

# Bio-Indices of Bacteria Loads in Water and Mangrove Oyster (*Crassostrea Gasar*) of Woji/Trans-Amadi Creek, Port Harcourt, Nigeria

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**Abstract:** - The bio-indices of bacteria load in water and mangrove oyster (*crassostrea gasar*) tissues of Woji/Trans-Amadi creek, Port Harcourt, Nigeria were studied between January and June, 2019. Water and oyster (*Crassostrea gasar*) samples were collected from three stations and analysed for bacteria using standard method. The data obtained were subjected to SPSS software version 20 for descriptive and inferential statistics using one-way analysis of variance and Duncan multiple range test. The results obtained showed that the water and the oyster samples contained 11 and 12 species of bacteria respectively with oyster samples containing more bacteria counts than the water. Bacteria counts in station 2 for both samples differed significantly from stations 1 and 3 at  $p < 0.05$ . Some of the species of bacteria identified were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella species*, *Enterococci sp*, *Klebsiella*. Shannon-Wiener index and other indices showed that the water and oyster tissues were heavily ( $0.928 \pm 0.01$ ) and moderately ( $1.044 \pm 0.09$ ) polluted microbially. The results suggest that Woji/Trans-Amadi creek is moderately to heavily polluted microbially hence adequate measure should be taken to prevent further discharge of organic wastes into the creek.

**Key words:** Bio-indices, bacteria load, water, oyster tissue, Woji/Trans-Amadi creek, Port Harcourt.

## I. INTRODUCTION

Diversity refers to the measure of the complexity of the community structure which increase or decrease with the environment (physical, chemical and biological factors). Diversity indices are in addition to their usefulness in assessing richness and evenness are important statistical measure used to characterize richness (the number of species) and evenness (how uniform abundant species are in a sample) of the species in the community (Magurran, 1988) and is used as a tool for determining the health and pollution status of an ecosystem (Norris and Georges, 1993; Schmitz and Nadel, 1995; Guerold, 2000). Czenlawska-kusza (2005) argued that bio-indices are suitable criteria for understanding the quality of aquatic environment using numerical expressions of quantitative values of species diversity with qualitative information on the ecological sensitivity of each taxon. Ecologists use various metrics and indices for ecological assessment of river ecosystem. They can be used to predict the response of an ecosystem to different water resources

management practices and water conditions. Hence diversity indices serves as good indicator of all pollution of water (Sandhya and Laxmi, 2016).

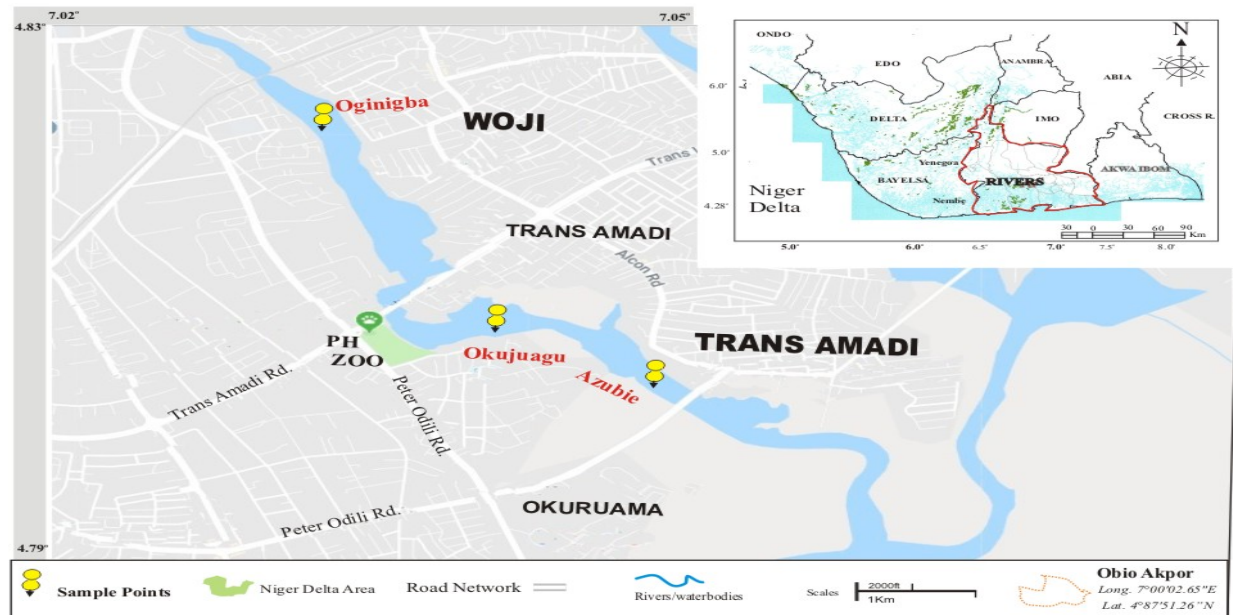
Seafood like oysters constitute an important food component for the teeming world population especially those living in coastal areas (Edema *et al.*, 2005). In recent years, Niger Delta environment have been exposed to both organic and inorganic contaminants from industries and domestic wastes especially oil related activities which are predominantly in the area thus enhancing the capacity of the ecosystem into harboring a sizeable population of microbes (Akinrotimi *et al.*, 2015). However, proper understanding of the transfer of micro-organisms through the food web is essential to predict the exposure of consumers of this seafood to possible health consequences associated with their consumption. Fisheries resources are conditioned by their environment such that if the growing and harvesting environment is chemically or microbiologically polluted then the fish are equally polluted. Studies on microbiological quality of shellfishes have shown that it harbors pathogens which have been implicated in outbreak of food-borne diseases such as typhoid fever, hepatitis and similar disorder of the digestive system and neurological disorder (Ukpong and Utuk, 1992, Huss *et al.*, 2003).

The Woji/Trans-Amadi creek plays so many vital roles in the lives of the inhabitant by providing ready incentives for capture fisheries, transportation of fuel, wood production and domestic waste disposal. These human intervention/activities are being seen by environmentalist as sources of threat (Otene and Alfred-Ockiya, 2019 a&b). The aquatic environments of Niger Delta has been widely studied in but very few microbiological (bacteriological) studies have been carried out on the brackish water ecosystem of this creek with respect to bio-indices (Otene *et al.*, 2019). This present paper is therefore designed to investigate the bio-indices of bacteria load of water and mangrove oyster of Woji/Trans-Amadi creek. The possible implication of bacterial absence and presence as well as its abundance were also examined/ investigated.

## II. MATERIALS AND METHODS

The various station of the study area falls within latitudes 4°48'44.285" N and longitudes 7°3'2.182" E from station one using the WGS-84 and 4°49'21.376" N, 7°2'45.861"E. Three

sampling stations were selected within the study area with a distance of 500m apart. The stations were chosen based on ecological settings and human activities in the area. The stations include Oginigba, Okujuagu and Azubie along Trans-Amadi axis (Figure 1).



A lot of effluents are received by these sites. At the Woji area, the river channel widened as it crosses the railway bridge. It maintained this broad width, though at a small divergent angle, through to Slaughter Bridge (Trans-amadi area), then into the Amadi Creek behind the Port Harcourt Zoo and finally joined the Bonny River, a trajectory of the Atlantic Ocean.

### *Samples Collection and Preparation*

Water samples were collected based on standard limnological method in triplicate for six months from the three stations in sterile containers and transported to the laboratory in an ice pack the same day where microbiological analysis were carried out immediately. Oyster samples were randomly cut with a sharp knife on the roots of mangrove at the river bank collected by hand picking at river bank intertidal zone (sediment) from the various sampling points through the help of the fishers in the areas and transported to the laboratory where they were sorted, rinsed, processed and kept in refrigerator for further analysis.

### *Total and Faecal coliform Examination*

**Presumptive test:** Enumeration of total coliform and faecal coliform were done by multiple tube fermentation tests (APHA,2005).

**Confirmed test:** This test was carried out by transferring a loopful of culture from a positive tube from presumptive test into a tube of Brilliant Green Lactose Bile broth with Durham tubes. The tubes which were incubated at 36°C for 37 hours

for total coliform and 44.5°C for faecal coliforms were observed for gas production.

**Completed test:** This test was carried out by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue agar plate for pure colonies. The plates that were incubated at 37°C for 30 hours to develop colonies on EMB agar.

**Isolation of Salmonella/Shigella species:** They were isolated using Salmonella/Shigella agar (SSA). The media was prepared following the manufacturer's instruction as described by (Cheesbrough, 2002).

**Isolates Identification:** The isolates in a pure culture were determined as per the procedures described in Bergey's manual (Schleifer, 1989).

### *Statistical Analysis*

SPSS software version 20 was used to carry out the statistical analysis of the bacteria density of the samples. One-way analysis of variance was carried out at P = 0.05, and ANOVA test was used to determine source of the observed differences.

### *Calculation of Bioindices*

These indices were used to obtain estimation of species diversity, species richness and species evenness.

1. Species richness (R1 and R2) obtained using the equation

$$R1 = (\text{Margalef, 1958}) \quad \frac{S-1}{\ln N}$$

$$R2 = (\text{Menhinick, 1964}) \frac{S}{\sqrt{\sum ni}}$$

Where,

R = Index of species richness

S = Total number of species

N = Total number of individuals

Ln = Natural logarithm

3. Shannon and Wiener (1949) and Simpson (1949) diversity index value were obtained by using the following equation:

$$(\text{Shannon's index}) - \sum_n \left( \frac{ni}{N} - \log_2 \left( \frac{ni}{N} \right) \right)$$

$$(\text{Simpson index}) \frac{\sum ni(ni-1)}{N(N-1)}$$

4. Species evenness was determined using the following expression.

Shannon's equitability (EH) was calculated with the equation:

$$\frac{\sum i \left( \frac{ni}{N} - \ln \left( \frac{ni}{N} \right) \right)}{\ln N}$$

Equitability assumes a value between 0 and 1 being complete evenness.

5. Dominance index is used to characterize most conspicuous and abundant species with its relative importance related to degree of influence it has on ecosystem components.

$$\text{Dominance index} = 1 - \left( \frac{\sum ni(ni-1)}{N(N-1)} \right)$$

6. The Berger – Parker Dominance Index is a simple measure of the numerical importance of the most abundance species.

$$\text{Berger – Parker Dominance Index} = \frac{n_{\max}}{N}$$

Where

nmax = maximum number of organisms

N = Total number of individuals

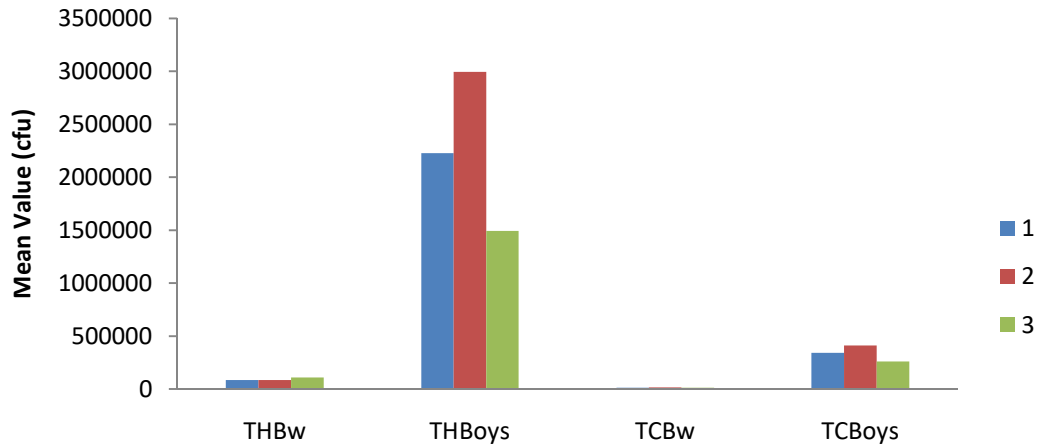
### III. RESULTS

Table 1 showed the spatial distribution and abundance of bacteria in the study area. The highest and lowest bacterial loads in both water and oyster tissues were observed in stations 2 and 3 respectively with statistical difference at p<0.05 with oyster tissues having more bacterial count than water (fig.2).

Table 1: Monthly and Spatial Values of Bacteria and Fungi in the Study Area

Month	Station	THBw	THBoys	TCBw	TCBoys
January	1	1.10x10 <sup>5</sup>	2.84x10 <sup>6</sup>	1.10x10 <sup>4</sup>	5.10x10 <sup>5</sup>
	2	1.23x10 <sup>4</sup>	3.25x10 <sup>6</sup>	1.50x10 <sup>4</sup>	5.20x10 <sup>5</sup>
	3	7.90x10 <sup>3</sup>	5.10x10 <sup>5</sup>	1.30x10 <sup>4</sup>	4.50x10 <sup>5</sup>
	<b>Total</b>	<b>1.302X10<sup>5</sup></b>	<b>6.60X10<sup>6</sup></b>	<b>3.90X10<sup>4</sup></b>	<b>14.80X10<sup>5</sup></b>
February	1	1.32x10 <sup>4</sup>	2.10x10 <sup>6</sup>	1.20x10 <sup>4</sup>	3.30x10 <sup>5</sup>
	2	2.10x10 <sup>4</sup>	3.30x10 <sup>6</sup>	1.10x10 <sup>4</sup>	5.00x10 <sup>5</sup>
	3	1.20x10 <sup>5</sup>	2.00x10 <sup>6</sup>	1.10x10 <sup>4</sup>	2.30x10 <sup>4</sup>
	<b>Total</b>	<b>4.62X10<sup>5</sup></b>	<b>7.40X10<sup>6</sup></b>	<b>3.40X10<sup>4</sup></b>	<b>8.53X10<sup>5</sup></b>
March	1	1.30x10 <sup>5</sup>	2.10x10 <sup>6</sup>	1.31x10 <sup>4</sup>	2.30x10 <sup>5</sup>
	2	1.23x10 <sup>5</sup>	2.31x10 <sup>6</sup>	1.40x10 <sup>4</sup>	2.40x10 <sup>5</sup>
	3	1.50x10 <sup>5</sup>	3.10x10 <sup>6</sup>	1.21x10 <sup>4</sup>	2.15x10 <sup>5</sup>
	<b>Total</b>	<b>4.03X10<sup>5</sup></b>	<b>7.51X10<sup>6</sup></b>	<b>3.92X10<sup>4</sup></b>	<b>6.85X10<sup>5</sup></b>
April	1	1.33x10 <sup>5</sup>	2.10x10 <sup>5</sup>	1.32x10 <sup>4</sup>	3.35x10 <sup>5</sup>
	2	1.20x10 <sup>5</sup>	4.90x10 <sup>6</sup>	1.50x10 <sup>4</sup>	5.50x10 <sup>5</sup>
	3	1.10x10 <sup>5</sup>	2.10x10 <sup>4</sup>	1.20x10 <sup>4</sup>	2.35x10 <sup>5</sup>
	<b>Total</b>	<b>3.63X10<sup>5</sup></b>	<b>5.32X10<sup>5</sup></b>	<b>4.02X10<sup>4</sup></b>	<b>11.20X10<sup>5</sup></b>
May	1	1.10x10 <sup>5</sup>	2.20x10 <sup>6</sup>	2.30x10 <sup>4</sup>	4.30x10 <sup>5</sup>
	2	1.20x10 <sup>5</sup>	2.20x10 <sup>6</sup>	2.30x10 <sup>4</sup>	4.30x10 <sup>5</sup>
	3	6.65x10 <sup>4</sup>	2.12x10 <sup>6</sup>	7.80x10 <sup>3</sup>	3.30x10 <sup>5</sup>
	<b>Total</b>	<b>2.96X10<sup>5</sup></b>	<b>6.52x10<sup>6</sup></b>	<b>3.50X10<sup>4</sup></b>	<b>11.90x10<sup>5</sup></b>
June	1	1.20x10 <sup>4</sup>	2.31x10 <sup>6</sup>	1.12x10 <sup>4</sup>	2.10x10 <sup>5</sup>
	2	1.10x10 <sup>5</sup>	2.01x10 <sup>6</sup>	1.21x10 <sup>4</sup>	2.35x10 <sup>5</sup>
	3	2.00x10 <sup>5</sup>	1.20x10 <sup>6</sup>	1.21x10 <sup>4</sup>	3.10x10 <sup>5</sup>
	<b>Total</b>	<b>3.22X10<sup>5</sup></b>	<b>5.52X10<sup>6</sup></b>	<b>3.54X10<sup>4</sup></b>	<b>5.65X10<sup>5</sup></b>

KEY: THBw=Total Heterotrophic Bacteria in Water. THBoys=Total Heterotrophic Bacteria in Oyster. TCBw = Total Coliform Bacteria in Water. TCBoys = Total Coliform Bacteria in Oyster.



**Fig. 2: Spatial Mean Value of Bacteria in Water and Oyster Tissues in the Study Area**

KEY: **THBw**=Total Heterotrophic Bacteria in Water. **THBoys**=Total Heterotrophic Bacteria in Oyster. **TCBw** = Total Coliform Bacteria in Water. **TCBoys** = Total Coliform Bacteria in Oyster.

The highest bacterial count was observed in the month of March. Some of the common bacteria among the 12 and 11 species isolated from oyster tissues and water respectively include, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella species*, *Enterococci sp*, *Enterobacta aerogenes*, *Pseudomonas species*, *Streptococci species*, *Klebsiella species*

and *Bacillus species* (Table 2. The groups of bacteria identified in the samples (oyster tissues and water) were heterotrophic and coliform (Fig.2). The most dominant species of bacteria in this study were *E. coli* (though completely absent from oyster samples in station 3), *Proteus sp.* and *Streptococcus sp.* while the least was *Bacillus sp.*

Table 2: Spatial frequency/distribution of Bacteria species in Water and Oyster tissue in the Study area

S/N	Bacteria Species	Stn 1		Stn 2		Stn 3	
		Water	Oyster	Water	Oyster	Water	Oyster
1	<i>E.coli</i>	+	+	+	+	+	-
2	<i>Proteus sp</i>	+	+	+	+	-	-
3	<i>Staphylococcus aureus</i>	+	+	+	+	+	+
4	<i>Enterococci sp</i>	-	+	-	+	-	+
5	<i>Enterobacta aeroens</i>	+	+	+	+	+	+
6	<i>Pseudomonas sp</i>	+	+	+	+	+	-
7	<i>Streptococcus sp</i>	+	+	+	+	+	+
8	<i>Klebsiella sp</i>	+	+	+	+	+	+
9	<i>Bacillus sp</i>	-	-	+	+	+	+
10	<i>Serrata sp</i>	-	-	+	+	+	+
11	<i>Shigella sp</i>	+	+	+	+	+	+
12	<i>Salmonella sp</i>	+	+	+	+	+	-

Key: Stn=Station + = species presence, - species absence, Sp=Species

**Biodiversity Indices of Bacteria in Water and Oyster Tissues**

The biodiversity indices of bacteria in water and oyster tissues are as shown in tables 3 and 4 respectively.

Margalef index of water ranged between 1.70 and 1.92 with the overall mean of 1.789±0.13 while that of oyster tissue

ranged between 1.71 and 2.70 with the overall mean value of 2.273±0.50 (Tables 3 and 4). Station 3 had the lowest Margalef values for both water (1.702) and oyster tissues while other stations remain uniform.

Table 3: Biodiversity indices of Bacteria in Water

Indices/Station	1	2	3	Mean	Minimum-Maximum
Margalef (d)	1.733	1.733	1.702	1.789±0.13	1.70-1.92
Menhinick (d <sub>1</sub> )	4.189	4.665	4.151	4.335±0.29	4.15-4.67
Shannon diversity (H <sup>1</sup> )	2.134	2.287	2.153	2.190±0.08	2.13-2.29
Shannon Wiener (H)	0.927	0.922	0.935	0.928±0.01	0.92-0.94
Evenness/Equitability (E)	0.971	0.922	0.980	0.958±0.03	0.92-0.98
Simpson Dominance(C)	0.125	0.113	0.121	0.120±0.01	0.11-0.12

Table 4: Biodiversity indices of Bacteria in Oyster tissue

Indices/Station	1	2	3	Mean	Minimum-Maximum
Margalef (d)	2.702	2.394	1.723	2.273±0.50	1.72-2.70
Menhinick (d <sub>1</sub> )	6.382	5.598	4.176	5.385±0.12	4.18-6.38
Shannon diversity (H <sup>1</sup> )	2.592	2.489	2.187	2.423±0.21	2.19-2.59
Shannon Wiener (H)	1.126	1.057	0.950	1.044±0.09	0.95-1.13
Evenness/Equitability (E)	0.982	0.982	0.996	0.958±0.04	0.91-0.98
Simpson Dominance(C)	0.078	0.911	0.113	0.367±0.47	0.08-0.91

Menhinick index of water ranged from 4.15 to 4.67 with the overall mean value of 4.335±0.29 while that of oyster tissues ranged from 4.18 to 6.38 with the overall mean value of 5.385±0.12. Margalef values for water and oyster tissues were lowest in station 3 but highest in station 2.

Shannon diversity index (H<sup>1</sup>) of water ranged between 2.13 and 2.29 with the overall mean value of 2.190±0.08 while that of oyster tissues ranged from 2.19 to 2.59 with the overall mean value of 2.423±0.21. Shannon diversity index of water was lowest in station 1 and highest in station 2 while that of oyster was lowest in station 3 but highest in station 1 (Tables 3 and 4).

Shannon Wiener index(H) of water ranged from 0.92 to 0.94 with the overall mean value of 0.928±0.01 (Tables 3 and 4). The values for water and oyster tissues were lowest in station 2 and 3 (0.922, 0.950) but highest in station 3 (0.935) and 1(1.126) respectively.

Evenness index (E) of water ranged between 0.92 and 0.98 with the overall mean value of 0.958±0.03 while that of oyster tissues ranged from 0.91 to 0.98 with the overall mean value of 0.958±0.04 (Tables 3 and 4) which are equal. The lowest evenness index for water was in station 2(0.922) with the highest value in station 3(0.980) while the lowest for oyster tissues was observed in stations 1 and 2(0.982) while the highest was observed in station 3(0.996).

Simpson dominance index(C) for water ranged from 0.11 to 0.12 with the mean value of 0.120±0.01 while that of oyster tissues ranged between 0.91 and 0.98 with the mean value of 0.958±0.03 (Tables 3 and 4).

#### IV. DISCUSSION

The highest number of bacteria count in station 2 than stations 1 and 3 which was statistically different at  $p < 0.05$  in this study confirmed the assertion by Ikpesu *et al*(2017) that areas with high bacteria load usually receive allochthonous materials (domestic and industrial wastes).The higher bacterial load in oyster tissues than water in this study is in line with the assertions that bivalve's mollusks of genus *Crassostrea thizophorae* generally have high microbial load since they build great communities both in estuary edges and stick to the substrate where there are numerous microbes (Sroczyńska *et al*, (2012), they filter roughly 100l of water per day and therefore absorb toxins, pollutants and high level of microorganisms from the water (Suplicy (2000) and that their concentration of biotic and abiotic elements is a reliable indicator of environmental conditions. The highest bacterial count observed in the month of March in this study is in line with the finding of Ikpesu *et al.*,(2017) where the highest number of bacteria were recorded in March and February. The group and species of bacteria identified in this study tallied with the observation of Ayo and Arotupin (2017) where fifteen species of bacteria were reported from River Owena in Niger Delta and Njoku *et al.*,(2015) where twelve bacteria were reported from fish pond water in Niger Delta. The dominance of bacteria species in this study by *E. coli*, *Proteus sp* and *Streptococcus sp* with *Bacillus sp* being the least is similar to the finding of Ayo and Arotupin (2017) but contrary to the finding of Ikpesu *et al.*,(2017) where the dominance of *Bacillus sp* was attributed to their ability to survive as aerobic or facultative anaerobic microbes.

The diverse groups of bacteria isolated from these water and oyster tissues are in line with the report of Okpokwasili and



Ogbulie (1999) who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principal source of bacteria of health importance and Dabbor (2008) who reported similar organisms in the microbiological study of El-quanter fish pond. This is also in line with the assertion that fishery products have been recognized as a major carrier of food-borne pathogens (Yucel and Balci, 2010, FAO,2011). The presence of pathogenic microorganisms especially *E.coli*, *Salmonella*, and *Shigella* can lead to the transmission of water and food-borne diseases such as, Typhoid fever, Cholera, food poisoning and gastroenteritis (Piet, 2009) on consumption of improperly cooked fish from this environment.

Fapohunda *et al.*,(1994) and Van- DUIJN(1973) opined that the presence of the coliform group of bacteria, mainly *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella* in fish and fish products presents a health hazard to humans. Allen and Hopher (1969) and Allen *et al.*,(1979) stated that most of the epidemics attributed to wastewater sources are from raw sewage gaining access to food eaten directly by man, or from contamination of water supply systems by untreated sewage. Olayemi *et al.*,(1991) reported earlier that the presence of faecal coliform in fish intended for human consumption may constitute a potential danger not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from non-aquatic sources. Some human pathogens such as *Aeromonas*, *Escherichia*, *Klebsiella*, *Pseudomonas* and *Salmonella* have been found to survive and multiply in the gut, mucus and tissues of fish and thus render fish a potential vector of human disease over long periods as opined by Allen and Hopher (1969).

A diversity index is a quantitative measure that reflects how many different types (such as species) that are in a dataset, and simultaneously takes into account how evenly the basic entities (such as individuals) are distributed among these types.

Ecological indices such as Margalef and Menhinick measure richness of species in an ecosystem. Margalef index has no limit value and it shows a variation depending upon the number of species hence used for comparison of sites (Kocatas 1992) and takes only one component of diversity (species richness) into consideration reflecting sensitivity to sample size. Menhinick index, like Margalef's index, attempts to estimate species richness but at the same time it is independent on the sample size. The higher values of Margalef, Menhinick's Shannon Wiener and all other indices except evenness indices of oyster tissue than water in this study could be attributed to more number of species in oyster tissues than water as confirmed by Ravera (2001) and Otene and Alfred-Ockiya (2019). The consistently higher values of Margalef and Menhinick's indices of water and oyster tissues in stations 1 and 2 in this study could be attributed to high level of microbial population and pollution resulting from

environmental degradation due to anthropogenic activities in the area.

Shannon and Weiner index (1949) represents entropy. It is a diversity index taking into account the number of individuals as well as the number of taxa. It varies from 0 for communities with only single taxa to high values for community with many taxa each with few individuals. This index can also determine the pollution status of a water body. Normal values range from 0 to 4. This index is a combination of species present and the evenness of the species. Examining the diversity in the range of polluted and unpolluted ecosystems, Wilham and Dorris (1968) concluded that the values of the index greater than 3 indicate clean water, values in the range of 1 to 3 are characterized by moderate pollution and values less than 1 are characterized as heavily polluted. In this present study, based on the above classification the water and the oysters are considered to be heavily and moderately polluted since their values were below one (1) and range of 1-3 respectively as confirmed by Davies and Otene (2009). The higher values of Shannon-Wiener index in this study with respect to bacterial load in oyster tissues across the stations (especially stations 1 and 2) satisfied the assertion by Davies and Otene (2009) that they are indicators of environmental pollution. Shannon-Wiener index obtained in this study is lower than the value (3.90) reported by Antal and Jospheh (2015) in great Kwa River, Cross River State which was attributed to difference in environmental factors.

The index when applied to the present study indicates that individuals of the community in the creek are not evenly distributed with values ranging from 0.92-0.98 and 0.91-0.98. The consistent fluctuation in value of evenness across station 2 especially in the water sample in this study could be attributed to presence of stress hence the presence of more of the species studied in the station.

The higher Simpson dominance index observed in stations 2 for oyster tissue and low value in station 1 for water clearly satisfied the assertion by Whitaka (1965) that Simpson diversity index is usually higher where community is dominated by less number of species and when the dominance is shared by large number of species.

## V. CONCLUSION/RECOMMENDATION

It could be concluded based on the result the creek system is moderately to heavily polluted microbially yet the water and oysters are sources of livelihood for the inhabitants. Therefore, there is need to avoid further contamination/pollution of the area.

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