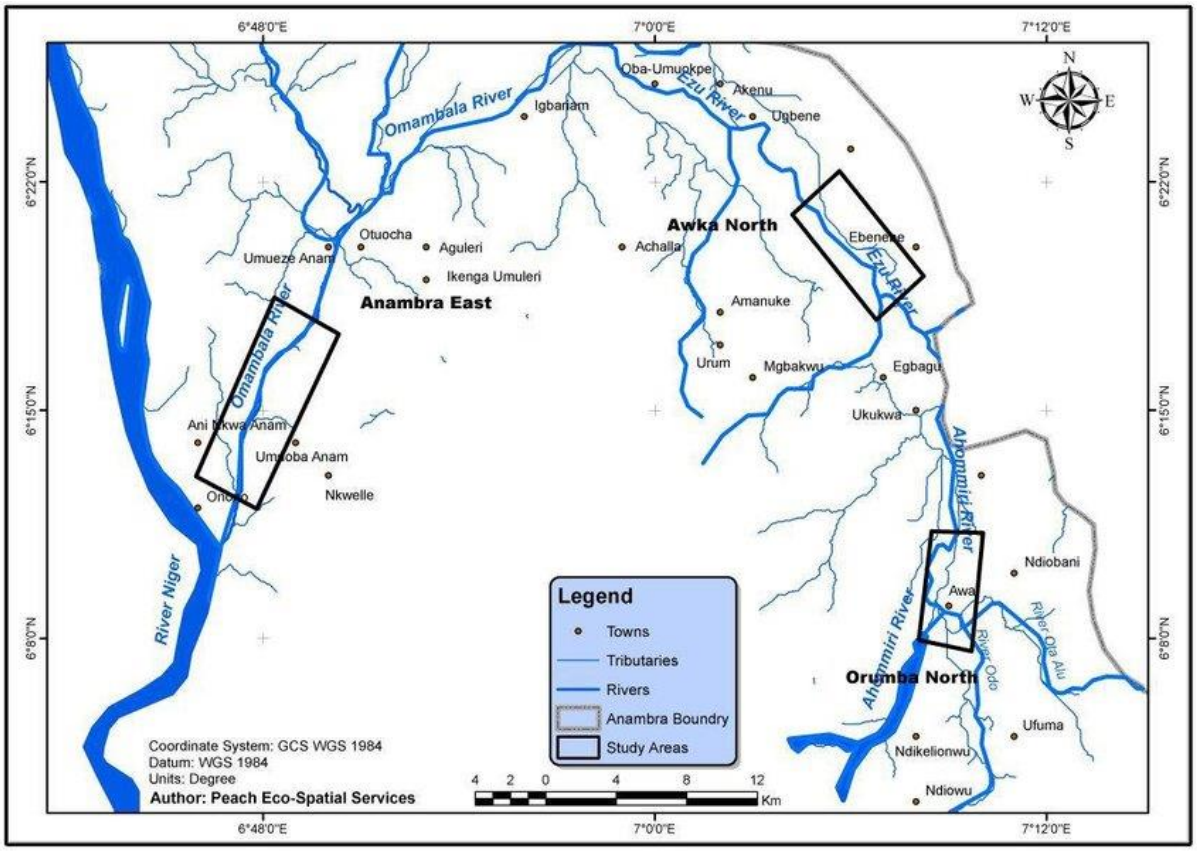

Fig.1:Map ofAnambra State showing the Sampling Location

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Sample Collection

In the course of a year, 144 different water samples were taken from the river. Every month, three samples were taken at the upstream, midstream, and downstream (approximately 120 meters apart) (about 115m away from the midstream). Using the Dida *et al*. (2015) approach, the samples were taken standing up from the riverbed. A clean, long-handled plastic dipper with a wide opening and a placement of 25 cm from the riverbed and 6 cm deep was used to collect the samples. The water samples were then put into sterile,

screw-capped vials, placed in ice-filled bags, and brought to the Microbiology Laboratory for analysis. By making a surface clearance, care was taken not to capture any floating organic debris. By clearing the surface film with the dipper's closed end, care was taken to avoid collecting any floatable organic materials.

Preparation of Culture Media for the Isolation of bacteria

Media used include Nutrient Agar, Saboraud Dextrose Agar (SDA) and McConkey Agar and peptone water. All were aseptically prepared in compliance with standard bacteriological procedures as described by Agada *et al*., (2022) and according to the manufacturer's instructions.

Isolation of Heterotrophic Bacteria

The heterotrophic bacteria were isolated using the pour plate method. According to Public Health England (2017). This was done aseptically to prevent contamination of the growing media. Following a ten (10)-fold serial dilution of the water samples, 0.1 ml of the diluents at a concentration of 10^4 was pipette in triplicate into sterile petri dishes. Molten plate count agar at 45 °C was then added to the petri dishes which were then swirled to mix and left to gel. The distinct colonies were then counted on the plates after 48 hours of incubation at 37°C. Pure cultures were prepared by sub culturing onto sterile nutrient agar, which were then kept in agar slants at 4°C for later use.

Coliforms

The river coliforms were isolated via serial dilutions of the water samples and 0.1ml of 10^4 dilution was introduced into sterile culture plates after which, sterile McConkey agar was poured into the plates, swirled gently to mix and then allowed to gel. This was then incubated at 37ºC for 24 hours.

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Hydrocarbon Utilizing Bacteria

However, a ten-fold serial dilution of regular saline was used to carry out the hydrocarbon-utilizing bacterial (HUB) count of the river. To create a 10-1 dilution, 1ml of diesel was pipette into test tubes containing 10ml of normal saline and 1ml from the stock was then transferred into the next test tube containing 9ml of normal saline. This was done up to number 103. The spread plate method, as provided by Apha (1998) and referenced by Abdallat *et al*. (2020), was used to inoculate aliquots (approximately 0.1 ml) of the diluents onto nutrient agar (NA) plates in triplicates. The plates were then incubated at 37 oC for 24 hours. For the bacteria that consume hydrocarbons, the count was done in triplicates on mineral salt agar (MSA) in accordance with the instructions provided by Mills *et al.* (1978) and adopted by Wokem e*t al.* (2017). The mineral salt agar (MSA) was created by dissolving 0.29g of KCl, 10g of NaCl, 0.42g of MgS04.7H20, 0.42g of NH4NO3, 1.25g of K2HPO4, 0.83g of KH2 PO4, and 15g of agar in one liter of distilled water. The mixture was then autoclaved at 121^0C for 15 minutes. Aliquots of 0.1 ml of diluent were used to inoculate onto MSA plates in triplicates using the spread plate method as demonstrated by APHA (1998) and adopted by Katya *et al.* (2019). Each plate's inner lid was aseptically covered with sterile filter papers (Whatman No. 1) that had been saturated with diesel. The plates were then inverted and incubated at 37^0C for 48 hours. During incubation, the only source of carbon and energy was the diesel; after that, the plates with colonial growths were counted.

Estimation of hydrocarbon utilizing Bacterial Biomass Using Optical Density

Mineral salts medium was prepared as described by *Mills et al. (1978)* and dispensed in 99ml quantities into thirty-five Erlynmeyer flasks. To each flask was added 1ml of diesel (1%) and autoclaved at 121^{o}C for 15 minutes and allowed to cool, after which, the bacterial isolates (2 loopfuls) were individually inoculated into the flasks which were then incubated under a static condition at 30^{o}C for 14 days. The optical density was

determined at 660nm wavelength at every 48 hours using a spectrophotometer (Unicam henos gamma and delta) while the pH determined using a pH meter (Jenwey 3510).

2.5 Isolation of Heterotrophic Fungi

For the isolation of this category of microorganisms, Isolation method described by Agada *et al*.,(2022) was used. 10-fold serial dilutions of the water samples were performed and from the 10^{-3} dilution, 0.1ml aliquot was pipette into triplicate sterile petri dishes and pour plates made with Sabouraud Dextrose Agar (SDA)fortified with 250mg/ml of chloramphenicol. On gelling, the plates were incubated at 25^{o}C for 72 hours. Discrete colonies were counted, sub-cultured and stored in slants for further use.

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Estimation of fungal biomass

The estimation of fungal biomass was done using the method of Amachukwu *et al*.(1998)where known volume of the broth in each flask (5ml) containing the fungus was passed through a filter paper whose weight with the crucible was already noted. The filter paper retained the fungal cells but allowed the passage of the medium. It was then transferred into the crucible and weighted. This was then dried in an oven at 70^{o}C until the weight became constant. The fungal biomass was obtained by the difference between the weight of the crucible and filter paper before filtration and their weight after drying.

$W_1 = (Co + Fo)$

$W_2 = Co + Fo + Lc$

Biomass $= (C+F+Lc) - (C+F) = \mathbf{W_2 - W_1}$

Where:

Co=Original weight of crucible

Fo=Original weight of filter paper

Lc= Weight of liquid culture

Isolation of Hydrocarbon Utilizing Fungi

Likewise, the fungal colonies that grew on the mineral salts medium fortified with chloramphenicolwere also sub-cultured onto Sabouraud dextrose agar (SDA) plates and then transferred onto SDA slants and stored at room temperature as described by Agada *et al*., (2021).

Estimation of Biomass Using Optical Density for Hydrocarbon-Utilizing Fungal Isolates

Mineral salts medium was prepared as described by Mills *et al*.(1978) and dispensed in 99ml quantities into thirty-five Erlynmeyer flasks. To each flask was added 1ml of diesel (1%) and autoclaved at 121^{o}C for 15 minutes and allowed to cool, after which, the fungal isolates (2 agar plugs of 1.6cm diameter) were individually inoculated into the flasks which were then incubated under a static condition at 30^{o}C for 14 days. The optical density was determined at 66onm wavelength at every 48 hours using a spectrophotometer (Unicam henos gamma and delta) while the pH determined using a pH meter (Jenwey 3510).

CHARATERIZATION AND IDENTIFICATION OF THE MICROBIAL ISOLATES

The suspected heterotrophic isolates were characterized using conventional biochemical tests as discussed below:

Gram Staining

The pure cultures stored in agar slants were sub-cultured onto nutrient agar plates and incubated for 24 hours after which the Gram staining procedure was carried out as described by Tripathi *et al*. (2020). The

glass slides were washed off using a wash bottle, blotted dry in the air and viewed under the microscope using oil immersion as also described by Altun *etal*. (2015).

Spore Test

This was carried out on the isolates after 24 hours of growth. A smear of the cultures was made on a slide, dried, and fixed with flame. The slide was then placed over a beaker of boiling water, with the smear (film) of the bacteria remaining on the topmost (face-up). 0.5% aqueous solution of malachite-green was used to flood the smear as large droplets condensed on the under-slide of the slide and left to act for one minute while the water continued to boil. The slide was then washed and counterstained with carbol fuchsin for 30 seconds. The slide was washed, dried, and viewed under the microscope. The presence of spores indicated a green colour stain while the negative bacilli stained red (Kent *et al*., 2016).

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The test organisms were grown using 0.5ml of glucose-phosphate-peptone water and incubated at 37ºC for 48 hours. Then five drops of methyl red were added and mixed. A positive result was indicated by the appearance of a bridge red colour while negative ones gave yellow/orange colour (Watterson *et al*., 2014).

Voges Proskauer Test

This was used in the differentiation of enterobacteria. The test organisms were inoculated unto gluclose – phosphate-peptone-water and incubated for 48hours at 37ºC as discussed by SMITA (2019).

One milliliter of 40% solution of potassium hydroxide and small amount of creatinine were also added. Then, 3ml of a 5.0% solution of α-naphtol in absolute ethanol were added. The acetoin produced from the fermentation of glucose was oxidized to diacetyl which formed a pink compound the creatinine in 2-5 minutes.

Motility Test

Sterile nutrient broth was inoculated with each of the bacterial isolates and incubated at 30^{o}C for 24 hours. A loopful of the broth culture was taken and dropped on the center of a clean grease – free cover slips and surrounded by a ring formed with a plasticine and turned over to obtain a hanging drop from the cover slip. The drop was then observed under the microscope for motility.

Catalase Test

A wire loop was used to transfer the test organism from the growth medium and into3 drops of3% hydrogen peroxide solution contained on a clean microscope glass slide. The production of bubbles indicated a positive result.

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Sugar Fermentation Test

The multiple tube fermentation technique has been implicated as a conventional way to detect coliform in water samples through the fermentation of lactose sugar with production of acid and gas. This technique was used to identify Gram-negative enteric bacteria present in the bacterial population of the river. It was carried out to determine the ability of the bacterial isolates to ferment sugars such as glucose/lactose using peptone water separately containing 1% of the sugar. Durham tubes were inverted into the nutrient broth with bromothymol blue as an indicator on cooling were inoculated with a loopful of the isolate. The sterile broth was inoculated on cooling and were incubated at 30^{o}C for 24 hours. Positive results were indicated by either acid production by colour change to yellow only or also with gas production in the Durham tubes.

Oxidase Test

A solution of tetramethyl-p-phenylene diamine dihydrochloride (1%) and used to soak Whatman No. 1 filter paper. The test organisms were collected using a glass rod and streaked on the presoaked filter paper. A positive result was indicated by a change to purple colour in 15 seconds.

Nitrate Reduction Test.

The test organisms were introduced into nitrate broth containing beef extract, 3g; peptone, 5g and potassium nitrate,1g per litre. This is used at a concentration of 0-9g in every 100ml of distilled water and incubated at 30^{o}C for 96hours after which, 0.1ml of sulfanilic acid mixed with α-naphthylamine was added to the nitrate broth culture and observed for colour change. Development of red colour within few minutes indicated the presence of nitrite showing nitrate reduction.

Indole Test.

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The test organisms were grown in peptone water containing tryptone for 3 days, after which, xylene (3 drops) was added and left for 30 minutes. This is followed by the addition of few drops of Kovac's reagent. Positive result was indicated by a red colour.

ISOLATION OF ENTERIC BACTERIA FROM EZU RIVER

Isolation and Identification of the Coliform Bacteria Present

Techniques such as multiple tube fermentation, plate counting, and membrane filtering were used for the identification and estimation of indicator organisms and other bacterial populations. The detection of the coliform index in water samples by presumptive, confirmatory, and finished tests were specifically applied

to multiple tube fermentation testing. The most probable number (MPN)was recorded as MPN/100 ml according to Balogun *et al.,* (2016) and served as the index value.

The coliform group was made up of gram-negative, non-spore-forming, facultatively anaerobic rod-shaped bacteria that fermented lactose with gas production as described by Rompré *et al*., (2002). In this study, a series of test tubes were filled with broth containing lactose and other nutrients, and these tubes were sterilized and inoculated with 1 ml sample in 10 ml medium. These tubes were incubated between at 37 °C, and gas production was monitored. The presumptive test, which was the initial step in the procedure, assumed the presence of coliforms in tubes with gas generation. Bacterial culture from positive tubes was injected on an Eosin Methylene Blue (EMB) agar plate for the confirmatory test. Purple colonies with black centers and green metallic sheen grew after being incubated for 24 hours at 37 °C and were used to identify *E. coli* bacterium.*Enterobacter aerogenes* exhibited pink colonies without a sheen in the finished test after a 24-hour incubation at 37 °C, and these colonies also produced gas in lactose broth [(APHA (1998), Himedia (2012), Dawangpa *et al*. (2021).

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THE PHYSICOCHEMICAL PROPERTIES OF EZU RIVER

Determination of The River Temperature (^{0}C)

The temperature of the river was taken. The spot chosen was moving with average depth of about 25cm.The thermometer was held out to calibrate to the ambient air temperature before it was submerged in the river and allowed to soak for1-minute. The river Temperatures were then taken from upstream, midstream and downstream for both seasons.

Determination of p^H

The determination of the hydrogen ion concentration of each sample was carried out using a pH tester model Jenwey 3510

Conductivity Measurement

The conductivity of all the samples collected all through the year was also measured using a conductivity meter, (Labtech model).

Biological Oxygen Demand (BOD)

The amount of oxygen consumed by the microbial flora (BOD) of the river while they decomposed organic matter under aerobic conditions at a specified temperature using the Winkler titration method as described by Mitchell *et al.* (1995) and as updated by Lamidi *et al*. (2019**)**. Biochemical oxygen demand is the amount of dissolved oxygen (DO) needed by aerobic organisms to decompose organic materials present in a water sample at a specific temperature for a specific amount of time (in this case 5days for BOD_5). BOD_5 is widely used as a stand-in for the level of organic pollution in water and is typically measured in milligrams (mg) of oxygen utilized per liter of the sample over a 5-day incubation period at 20°C without light.

BOD was determined using the formula $BOD = (DO_1 – DO_2)$ mg/l.

Where DO_1 represents the initial concentration of dissolved oxygen and DO_2 represents the concentration of dissolved oxygen at the end of incubation [Al-Bayatti *et al*., 2012].

Where: DO = Dissolved Oxygen

DO_1 = Initial DO (mg/l) in the sample

D_2 = DO (mg/ l) in the sample after 5 days

P = Decimal volume fraction used

Statistical Analysis

Data generated were analysed using the statistical package for social sciences (SPSS) software, version 21.0. One-way Analysis of Variance (ANOVA) was the statistical tool employed to determine if there was any significance among the variations and a p-value of $p < 0.05$ was considered statistically significant.

Results

For total heterotrophic bacteria, nutrient agar plates were used while MacConkey agar plates were used to isolate and count the Gram-negative enteric bacteria as described by Bhadra *et al.* (2003).

heterotrophic bacterial count of the river under study had a higher mean value of 12.78×10^4cfu/ml in the rainy season while a lesser mean value of 6.50×10^4 cfu/ml was obtained during the dry season with a p-value of 0.007 making the seasonal change in heterotrophic bacterial population significant.

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The population of heterotrophic bacteria in both dry and rainy seasons is represented in Table 1. There was a clear indication of high populations within and around May at the midstream with a decrease in population by February which was peak of dry season. The distribution is as illustrated in Fig. 1.

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Table 1: Heterotrophic bacterial count of Ezu River (x10^{4}cfu/ml)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	3.8	19.0	14.0	12.26667
	May	8.7	20.0	18.0	15.56667
	June	10.0	19.0	16.3	15.10
	July	12.0	18.0	14.0	14.66667
	August	5.6	9.7	7.0	7.43333
	September	10.0	14.0	11.0	11.66663
Dry season	October	6.4	10.5	10.0	8.966667
	November	1.7	10.0	8.5	6.73333
	December	3.6	8.3	7.0	6.3
	January	5.2	6.7	6.3	6.066667
	February	4.1	5.4	4.8	4.766667
	March	3.4	8.6	8.2	6.73333

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Season	Mean
Rainy	12.78
Dry	6.50
P-value	0.007

S Season	Mean	SD	Mean
R Rainy	12.78	12.939	12.78
DDry	6.50		6.50
P-P-value	0.007	Sig.	0.007

April – September = Rainy Season

March – October = Dry Season

The biochemical characterizationof the heterotrophic bacterial isolates is as shown in Table 2. The isolates include *Salmonella, Streptobacillus, Yersinia, Proteus, Micrococcus, Streptococcus, Pseudomonas, Escherichia coli, Vibrio, Flavobacterium, Klebsiella, Bacillus, Citrobacter, Enterobacter and Staphylococcus spp.*

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Table2:Biochemical Characterization and Identification of Heterotrophic Bacterial Isolates from Ezu River

	Gram Stain	Cell	Morphology	Catalase	Oxidase	Indole	Citrate	Motility	Spore test	Glucose	Maltose	Lactose	Voges –P	Methyl – red	Probable Identity
1	-	Rod		+	-	-	+	+	-	A	A/G	-	-	+	*Salmonella. Sp*
2	-	Rod		-	-	-	+	-	-	A	A	A	-		*Streptobacillus sp*
3	-	Rod		+	-	+	-	+	-	A	A	A	-	+	*YersEnteroinia sp*
4	-	Rod		+	+	+	+	+	-	-	-	A	-	-	*Pseudomonas sp*
5	-	Rod		+	-	+	-	+	-	A/G	+	A/G	-	+	*Escherichia*

														coli
6	-	Comma Rod	+	+	+	+	+	-	A	A	A	+	+	*Vibrio sp*
7	-	Rod	+	+	-	+	-	-	A	A	-	-	-	*Flavobacterius sp*
8	+	Rod	+	+	-	+	+	-	+	-	A/G	-	-	*Bacillus sp*
9	-	Rod	+	-	-	+	+	-	A/G	A/G	A/G	-	+	*Citrobacter*
10	+	cocci in cluster		-	+	-	+	-	A/G	A	A	+	+	
11	+	Cocci in chain	+	-	+	+	-	-	A	A	A	-	+	*Streptococcus sp*
12	-	Rod	+	-	+	+	+	-	A/G	A/G	A/G	+	+	*Proteus sp*
13	+	Cocci	+	-	-	+	+	-	A/G	A	A	+	+	*Micrococcus sp*
14	-	Rod	+	-	-	+	-	-	A/G	A/G	A/G	-	-	*Klebsiella sp*
15	-	Rod	+	-	-	+	+	-	A/G	A/G	A/G	+	-	*Enterobacter sp*
16	+	Cocci in clusters	+	-	-	-	+	-	A/G	A	A	+	+	*Staphylococcus sp*

+ = Positive to test: A = Positive with acid production

= Negative to test: A/G = Positive with acid and gas production

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The distribution of heterotrophic bacteria in both dry and rainy seasons is as represented in Fig.2. There was a clear indication of high populations within and around May at the midstream with a decrease in population by February which was peak of dry season.

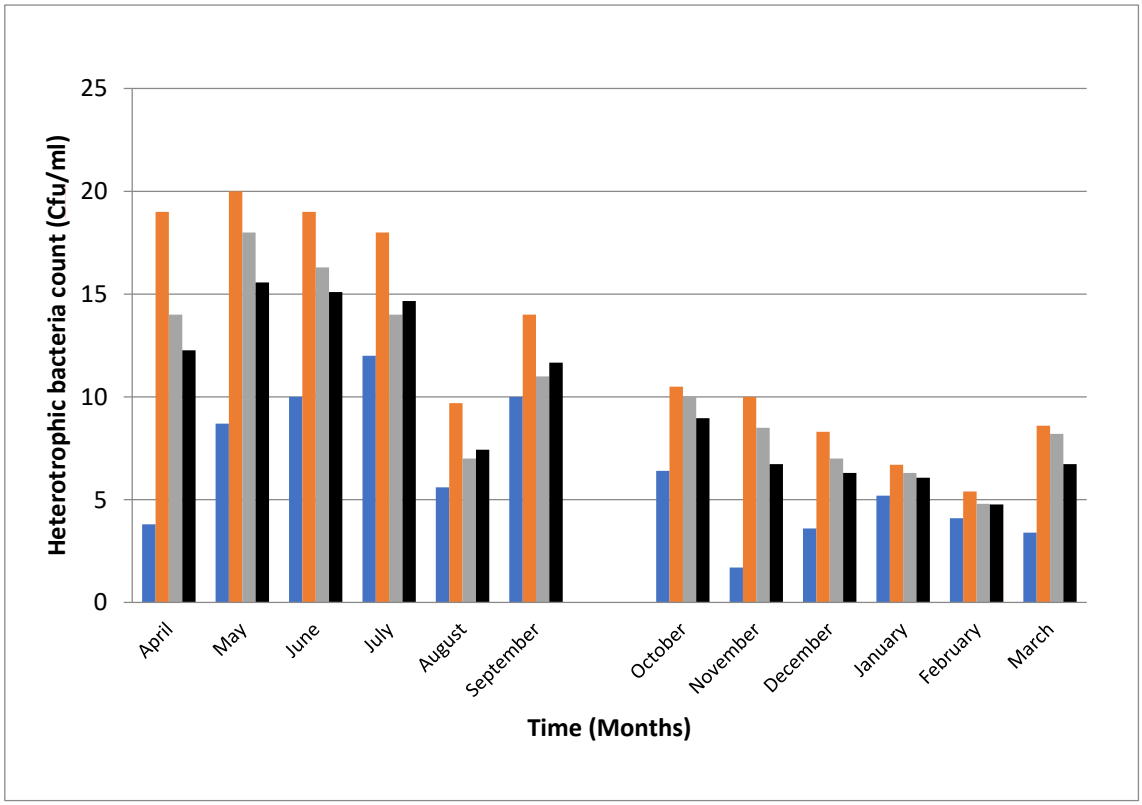

Fig. 1: The heterotrophic bacterial distribution of Ezu River (x 10^4 cfu/m)

Key:

- Upstream
- Midstream
- Downstream
- Means

The coliforms were also enumerated as shown in Table 3 with the highest mean value of 16.11 x 10^4 cfu/ml obtained during the rainy season as against 8.94 x 10^4 cfu/ml obtained during the dry season while the P- value was significant at of 0.037.

Table 3:Total Coliforms (x10^4 cfu/ml) of Ezu River

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	3.2	15	20	12.73333
	May	9.4	17.5	24	16.96667
	June	10	18	25	17.66667
	July	11	29.5	25	21.83333
	August	0.4	12.5	13.5	8.8
	September	10	27	19	18.66667
Dry season	October	9	25	22.5	18.83333
	November	2.8	5.5	12.5	6.933333
	December	3.6	9	12.5	8.366667
	January	2.2	2.5	4	2.9
	February	2.2	9.6	4	5.266667
	March	2.6	14	17.5	11.36667

Season	Mean	SD
Rainy	16.111	4.632
Dry	8.944	5.623
P. Value	0.037	sig.

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April – September = Rainy Season

October – March = Dry Season

Table 4 shows the results of the characterization and identification of the coliforms obtained from the river samples. The isolates include *Proteus sp Citrobacter sp,Klebsiella sp,Enterobacter sp* and *Escherichia coli* while their distribution along the river is as presented in Fig 2.

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Table 4: Characterization and identification of coliform isolates from Ezu River

	Gram Stain	Cell Morphology	Catalase	Oxidase	Indole	Citrate	Motility	Glucose	Maltose	Lactose	Voges –P	Methyl – red	Probable Identity
1	-	Rod	+	-	+	-	+	A/G	+	A/G	-	+	*Escherichia coli*
2	-	Rod	+	-	-	+	+	A/G	A/G	A/G	+	-	*Enterobacteria sp*
3	-	Rod	+	-	-	+	-	A/G	A/G	A/G	-	-	*Klebsiella sp*
4	-	Rod	+	-	-	+	+	A/G	A/G	A/G	-	+	*Citrobacter*
5	-	Rod	+	-	+	+	+	A/G	A/G	A/G	+	+	*Proteus sp*

+ = Positive to test; A = Positive with acid production

- = Negative to test; A/G = Positive with acid and gas production

Screening Test for Hydrocarbon Utilizers with Diesel as the only source of Carbon

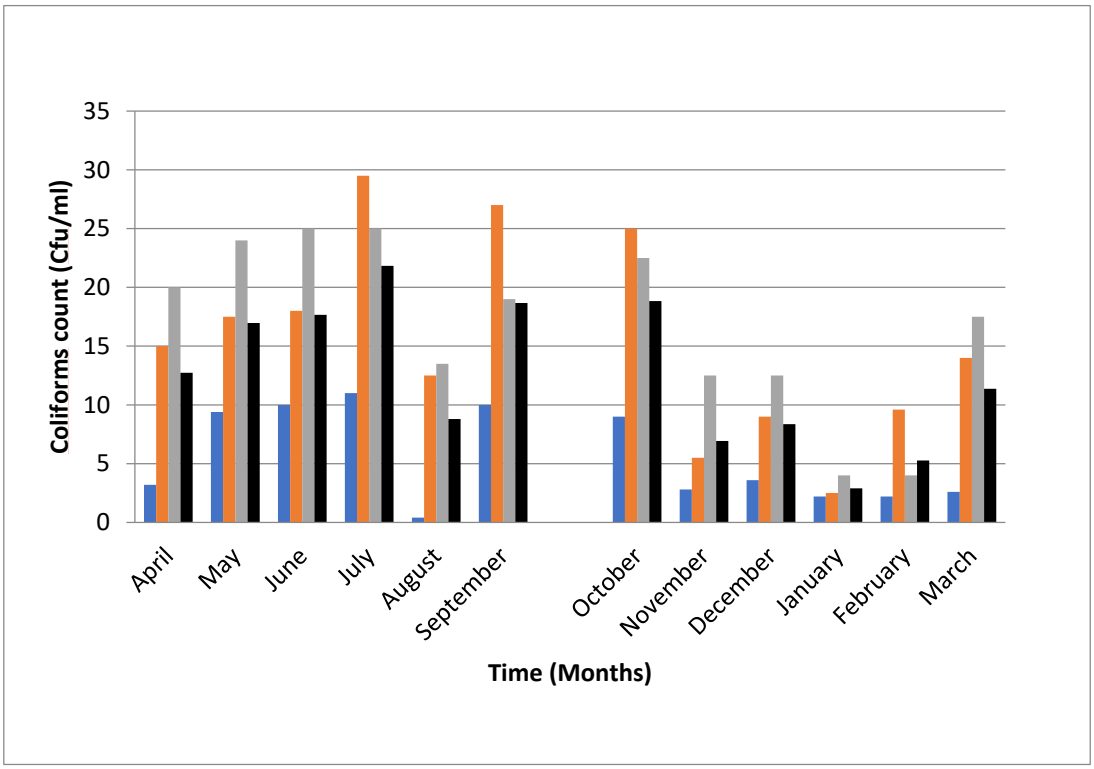

Fig. 2: The Total Coliforms of Ezu River (x10^{4}cfu/ml)

Table 5shows the population of the hydrocarbon- utilizing bacteria with the mean value of 12.1x 10^4cfu/ml for the rainy season and 4.2x10cfu/ml during the dry season, while the P- value of 0.007 was also recorded with a significant change. Table 5 shows the characterization and identification ot the hydrocarbon-utilizing bacterial isolates. The isolates include *Pseudomonas sp., Bacillus sp, Micrococcus sp, Klebsiella sp, Corynebacterium sp* and *Flavobacterium sp*

Table 5: Hydrocarbon Utilizing Bacterial Count (x10^4cfu/ml)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	2.6	5.1	5	4.233333
	May	10	26	9.4	15.13333
	June	11	27	10	16
	July	12	30	11	17.66667
	August	11.2	10.3	6.6	9.366667
	September	11.6	11	8	10.2

	October	6.9	13	6.8	8.9
	November	3.1	6.5	2.8	4.1333333
	December	2.2	8.8	4	5
	January	0.2	2.5	2.2	1.633333
Dry season	February	0.6	4	2.6	2.4
	March	1.2	4.5	4	3.233333

April – September = Rainy Season

October – March = Dry Season

Season	Mean	SD
Rainy	12.100	5.0668
Dry	4.2167	2.589
P. Value	0.007	sig.

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However, Table 6 *represents* the characterization and identification of hydrocarbon -utilizing bacterial isolates while Fig. 3 is the histographypresentation of the distribution of the Hydrocarbon-utilizing bacteria.

Table 6: Characterization and Identification of Hydrocarbon -utilizing bacterial isolates

	Gram	Cell	Catalase	Oxidase	Indole	Citrate	Motility	Spore test	Glucose	Maltose	Lactose	Voges –P	Methyl –	Probable Identity
1	-	Rod	+	+	+	+	+	-	-	-	A	-	-	*Pseudomonas sp*
2	+	Rod	+	+	-	+	+	-	A/G	A	A	-	-	*Bacillus sp*
3	+	Cocci	+	-	-	+	+	-	A/G	A	A	+	+	*Micrococcus sp*
4	-	Rod	+	-	-	+	-	-	A/G	A/G	A/G	-	-	*Klebsiella sp*
5	+	Rod	+	+	_	+	-	-	A/G	A	A	-	-	*Corynebacterium sp*
6	-	Rod	+	+	-	+	-	-	A	A	-	-	-	*Flavobacterium sp*

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+ = Positive to test; A = Positive with acid production

- = Negative to test; A/G = Positive with acid and gas production

As shown above, the organisms isolated include *Pseudomonas sp, Bacillus sp, Micrococcus sp, Klebsiella sp, Corynebacterium sp, Flavobacterium sp The histogram in Fig.3 shows the distribution of these organisms along the river duringboth rainy and dry seasons.*

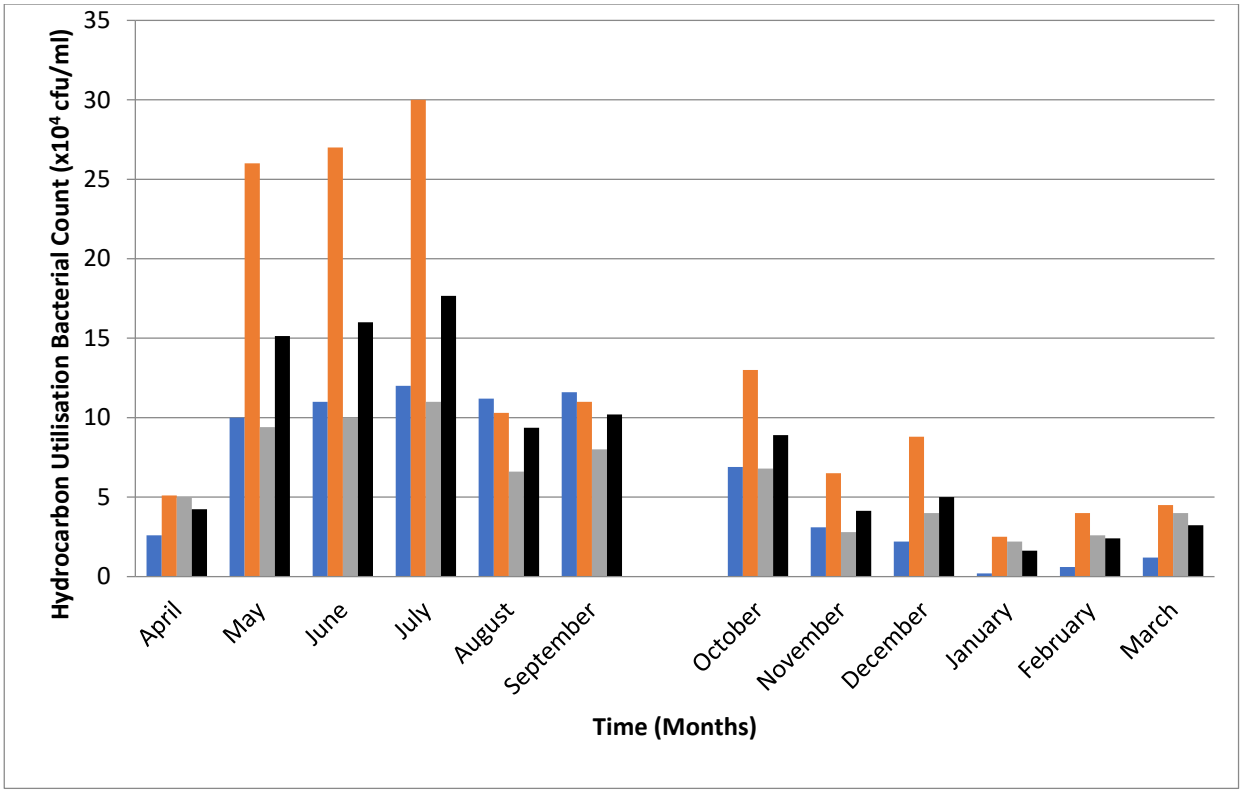

Fig 3: Hydrocarbon - Utilizing Bacterial Count (x10^4 cfu/ml)

© GSJ

- Upstream
- Midstream
- Downstream
- Means

The heterotrophic fungal count obtained are as shown in table 7 with the highest mean values of 57.17 x 10^4cfu/ and 28.00 x 10^4 at both the rainy and dry seasons respectively with a P-value of 0.003 making the change significant.

Table 7: The Heterotrophic Fungal Count (x10^4cfu/ml)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	29	54	40	41
	May	46	80	73	66.33333
	June	59	72	82	71
	July	65	81	58	68
	August	31	62	41	44.66667
	September	41	70	45	52
Season	October	29	80	40	49.66667
	November	19	56	39	38
	December	11	21	13	15
	January	7	46	16	23
	February	4	34	9	15.66667

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March	12	48	20	26.66667

Season	Mean	SD
Rainy	57.167	12.939
Dry	28.00	13.545
P. Value	0.003	Sig.

April – September = Rainy Season

October – March = Dry Season

The characterization and identification of heterotrophic fungal isolates of the river within the seasons are as shown in Table 8 below.

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Table 8: Characterization and Identification of Heterotrophic Fungal Isolates

Colour of hyphae	Kind and shape of spores	Nature of hyphae	Appearance of spore Head	Appearance of Conidiophores or Sparangiophore	Probable Identity
Greenish-black	Conidia – oval and green	Multinucleate branched, septate hyphae	Multinucleate vesicle	Conidiophore long, Septateand erect	*Penicillium sp*

Green	Conidia oval and greenish	Hyphae septate, multinucleate and branched	Multinucleate vessicle	Conidiophore long and erect	*Aspergillus , fumigatus*
Gray	Black oval spores Brown, oval conidia	Non-septate hyphae Septate hyphae multinucleate and branched	Zygospores present Multinucleate	Non Septate Long Conidiosphore	*A.Tamarii*
Pink	Large and sickle macro conidia	Septate and multinucleate hyphae	White and cottony conidia	Multiseptate conidiophore	*Fusarium sp*
Greenish-yellow	Green oval conidiophore	Septate and multinucleate	Fluffy conidia	Conidiophore long and erect	*A. flavus*

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The isolates include *Penicillium sp, Aspergillus fumigatus, A. tamari, A.flavus and Fusarium spp.* as displayedabove and while distribution within the river atboth wet and dry seasons are as shown in Fig.4 below.

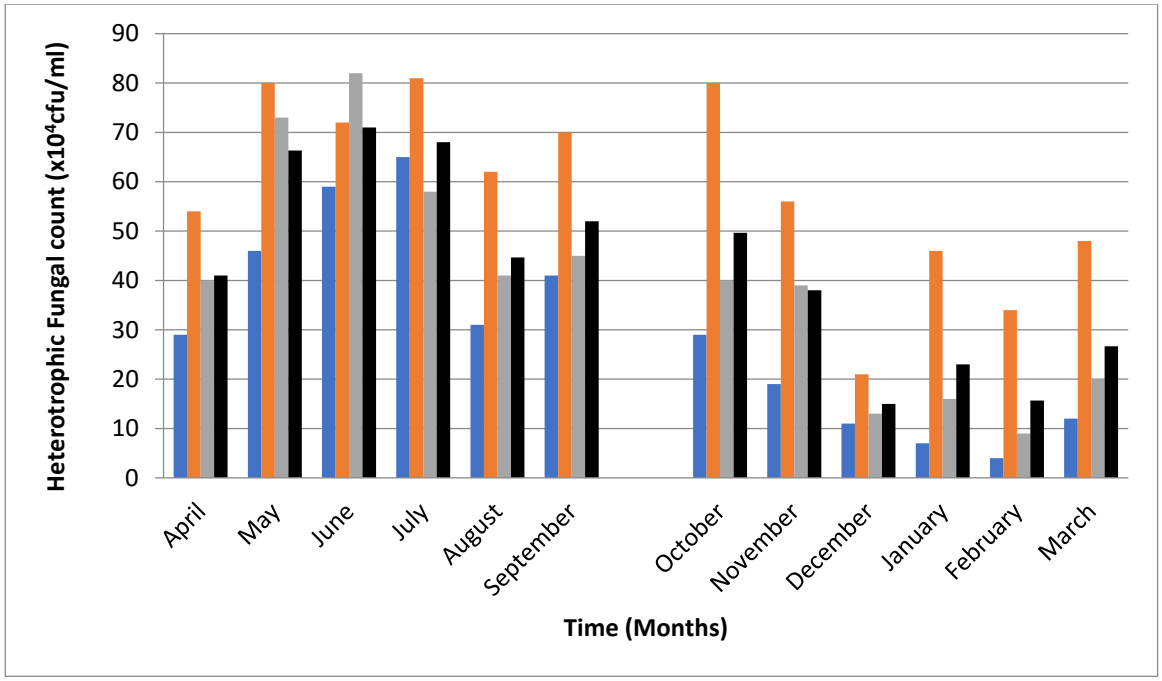

Fig. 4: The Heterotrophic Fungal Count (x10^4 cfu/m)

© GSJ

Upstream

Midstream

Downstream

Means

Table 9 shows the yeast population of the river with the highest mean values of 12.100 x 10^4cfu/ml in the rainy season against 4.2167 x10^4 cfu/ml during the dry season at a P-value of 0.008 making the change in seasonal count significant.

Table 9: The Yeasts Count (x10^4cfu/ml)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	2.6	5.1	5	4.233333
	May	10	26	9.4	15.13333
	June	11	27	10	16
	July	12	30	11	17.66667
	August	11.2	10.3	6.6	9.366667
	September	11.6	11	8	10.2
Dry season	October	6.9	13	6.8	8.9
	November	3.1	6.5	2.8	4.133333
	December	2.2	8.8	4	5
	January	0.2	2.5	2.2	1.633333
	February	0.6	4	2.6	2.4
	March	1.2	4.5	4	3.233333

Season	Mean	SD

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Rainy	12.100	5.0668
Dry	4.2167	2.589
P. Value	0.007	sig.

April – September = Rainy Season

October – March = Dry Season

The biochemical characteristics for the identification of the isolates are as shown in Table 10.

Table 10: Properties and Identification of the yeast isolates

Isolate	Gram	Cell	Citrate	Catalase	Starch	Growth at	Mannitol	Lactose	Maltose	Glucose	Ethanol	Probable Identification
1	+	Oval	+	+	-	+	A/G	-	A	A/G	+	*Candida sp*
2	+	Oval	+	+	-	+	-	A	A/G	A/G	+	*Saccharomyces sp*

+ = positive to test; A= positive with acid production

- = Negative to test; A/G = positive with acid and gas production

Fig. 5 shows the distribution of the yeast isolates along the riverwith the highest population shown in the month of July.

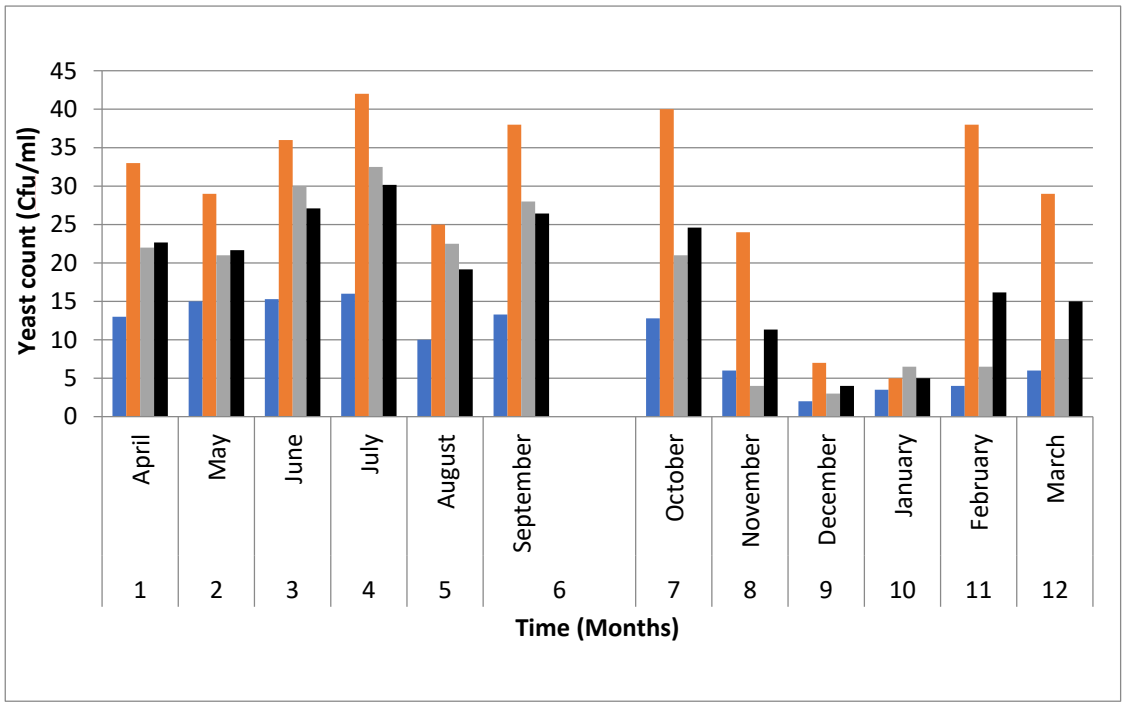

Fig. 5:The Yeasts Count (x10-4 cfu/ml)

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The enumeration of the hydrocarbon-utilizing fungi is as shown in Table 11 with the mean value of 25.46 $x10^3$cfu/ml during the rainy season while that for the wet season was $7.37x10^3$cfu/ml andwith a P-valu of 0.002, recording a significant change in their population.

Table 11: Hydrocarbon -Utilizing fungal count ($x10^4$ cfu/ml)

Months	Upstream	Midstream	Downstream	Means

Rainy Season	April	12	18.2	18	16.06667
	May	13	31	18.6	20.86667
	June	18	36.8	29.2	28
	July	25	63.5	32.2	40.23333
	August	11	28	23.5	20.83333
	September	18	42.2	20	26.73333
Dry season	October	11	21	13.4	15.13333
	November	3	9	6.2	6.066667
	December	2.2	5	3.1	3.433333
	January	1	3	2.5	2.166667
	February	1	5	3	3
	March	12.1	17.1	14	14.4

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Season	Mean	SD
Rainy	25.456	8.4506
Dry	7.367	5.8832
P. Value	0.002	sig.

The hydrocarbon-utilizing fungal isolates obtained at both seasons include *Aspergillus sp, Penicillium sp, Fusarium sp* and *Mucor sp* as characterized and identified in Table 12.

Table 12: Identification of Hydrocarbon – utilizing Fungal Isolates

Isolates	Colour of hyphae on nutrient medium	Pattern and shape of spores	Appearance and shape of conidiophores/ sporangiophore	Characteristics of spore head	Nature of hyphae	Probable identity
1	Dark green	Oval greenish conidia	Erect, septate and long conidia	Multinucleate	Septate, multinucleate, branched hyphae	*Aspergillus sp*
2	Green	Oval, brown conidia in chains	Erect long conidiophores	Long chains of conidia	Branched septate and multinucleate	*Penicillium sp*
3	Sharp pink	Large and sickle	Simple, short conidia	Brilliant white and	Septate and	*Fasarium sp*

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		macro conidia		cottony conidia	multinucleate	
4	Grey	Black sporangia	Sporangiophore long, branched and erect	Zygospores present	Non s-septate and branched	*Mucor sp*

Fig.6 shows the distribution of the hydrocarbon-utilizing fungal isolatesin the river.

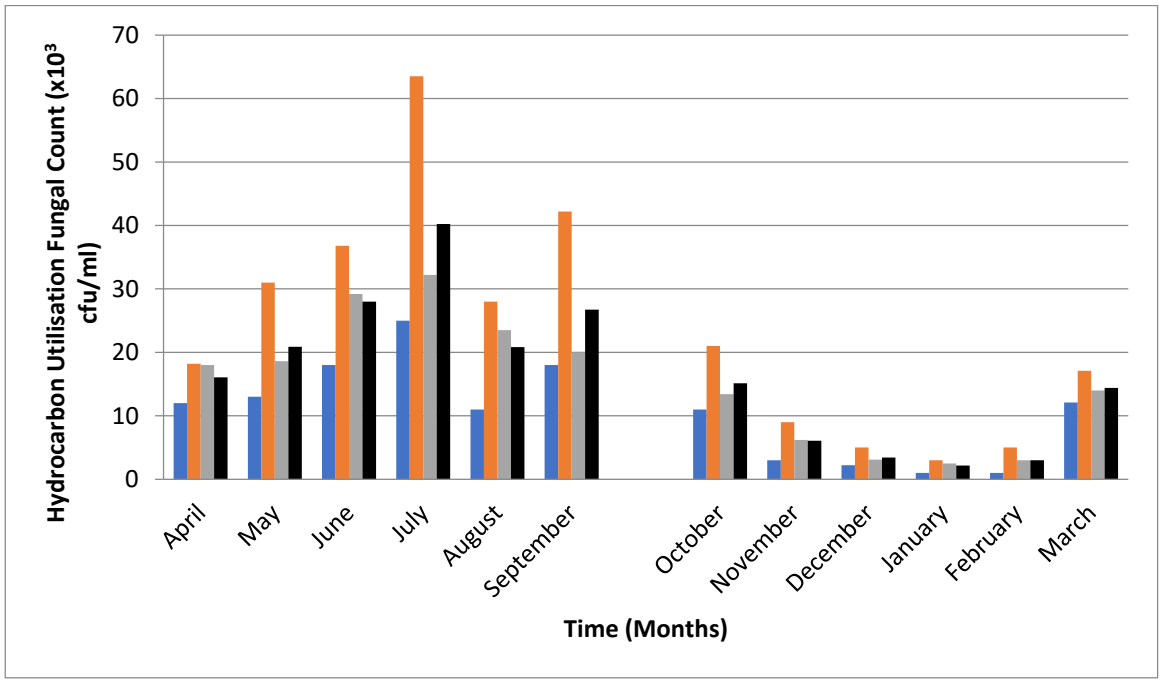

Fig 6: Hydrocarbon - Utilizing Fungal Count (x10^4 cfu/ml)

- Upstream
- Midstream
- Downstream
- Means

The Physicochemical parameters of Ezu river were also studied and include describing the relationship between the isolates and their microenvironment.

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Biochemical Oxygen Demand

The amount of oxygen, expressed in mg/litre or parts per million(ppm), is known as biochemical oxygen demand (BOD). When bacteria oxidize organic stuff, they remove a certain amount of oxygen from the water (Clifford et al., ND) and adopted by Lamidi *et al.*, (2019). The World Health Organization ((WHO, 2016) interprets BOD levels as shown below:

BOD Level (ppm **or** mg/l) of Water

1 - 2	Very Good
3 - 5	Fair or Moderately Clean
6 – 9	Poor: Somewhat Polluted
10 or greater	Very Poor: Very Polluted Contains organic waste

However, the BOD (mg/l) values obtained are as shown in Table 13with higher mean values of 9.7 x 10^2 mg/l obtained during the rainy season in the month of September(rainy season) and least mean value of 2x10^2mg/l in the month of February(dry season). There was also a clear indication of highest microbial activity on oxygen demand observed at the downstream witha P- value of 0.614.This may be due to increased microbial activities at catchment areas downstream because of increased deposition and sedimentation of organic materials within the area through run-off which may have influenced the aerobic bacteria.

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Table 13: BOD (mg/l) of Ezu River (x 10^2)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	4.73	5.2	6.42	5.45
	May	3.8	4.8	4.8	4.466667
	June	4.87	7.8	8.1	6.923333
	July	4.1	5.4	5.6	5.033333
	August	2.1	3.8	4.3	3.4

	September	8.26	14	16.2	12.82
	October	6.3	8.2	14.7	9.733333
	November	5.5	6.1	6.7	6.1
	December	2.7	3.9	4.6	3.733333
	January	1.5	3.54	4.2	3.08
Dry season	February	2.8	2.88	3.3	2.993333
	March	4.4	7.8	8.7	6.966667

Season	Mean	SD
Rainy	6.3489	3.3759
Dry	5.4344	2.6709
P. Value	0.614	Not sig.

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April – September = Rainy Season

October – March = Dry Season

The biochemical oxygen demand in five dayswas performedat the three different points of the stream and the result are as displayed in the histogram as shown below.

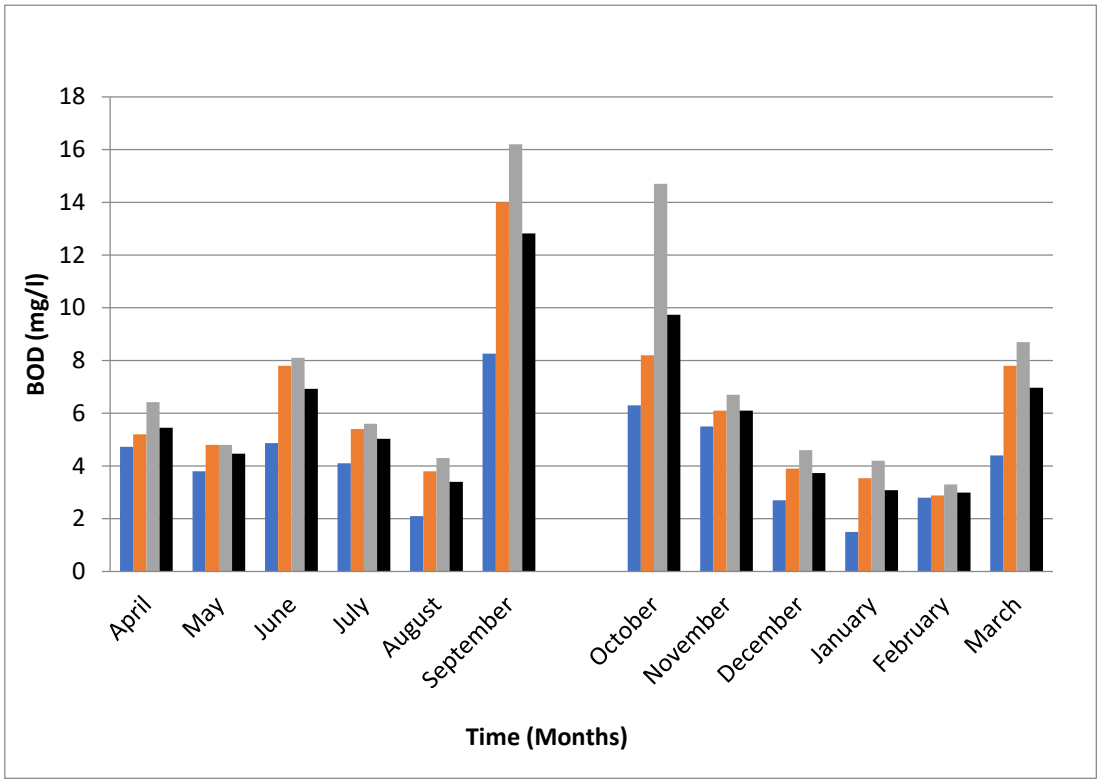

Figure 7: BOD_5 (Mg/I)

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- BOD_5 (Mg/I) Upstream
- BOD_5 (Mg/I) Midstream
- BOD_5 (Mg/I) Downstream
- BOD_5(Mg/I) Means

Temperature (^{o}C)

The temperature of the river was observed to be higher during the dry season and lower during the rainy season as shown in fig.3 with mean values of 23.5^{o}C during the rainy season and 25.6^{o}C during the dry season, while the P-value obtained *was* 0.000 making the seasonal change in temperature significant.

Table 14: Temperature (^{0}C)

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	23.2	23.4	23.3	23.3
	May	23.1	23.8	23.6	23.5
	June	23	23.7	23.3	23.33333
	July	22.4	23.7	23.1	23.06667
	August	24.1	24.8	24.4	24.43333
	September	23.3	23.7	23.7	23.56667
Dry season	October	25.4	25.8	25.8	25.66667
	November	25	25.7	25.3	25.33333
	December	25.2	25.6	25.4	25.4
	January	26.1	26.8	26.6	26.5
	February	26.3	26.7	26.6	26.53333

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March	24.1	24.4	24.2	24.23333

Season	Mean	SD
Rainy	23.533	0.4742
Dry	25.611	0.856
P. Value	0.000	sig.

April – September = Rainy Season

October – March = Dry Season

The temperature of the river all year round is as shown below in Fig.8. with the highest temperature manifest in January.

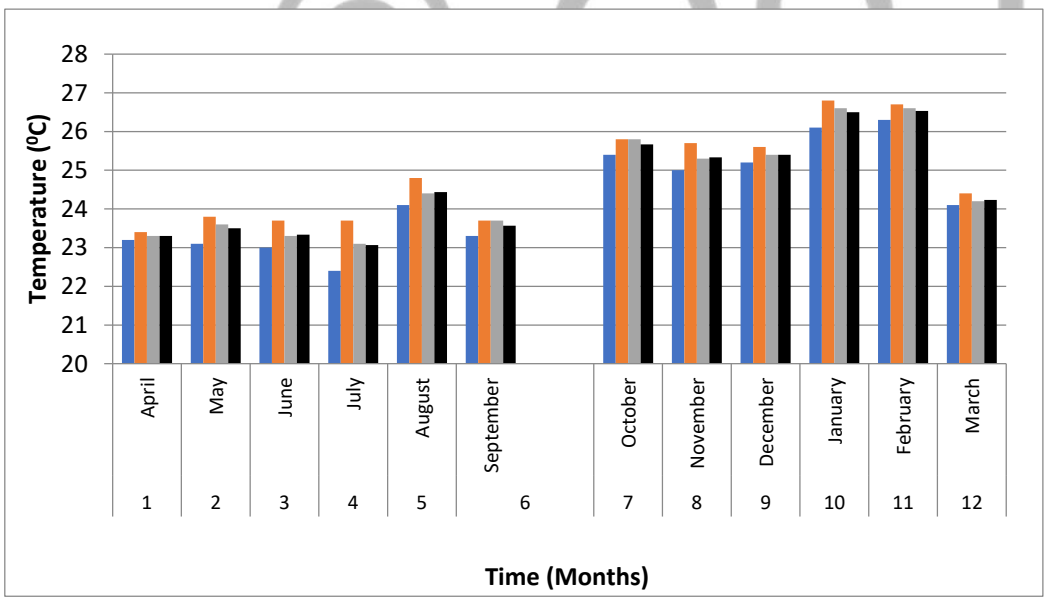

Fig. 8: Monthly Temperature(^{0}C)

- Temperature Upstream
- Temperature Midstream

- Temperature Downstream
- Temperature Means

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Conductivity (μs/cm)

This measures how much dissolved substances, chemicals, and minerals are present in a water sample. Higher amounts of these impurities will lead to a higher conductivity. The conductivity tests conducted on the samples were higher during the rainy season and lower during the dry season as shown in Table 15 with the mean value of 22.82 x 10^2μs/cm during the rainy season and 13.03 x 10^2μs/cm during the dry season.The P-value of the change in both seasons was 0.009 which implies a significant change.

Table 15: Conductivity µs/cm

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	18.222	23.411	24.381	22.00467
	May	18.4	24.48	28.661	23.847
	June	27.24	29.11	29.79	28.71333
	July	28.961	31.313	36.721	32.33167
	August	9.401	12.426	14.101	11.976
	September	16.052	18.8	19.203	18.01833
	October	14.111	16.333	17.008	15.81733
Dry season	November	8.122	11.128	14.479	11.243
	December	11.009	13.875	15.004	13.296
	January	9.42	11.812	16.47	12.56733
	February	9.409	12.638	15.436	12.49433
	March	9.946	12.621	15.712	12.75967

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Season	Mean	SD
Rainy	22.815	7.3153
Dry	13.029	1.5236
P. Value	0.009	sig.

April – September = Rainy Season

October – March = Dry Season

The results were as well displayed in a histogram as shown in Fig.9 and can be observed to be higher in July. the peak of rainy season.

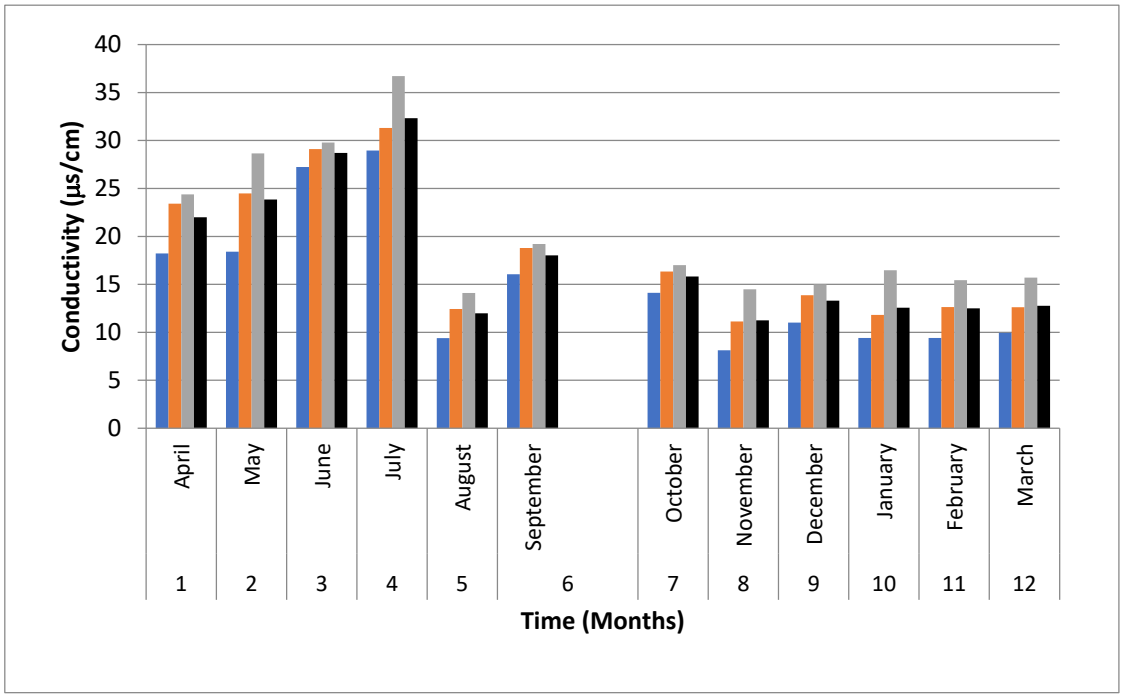

Fig. 9: Conductivity (µs/cm)

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The p^H of Ezu river measured the degree of acidity or alkalinity of the river during both seasons. Table 16 shows the mean p^H value of 8.2 during the rainy season and 6.96 in rainy season. The P-value was 0.04 and so the seasonal change in this parameter was significant.

Table 16: p^H of Ezu River

	Months	Upstream	Midstream	Downstream	Means
Rainy Season	April	7.6	8.4	8.1	8.033333
	May	7.9	8.7	8.9	8.5
	June	8.1	8.3	8.3	8.233333
	July	8	8.3	8.9	8.4
	August	6	8.4	8	7.466667
	September	8.2	8.9	9.2	8.766667
Dry season	October	8	8.8	8.5	8.433333
	November	7.8	6.6	6.2	6.866667
	December	6.2	6.5	6.8	6.5
	January	4.5	5.4	6.6	5.5
	February	5.2	6.5	6.6	6.1
	March	8	8.8	8.4	8.4

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Season	Mean	SD
Rainy	8.233	0.4497
Dry	6.967	1.211
P. Value	0.037	Sig.

April – September = Rainy Season

March – October = Dry Season

The degree of the acidity or alkalinity(P^H) of the river is as shown in in Fig

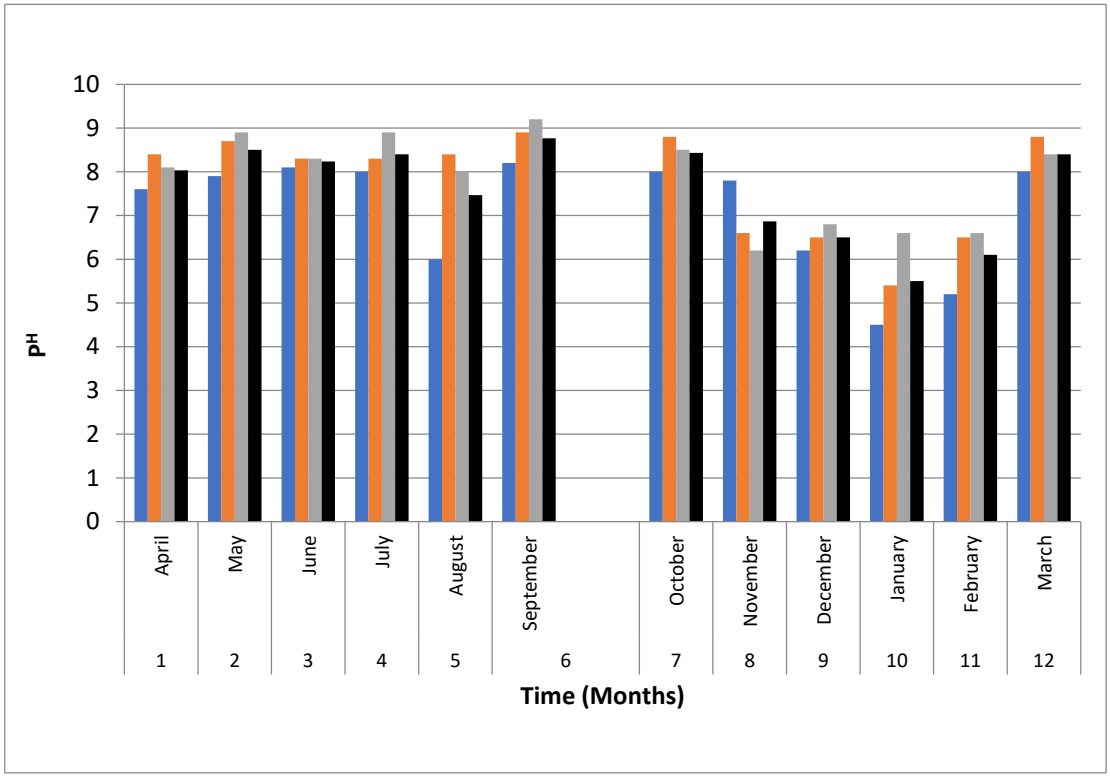

Fig. 10: p^H

4.Discussion

During the experiment, groups of microorganisms isolated included: the heterotrophic bacteria, fungi and yeasts due to the introduction of excess nutrients through runoff from farms, industries, and other busy sites. This agrees with the work of Chislock (2013) that eutrophication is also associated with major changes in aquatic community structure as well as with USEPA (2022) that runoff picks up fertilizer, oil, pesticides, dirt, bacteria, and other pollutants as it makes its way through storm drains and ditches - untreated - to our streams, rivers, lakes, and the ocean and is of the greatest threat to clean water.

Samples were collected from three distinct areas of the river namely: upstream, midstream, and downstream for a period of twelve months. These samples were investigated for the effect of season on both the physicochemical features and microbial flora of the river. The microbial counts were used to assess some microorganisms present together with their probable loads. The study unveiled marked variations in both the sampling areas of the river and the seasons. This is as presented in Table 1 which indicates that the total heterotrophic bacteria were higher during the rainy season than during the dry

seasonpossibly due to influx of nutrients from runoff water into the river. The heterotrophic bacterial population was 12.78 x 10^5cfu/ml in the rainy season with lesser mean value of 6.50 x 10^5 cfu/ml during the dry season. The increase in the heterotrophic bacterial population was significant with a p-value of 0.007.Thisblends with the report by Bartosz *et al.* (2019) that eutrophication may lead to serious changes in microbial community composition, affecting the functioning of the microbial loop and thus the entire aquatic food web. However, it was observed generally that there was reduced microbial population in the month of August due to the usual break in raining with associated scanty runoff due to reduced eutrophication.The total heterotrophic bacteria was more aggravated during the rainy season than in the dry season possibly due to influx of nutrients from runoff water into the river. This agrees with the report of Shepard (2015) that the availability of nutrients during rainy season is due to eutrophication of the organic materials from the catchment areas.

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Pathogenic organisms are almost never more numerous than the typical intestinal excremental organisms, which can serve as a sign of fecal pollution and are simpler to spot. This agrees with the findings of Wanjugi *et al*. (2016) and Dongzagla *et al.*,(2020) that if indicator organisms are not found in water, disease-causing microbes must not be present. Some bacterial groups have been frequently known as an indication of water pollution in microbiology. Organisms such as *E. coli* or fecal Streptococci are two common bacteria that signal sewage pollution of the study area. The total coliform count is one of the best markers of water pollution. Numerous types of organisms from the family Enterobacteriaceae are included in the coliform category. *E. coli* is well-known members of the coliform group and are often found in polluted water. This is in harmony with the report of the study by McLarnan (2017), and Messner *et al.* (2017) that *Escherichia coli* is a highly reliable indication of fecal pollution in water because it is a common bacterium in human excreta. *E. aerogenes*, on the other hand, and *E. aerogenes*is referred to as typical bacterial flora of soil and vegetation and non-fecal coliform.

The development of parameter-based technologies for the assessment of the quality of water as well as basic and applied research in aquatic microbial ecology both heavily rely on the enumeration of the microbial flora in both dry and wet seasons.

Other bacterial species isolated include *Streptobacillus* species which may be implicated in rats and other rodents present within and around the river . This agrees with the report of Ogawa (2018) that human infections involving *S. notomytis* can have rats and other animal species as reservoir while Eisenberg (2016) also reported that a zoonotic potential can be assumed by these species of organisms. This clearly implies that the river is not safe for both domestic and farm animals. *Species such as Yersinia.were also reported as a threat to rodents* occasionally infecting man causing plague while some species are known to cause gastroenteritis through water transmission while the isolation of *Citrobacter spp.*have been reportedby Sekhi *et al*. (2022) as disease-causing species from fish, animals, humans, soil, water, and food thereby reducing both the domestic and recreational values of Ezu river. This also agrees with the findings of Korajkic *et al*. (2018) that fecal pollution of recreational waters can cause scenic blight to crop and pose a threat to public health, resulting in beach advisories and closures.

As a result of the discharge of effluents from the nearby abattoir, the isolation of *Streptococcus sp*. and *Enterobacter sp*., were not out of place since Spellerberg (2015) also isolated the *species* from the oral cavity and gastrointestinal tract of various mammals and as well reported by WHO (2011), that these organisms can cause diverse human ailments which implies that water from Ezu River is not safe for drinking.However, WHO has directed that the permissible limits of coliform and *E. coli* should be 0/100 ml for drinking water and 126 CFU/100 ml for domestic and recreational water as agreeably explained by Gunda *et al* (2016). In the same vein, the presence of Micrococcus species may have been due tohuman activities such as swimming. This is in congruent with the findings of Eunice *et al*. (2018) that Micrococcus sp. are normal flora of human skin and oral cavity hence the possibility of swimmers contaminating the river. *Hossan et al. (2021) also isolated Klebsiella,Staphylococcus, Bacillus and*

Pseudomonas, Flavobacterium species from water environments with *Staphylococcus* spp. known to be among the most important causative agents of acute and chronic bacterial infections in humans as well as in animals. Some of the flora isolated, characterized and identified showed their ability to utilize and degrade hydrocarbons by their growth on mineral salts medium using diesel as a source of carbon and energy. The hydrocarbon utilizers after characterization, were identified as *Pseudomonas, Klebsiella, Bacillus, Flavobacterium, Micrococcus, and Citrobacter spp.*as also reported by Mohanty *et al*. (2016), who isolated some species of heterotrophic bacteria from oil polluted brackish beach ridge and freshwater soils which had hydrocarbon- utilizing capabilities. It also agrees with the work of Haritash (2016) that the growth of oil- degrading bacteria may involve growth on a medium that contains the hydrocarbon as the selective substrate. The increase in the population of this category of organisms from a river polluted with hydrocarbons such as kerosene used in roasinf of animal skin from the abattoir agrees with the findings of Shepard (2015) with the explanation that increase in the bacterial population may be due to the stimulatory effect of the additional carbon and energy sources such as crude oil which leads to an enrichment of the hydrocarbon-degrading microbial population.

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The state of Ezu river with respect to its rise in microbial flora and the subsequent variations in its physicochemical properties during the rainy season specifically at the midstream due to increased human activitiesis a clear example of microorganism-mediated water pollution which is known to be one of the greatest global concerns to the aquatic environment and general public health.The most probable number (MPN) test is used to measure potability of water and *Escherichia coli*, is considered as the indicator organism of fecal contamination in water.These findings are in harmony with the findings of Sudip et al.(2021)who reported that the effluent of fecal matter, industries, hospitals and cattle farms increased the bacterial load in a water body and that among the coliforms which include *Streptococcus , Shigella and Proteus spp., E.coli* is the known indicator of fecal contamination. Interestingly this agrees with the finding of Onyango *et al.,* (2009) that the potability of water is

measured by the absence or presence of coliform bacteria within the permissible limit with reference to the most probable number index value per 100 ml of water.

The standard plate count (SPC) of heterotrophic bacteria, biochemical oxygen demand (BOD), degree of acidity or alkalinity (P^H), Temperature and conductivity techniques also determine the bacterial and organic pollution load in a water sample.

However, the bacteriological analysis of the water samples from Ezu river indicated that the water body was heavily polluted by sewage during the rainy season with higher populations identified at the midstream due to possible increase in human activities. The degree of pollution revealed that water from the river is unsuitable for both drinking and recreational purposes.

5. Conclusion

Several contaminants, including farm fertilizers, animal and human waste, industrial and residential was te, provided the microorganisms of the Ezu River with both carbon and energy. However, this explains why the eutrophication of the river caused by runoff and human activities during the rainy season had a significant impact on the microbial flora.One of the main problems affecting the sanitary quality of river and recreational water is microbial pollution in the water body. Numerous enteric outbreaks are caused by the spread of harmful microorganisms. To safeguard the community from severe waterborne infections, prompt affirmative action is required to arrest increasing debility and enhance water quality. Wastewater such as that from the abattoir needs to be treated before it can reach a natural body of water. For sustainable agriculture, a system of irrigation should employ recycled water. Microbial pollution in the water body is one of the key issues influencing the hygienic quality of drinking, domestic and recreational water. Before it can get to a natural body of water, sewage and industrial effluents must be treated and recycled water should be used in irrigation systems for sustainable agriculture to reduce the microbial load of run-off water during the rainy season

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