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Spatial distribution and abundance of bacteria and phytoplankton in Calabar River, cross river state, Nigeria

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Abstract
Spatial distribution of bacteria and phytoplankton in Calabar River was studied for three months (January to March, 2015). Water samples were collected from the three sampling stations (Nsidung, Adiabo and Esuk-Utan) and preserved in the laboratory for phytoplankton and bacteria analysis. A total of thirty five (35) species of phytoplankton were recorded during the studies with Bacillariophyceae having the highest numerical abundance followed by Cyanophyceae, Chlorophyceae, Dinophyceae, Euglenophyceae, Xanthophyceae and Chrysophyceae. There was significant variation ($p<0.05$) in distribution of phytoplankton in the three stations of the River. A total of eighteen (18) species of bacteria were isolated (Cyanobacteria 9 and Heterotrophic bacteria 9). Heterotrophic bacteria were higher in numerical abundance than Cyanobacteria. There was significant variation ($p<0.05$) in distribution of bacteria in the three stations of Calabar River. A positive correlation ($r^2 = 0.4$, $p<0.05$) was observed between composition of phytoplankton and bacteria in Calabar River with correlation coefficient $r = 0.63$. Based on these findings, it was observed that phytoplankton diversity in the River system can contribute significantly to the sustenance of fishery. Control of human activities and prevention of sewage entry into water bodies are to be key measures to avoid fecal pollution and transmission of water borne related infections.

Keywords: Spatial distribution; Abundance; Bacteria; Phytoplankton; Calabar River; Nigeria.

1. Introduction
Bacteria are microorganisms whose single cells have neither a membrane-enclosed nucleus nor other membrane-enclosed organelles like mitochondria and chloroplast. In aquatic ecosystem, bacteria occur naturally but are unable to manufacture their own food, hence are dependent on organic compounds as a source of energy (Imshenetski, 2010) [25]. They reproduced more rapidly in favourable environment, rich in organic nutrients from anthropogenic contaminant (Martin and Blanchi, 1980) [32].

In natural setting, organic compounds are also fixed by phytoplankton, photosynthesizing microscopic aquatic flora that are found drifting on the euphotic zone of the river water. Degradation of this organic matter contributes to the purification of the ecosystem and is, therefore a major process controlling water quality (Mokbel and Yamakanamerdi, 2008) [33].

The role of the microbial loop in aquatic environment is of utmost importance. Bacteria as the first biological component of the microbial loop are controlled either by substrate limitation or by grazing pressure primarily from protists. Thus, the investigation of the relationship between the members of the microbial loop-phytoplankton, bacteria, micro zooplankton allows us to understand better the transfer of energy in the marine ecosystem where the microbial food web is of importance atleast for a certain period of the year.

Numerous studies on coastal water bodies in Nigeria have been investigated. The distribution of bacteria in Nigerian river system has been reported by (Olayemi, 1994; Edun and Efiuvwevuere, 2012; Adesalu *et al*., 2010; Omoigberale *et al.*, 2013) [35, 17, 1, 4]. Studies on phytoplankton distribution and diversity has been reported by several authors (Eyo *et al*., 2013; Dimowo, 2013; Eni *et al.*, 2014; Ewa *et al.*, 2013; Ekwu and Sikoki, 2006; Ezekiel *et al.*, 2011) [23, 16, 21, 22, 20, 24]. Relationships between bacteria and phytoplankton in aquatic ecosystem have been reported by (Bird and Kalff, 1984; Cole *et al*., 1988; Coffin and Sharp, 1987; Kirchman and Hoch, 1988; Ducklow and kirchman, 1985; Plummer *et al*., 1987; Bent and Goulder, 1981; Bell and Albright, 1981; Cammen and Walker, 1982; Albright, 1983) [9, 14, 13, 30, 15, 37, 8, 7, 11, 5].

In Calabar River, little or no work has been reported on bacteria-phytoplankton relationship. The present research opts to bridge this gap and provide baseline knowledge on spatial distribution of bacteria and phytoplankton in the Calabar River.

2. Materials and Method

2.1 Study Area

The study area lies geographically between latitude 4º50'N and longitude 8º10'E, located in Cross River System, South Eastern Nigeria. It encloses Esuknsidung in Calabar South and Adiabobridge in Odukpani local government area. The Calabar river takes its rise from the Oban hills in Akampa, Nigeria and flows southwards through the high rain forest of the south east coast of Nigeria before discharging into the Cross River Estuary at Calabar (Ewa *et al*., 2013) [22].

Climate of the area is characterized by a long wet season from April to October and dry season from November to March. Mean annual rainfall is about 2000mm (Akpan and Offem, 1993) [4]. A short period of drought occurs in the wet season around August/September which is called the August drought. There is a cold dry and dusty period between December and January referred to as the Harmattan season. Human activities in the area include timber logging, fishing and sand mining (Fig. 1).

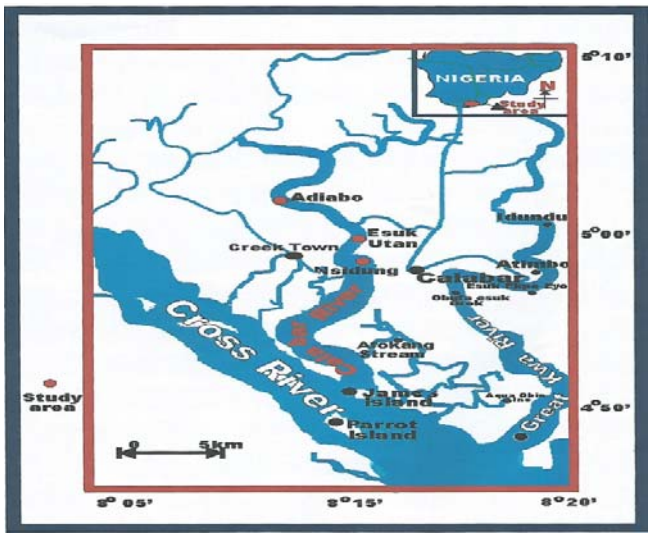

Fig 1: Map of the Study Area

2.2 Sampling Locations

Three sampling sites were used for the studies namely - Nsidung (Latitude 4º 20' N and longitude 8º 20'E), Adiabo (Latitude 4º 56' N and longitude 8º 17' E) and Esuk-Utan (Latitude 4º 58' N and longitude 8º 18' E).

2.3 Collection of Samples

Water samples were collected from the three sampling stations (Nsidung, Adiabo and Esuk-Utan) of Calabar River, twice in a month for continuous three months (January-March, 2015). Sampling was carried out between 07.00 to 11.00 hrs on each sampling day.

2.3.1 Water Samples for Bacteria Analysis

Sterile polyethylene bottles were used to collect sample water from the river at the three studied stations (Nsidung, Adiabo and Esuk-Utan). The samples bottles were plunged down below the water surface, some space was allowed for the mixing of the sample before capping. All samples were stored in an ice chest and transported to the laboratory within 3 hours to avoid any multiplication of the microorganism due to long intervals between the sampling and the laboratory analysis.

2.3.2 Water Samples for Phytoplankton Analysis

Quantitative phytoplankton samples were collected by filtering 100 liters of water fetched with a rubber bucket through 55 µm mesh standard plankton hydrobios. Phytoplankton samples were preserved in 4% buffered formalin solution and stored in 500ml plastic sample bottle before transporting them to marine biology laboratory, University of Calabar for phytoplankton analysis.

2.4 Laboratory Analysis

2.4.1 Bacterial Analysis

Nutrients Agar was used in isolating bacteria from the water samples. 28 grams of the agar were suspended in 1 liter of distilled water in a conical flask. This dissolved completely by boiling over a flame and sterilizing by autoclaving at a temperature of about 21ºC at 15 Ib pressure per square inch for 5 minutes (Cheesebrough, 2000) [12].

2.4.2 Isolation of Pure Cultures (Pour-Plate Method)

Serial- dilutions of water samples were made using sterilized water. Dilutions of 10^{-3} were used to prepare pour-plate in triplicate. Thus 1ml of dilution for each sample was placed on 3 petri-dishes using a pipette. 10 ml of the liquefied culture medium cooled to 45^0C were added after first flaming the mouth of the test tube. For, this purpose, the lid of the petri-dish were raised but not completely removed. The culture medium and water were mixed by circular movements of the dish, then the dish was stored on the horizontal surface and the layer of the culture medium was allowed to set. The plate was later incubated in the incubator at the temperature of 30^0C for 48 hours.

2.4.3 Counting of Colonies

Colonies observed at the end of incubation were enumerated using a Gallenkamp electronic colony counter (model NO. 3340910E) at the end of which the number of colonies per milliliter of samples was recorded.

2.4.4 Identification of Bacteria Isolates

The bacteria isolates were subjected to various tests such as growth morphology in different agar media and different microbiological identification tests such as gram staining and motility tests and biochemical identification tests such as catalase, coagulase, oxidase, citrate utilization, urease, indole production, hydrogen sulphide production, nitrate and nitrite reduction, methyl red, vogesproskeur and sugar fermentation tests.

2.4.5 Phytoplankton Analysis

In the laboratory, quantitative sample from the three stations were concentrated to 10ml. 1ml from each sample was taken and all individual taxa present were counted. Specimens were sorted, counted using Zeiss binocular microscope at different magnifications **(**x40, x100 and x400). Lugol's solution was used for staining the samples to enhance proper discernment of the phytoplankton species based on morphological features, as individual species normally takes up the stain, thereby exposing the organelles for proper identification according to Akpan, (1994) [3]. Phytoplankton was identified using relevant

literatures (Botes, 2003; John *et al*., 2003) [10, 26].

2.5 Data Analysis

Data obtained from each bacteria and phytoplankton group were empirically analyzed using the formula:

$\% R_a = {}^{n}/_{N} \times 100$ (Ali *et al*., 2003) [6].

Where: $\%_{Ra}$ = relative abundance

N = number of individuals

N = total number of all individuals.

Margalef's diversity index d was used in determining the very current ecological status of the river using the formula (Margalef 1978; Ogbeibu, 2006) [31, 34].

$$d = \frac{S-1}{\ln(N)}$$

Where: S = number of taxa in each phytoplankton and bacteria family

N = total abundance in each phytoplankton and bacteria family

ln = natural or Naperian logarithm ($\log_e$).

Shannon-wiener index was used to determine the species density of the bacteria and phytoplankton species using the formula (Ogbeibu, 2006) [34].

$$H = \frac{N \log N - fi \log fi}{N}$$

Where H = Shannon-wiener index

N = Numerical abundance of all phytoplankton and bacteria assemblages/families

Fi = number of each phytoplankton and bacteria family.

One-way analysis of variance (ANOVA) powered by (SPSS, version 20.0) was used to test for significant spatial variation in the distribution pattern of bacteria and phytoplankton in Calabar River using data collected from the three stations. Pearson's moment product correlation analysis was used to ascertain the relationship between abundance of bacteria and phytoplankton in order to establish how the presence of one variable affects the other.

3. Results

3.1 Distribution of Phytoplankton

During the three months survey, a total of thirty five (35) species of phytoplankton of Calabar River was recorded as shown in Table 1 and 2. The dominant phytoplankton were the Bacillariophyceae with 98 individuals representing (33.79%), followed by Cyanophyceae with 70 individuals (24.14%), Chlorophyceae with 50 (17.24%), Dinophyceae with 30 (10.34%), Euglenophyceae with 23 individuals representing (7.93%), Xantophyceae with 13 (4.48%) and the least been Chrysophyceae with 6 individuals representing (2.07). Margalef's diversity index d, ranged between 0.39 and 1.74 with a mean of 1.09, while Shannon-wiener index ranged between 1.79-2.45, with a mean of 2.21 indicating that the phytoplankton were densely distributed in the river (Table 2). There was significant variation ($P<0.05$) in distribution of phytoplankton in the three stations of Calabar River.

3.2 Distribution of Bacteria

A total of 18 species of bacteria were isolated during the study (Cyanobacteria 9 and Heterotrophic bacteria 9) as shown in table 3 and 4. Heterotrophic bacteria were higher in numerical abundance with 174 individuals representing (65.66) than Cyanobacteria which had 91 individuals representing (34.34). Margalef's diversity index d, ranged between 1.55 and 1.77 with a mean of 1.66, while Shannon-wiener index ranged between 0.95-1.75, with a mean of 1.35 indicating that the bacteria were densely distributed in the river (Table 4). There was significant variation ($P<0.05$) in distribution of bacteria in the three stations of Calabar River. Total viable count of bacteria during the study ranged from 2.03×10^5 to 3.67×10^5 cfu/ml in Nsidung, 3.43×10^5 to 5.85×10^5 cfu/ml in Adiabo and 5.35×10^5 to 6.97×10^5 cfu/ml in Esuk- Utan. All values were higher than WHO standard of 1.0×10^5cfu/ml.

3.3 Relationship between Phytoplankton and bacteria abundance in Calabar River

A positive relationship ($r^2= 0.4$, $p<0.05$) was observed between composition of phytoplankton and bacteria in Calabar River with correlation coefficient $r= 0.63$. This shows that the abundance of bacteria does not depend only on the presence of phytoplankton alone but also from anthropogenic source. However, both phytoplankton and anthropogenic sources contributed to the total burden of bacteria in Calabar River.

Table 1: Spatial Distribution of Phytoplankton in Calabar River, Nigeria (January – March, 2015).

	Species	Nsidung (ST.1)	Adiabo (ST.2)	EsukUtan (ST.3)	Numerical Abundance (n)	Relative Abundance (%n)
Bacillariophyceae (Diatoms)	*Actinocyclus species*	-	5	7	12	12.24
	Bacillaria paradoxa	-	6	8	14	14.29
	Cydotallacomta	-	5	4	9	9.18
	Melosira granulate	-	5	5	10	10.20
	Surirella oblonga	-	5	3	8	8.16
	Surirella striatula	-	4	6	10	10.20
	Nitzetriasigmoidae	5	6	8	19	19.39
	Nitizohia species	-	-	6	6	6.12
	Fragilaria species	-	6	4	10	10.20
Total abundance (N)					98	99.98
Chlorophyceae (Green algae)	*Staurastrum apiculatus*	-	-	3	3	6.0
	Eudorina elegans	4	3	5	12	24.0
	Closterium gracile	-	-	4	4	8.0

	Chlorocoum species	3	6	2	11	22.0
	Acanthosphaera	-	12	8	20	40.0
Total abundance (N)					50	100.0
Cyanophyceae (Blue green algae)	*Anabaena affinis*	-	5	6	11	15.71
	Anacystis cyanea	4	-	2	6	8.57
	Oscillatoria sancta	8	11	8	27	38.57
	Phormidium ambiguum	-	4	8	12	17.14
	Phormidium Cincinnati	-	-	4	4	5.71
	Microcystic species	2	3	5	10	14.29
Total abundance (N)					70	99.99
Dinophyceae	*Gymnodinium species*	-	4	3	7	23.33
	Gyrodinium species	8	-	4	12	40.0
	Peridinium species	-	2	3	5	16.67
	Ceratium hirundinella	-	6	-	6	20.0
Total abundance (N)					30	100.0
Euglenophyceae(Green flagellates)	*Euglena acus*	-	-	7	7	30.43
	Euglena gracilis	-	-	2	2	8.70
	Phacus caudate	-	2	1	3	13.04
	Phacuslongicaudata	2	3	-	5	21.74
	Cyptoglena species	-	-	4	4	17.39
	Trachelomonas species	-	-	2	2	8.70
Total abundance (N)					23	100.0
Xanthophyceae	*Chlorocloster species*	2	2	3	7	53.85
	Monocilia species	-	-	6	6	46.15
Total abundance (N)					13	100.0
Chrysophyceae	*Pyaetharminon species*	-	3	1	4	66.67
	Chrysops species	-	-	2	2	33.33
Total abundance (N)					6	100.0

Table 2: Summary of the Spatial Distribution of the Major Phytoplankton in the Calabar River, Nigeria during the Study Period (January – March, 2015).

S/N	Phytoplankton families	Numerical Abundance (n)	Number of species (S)	(%n)	D	H
1.	Bacillariophyceae	98	9	33.79	1.74	1.79
2.	Chlorophyceae	50	6	17.24	1.28	2.17
3.	Cyanophyceae	70	6	24.14	1.18	2.02
4.	Dinophyceae	30	4	10.34	0.88	2.31
5.	Euglenophyceae	23	6	7.93	1.59	2.35
6.	Xanthophyceae	13	13	4.48	0.39	2.41
7.	Chrysophyceae	6	2	2.07	0.56	2.45
	Total Abundance (N)	290	35	99.99	ΣD=7.62	ΣH=15.5

Table 3: Spatial Distribution of Bacteria in Calabar River, Nigeria (January – March, 2015).

Taxonomy	Species	Nsidung (ST.1)	Adiabo (ST.2)	EsukUtan (ST.3)	Numerical Abundance (n)	Relative Abundance (%n)
Cyanobacteria	*Anabaena affinis*	4	5	-	9	9.89
	Anabaena spiroides	-	6	4	10	10.99
	Anacystis cyanea	-	4	-	4	4.40
	Chroococcus species	6	5	3	14	15.38
	Gomphospharia aponia	-	2	-	2	2.19
	Oscillatoriaterius	3	4	2	9	9.89
	Oscillatoria santa	-	3	-	3	3.30
	Spirulina major	4	6	10	20	21.97
	Phormidium ambiguum	6	8	6	20	21.97
Total abundance (N)					91	99.98
Heterotrophic bacteria	*Staphylococcus species*	12	8	11	31	17.82
	Escherichia coli	8	6	4	18	10.34
	Micrococcus species	-	5	6	11	6.32
	Streptococcus faecalis	6	8	8	20	11.49
	Bacillus species	8	6	-	14	8.05
	Salmonella species	12	14	6	32	18.39
	Pseudomonas species	6	4	-	10	5.75
	Aeromonas species	12	8	8	28	16.09
	Achromobacter species	-	6	4	10	5.75
Total abundance (N)					174	100.0

Table 4: Summary of the Spatial Distribution of Bacteria in the Calabar River, Nigeria during the Study Period (January – March, 2015).

S/N	Bacteria Families	Numerical Abundance (n)	Number of species (S)	(%n)	D	H
1.	Cyanobacteria	91	9	34.34	1.77	1.75
2.	Heterotrophic bacteria	174	9	65.66	1.55	0.95
	Total Abundance (N)	265	18	100.0	ΣD=3.32	ΣH=2.7

Table 5: Total Viable Counts (TVC) (cfu/ml) of Calabar River, Nigeria during the Study Period (January – March, 2015).

Sampling Stations	January	February	March	WHO 1993
Nsidung (ST.1)	2.40×10^5	2.03×10^5	3.67×10^5	1.0×10^2
Adiabo (ST.2)	6.44×10^5	5.35×10^5	6.97×10^5	1.0×10^2
Esuk- Utan (ST.3)	4.77×10^5	3.43×10^5	5.85×10^5	1.0×10^2

4. Discussion

Results obtained in the present study clearly indicate that phytoplankton species diversity varied with sampling points as observed in the results. This observation is similar to findings of Sekadende *et al*., (2004) [38] who reported that phytoplankton diversity varied with sampling stations and season in the satellite lakes of Lake Victoria basin.

Frequency of occurrence of phytoplankton showed that the group Bacillariophyceae (diatoms) had nine (9) species, followed by Chlorophyceae, Cyanophyceae and Euglenophyceae which had six (6) species each respectively. Dinophyceae had four (4) species with two species each recorded for Xantophyceae and Chrysophyceae.

The variations in frequency of occurrence of phytoplankton in Calabar River could be attributed to the facts that most diatoms are capable of surviving under fluctuations in salinity. A total of thirty five (35) phytoplankton species recorded for seven families in this study is different from findings of Ekwu and Sikoki, (2006) [20] who recorded a total of 105 species of phytoplankton, belonging to five (5) families during the study in the Cross River Estuary. However, percentage numerical abundance of Bacillariophyceae in the present study is in consistent with the findings of Ekwu and Sikoki (2006) [20] who recorded the family Bacillariophyceae (diatom) as the most abundant with 63 species in the Cross River Estuary. Several authors including Akpan (1993, 1994) [2, 3]; Ekeh and Sikoki (2004) [19] have reported similar findings of Bacillariophyceae abundance in Nigerian Coastal waters and other water bodies. According to Egge and Aksnes, (1992) [18], growth of diatoms depends on the presence of silicates which is evident in the siliceous cell wall found in diatom. These findings indicate the presence of silicates in three sampling stations in the Calabar River. These findings were in accordance with the findings of Ekwu and Sikoki (2006) [20] which attributed the abundance of diatoms in the Cross River Estuary to higher concentration of silicates in this zone and also corroborates with findings of Akpan (1993, 1994) [2, 3], who reported strong correlation between silicates and diatom abundance.

In this study, the family Cyanophyceae was the second most dominant family with seventy (70) individuals, followed by Chlorophyceae, Dinophyceae, Euglenophyceae, Xantophyceae and Chrysophyceae. Our findings were in contradiction to the observation of Kebede and Belay (1994) [29]; Kadiri (1999) [27]; Kadiri and Omozusi (2002) [28], who observed more Chlorophyta than diatoms and very few Cyanophytes in some tropical water bodies. Values obtained for percentage occurrence of phytoplankton in the three sampling stations were similar to results reported by Eni *et al.*, (2012) [21] who attributed such findings to good ecological condition arising from important factor governing the abundance and distribution of the phytoplankton communities such as food availability.

Eighteen bacteria species were isolated in the River which belong to two families; Cyanobacteria and Heterotrophic bacteria. The presence of fecal coliform bacteria such as *Staphylococcus species*, *Escherichia coli, Streptococcus faecalis* and *Salmonella species* in Calabar River signals fecal contamination from human discharge into the river. This finding agrees with the work of Omoigberale *et al*., (2013) [36] who reported similar findings in Ebute River.

The total bacterial counts (TVC) for all the water samples obtained from the three stations were generally high exceeding the WHO limit of 1.0×10^2 cfu/ml which is the standard limit of TVC for drinking water (WHO, 1993) [39]. TVC is indicative of the presence of high organic and dissolved salts in the water. The primary sources of these bacteria in water are animal and human activities. Adiabo station showed the highest TVC value which is an indicative of high human and animal activity. This result is in accordance with the findings of Olayemi, (1994) [35].

A positive relationship was observed between composition of phytoplankton and bacteria in Calabar River. This shows that the abundance of bacteria does not depend only on the presence of phytoplankton alone but also from anthropogenic source. However, both phytoplankton and anthropogenic sources contributed to the total burden of bacteria in Calabar River. This finding is in consonance with Bird and Kalff (1984) [9] and Cole *et al*., (1988) [14], who reported significant correlation between bacteria and phytoplankton variables both in fresh and marine waters, thus suggesting the ubiquity of a functional relationship between bacteria and phytoplankton. Several researchers have suggested a trophic relationship between bacteria and phytoplankton in estuarine ecosystem (Coffin and Sharp, 1987; Kirchman and Hoch, 1988) [13, 30].

5. Conclusion

Based on these findings, it is therefore concluded that phytoplankton diversity in Calabar River can contribute significantly to the sustenance of fishery. Also the presence of feacal bacteria is an indication of fecal pollution. It is evident that water borne diseases are due to improper disposal of refuse and contamination of water by sewage and surface runoff. Control of human activities to prevent feaces and refuse from entering water body is the key to avoiding feacal pollution of water bodies. This study strongly recommends that government and other stakeholders should provide sanitary facilities especially in the rural areas to control River pollution. Also appropriate water treatments or safe portable water sources should be provided in the area to improve the welfare of the riverine dwellers. There is also need to educate the villagers on how to handle and locally treat water for

domestic use. The government should evolved sanitation programmes and propagates these through environmental education throughout the communities in the River catchments areas to prevent pollution of water bodies and consequent transmission of water- related diseases.

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