IMPACT OF HUMAN ACTIVITIES ON THE ABIOTIC CONDITIONS AND MYCOFLORA OF THE WATER

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ABSTRACT

New Calabar River and the Omuihuechi Stream are important sources of water in Southern Nigeria. They receive urban contaminants and wastes from automobile and marine fuels, heavy oils, spent lubricants and other petroleum products, untreated sewage, human and animal faeces and various kinds of domestic, agricultural and industrial waste. The impact of human activities on the abiotic quality and mycoflora of the New Calabar River and the Omuihuechi Stream were investigated for a period of seven months. Sampling and measurements were conducted within three designated zones. Five sampling stations (A, B, C, D and E) along the course of the River are grouped in Zone I. Station F is in Zone II while station G (control) constitutes Zone III The samples were assessed for Temperature, Flow Velocity, Secchi-disc Transparency, Sulphate, Phosphate, Dissolved Oxygen, BOD, total organic carbon, Oil and Grease concentrations. The forest stream acidity (pH 5.33 ± 0.29) was not significantly different from the River acidity (pH 5.47±0.37). The River flow velocity was 0.213 ± 0.015ms^{1}. The following fungal genera were identified: *Aspergillus, Byssochlamys, Candida, Cephalosporium, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Saccharomyces, Sporobolomyces and Trichoderma*. Zone II was observed to show a high tendency of eutrophication than zone 1 while zone III (control) was free from such influences. There were significant differences in Fungal count, pH, Organic carbon, BOD_5 and Transparency (P=0.01) between the Zones. The human activities occurring at specific points (stations) along the river course appear to have influenced both biotic and abiotic parameters of the aquatic ecosystem.
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Keywords: Calabar river, Nigeria, fungal population, human activities, Physico-chemistry.

INTRODUCTION

The new Calabar River is a brackish water located in Rivers State of Nigeria (Erondu and Chinda 1991). In spite of its importance there is general dearth of information on its water quality (Nwadiaro and Ezefili 1986; Erondu and Chinda 1991). Considering the arrays of companies sited within this area and the activities carried out by the populace, the river receive contaminants and wastes.
The Omuihuechi stream experiences domestic activities such as bathing, washing of clothes, household utensils and motorbikes. It also serves as source of drinking water for the community. It is important to carry out physicochemical and mycological studies of the New Calabar River and its influent streams to ascertain their water qualities. In addition, fungi are chosen as the biological end-point following a general neglect of fungal studies over the years. Apart from that, fungi are also very important in biogeochemical cycle of nutrients and contribute extensively to the atmospheric content of some gases such as CO, CO_2, SO_2 and halomethane (Harper and Hamilton, 1988; Cracker and Manning, 1970).

Microbial populations are nutrient dependent (Obire *et al.,* 2008). Hence it is necessary to study changes in fungal frequencies with the associated mineral nutrients in the River. The work is therefore investigate physico-chemical parameters (temperature, turbidity, total dissolved solids and pH), anions (sulphates and phosphates); Biological Oxygen Demand, Total Organic Carbon, and Total Oil and Grease. The work will also isolate fungi only since other workers (Odukuma and Okpokwasili, 1993) have looked into the effect of bacteria, faecal and coliforms and determine the contribution of anthropogenic inputs to the quality of these water bodies.

MATERIALS AND METHODS

Description of the study area

The River is one of the major rivers within the Niger Delta region of Nigeria (Fig. 1). It is used as routes and harbor for marine transport boats and barges from oil companies, Oil servicing companies, and marine transport companies. Notable among these companies are Tidex Nigeria Limited, Wilbros Nigeria Limited, Horizon Fibers limited, West Africa oil field services (WAOS), Limited and Trans Coaster Limited.

Description of sampling stations

Following the preliminary survey of the New Calabar River by boat cruise, the Choba-Aluu stretch of the river was chosen as the sampling area for this study (Fig. 2). Seven sampling stations were carefully designated and grouped as Zones I, II and III respectively. Five sampling stations (A, B, C, D and E) along the course of the River are grouped in Zone I. Station F is in Zone II while station G (control) constitutes Zone III

Station A - Horizon Fibers Sampling Station, a Sawmill and Abattoir.
Station B - Wilbros Nig limited Sampling Station, presence of Wilbros Marine boats and oil films on the surface of stems and roots of mangrove trees.
Station C - University of Port Harcourt Delta Park Sampling Station
Station D - Rural Development Centre Sampling Station between two jetties.
Station E - Africa Regional Aquaculture Fish Farm Sampling Station between two

furrows (drainages) which serves as inlet for Fresh River water into the fish farm during high tide.

Station F- Omuihuechi Stream Sampling Station of domestic activities.

Station G - Omuihuechi Stream Sampling Station upstream of station F (Control)

Fig. 1: MAP OF THE LOWER NIGER DELTA SHOWING THE POSITION OF THE STUDY AREA

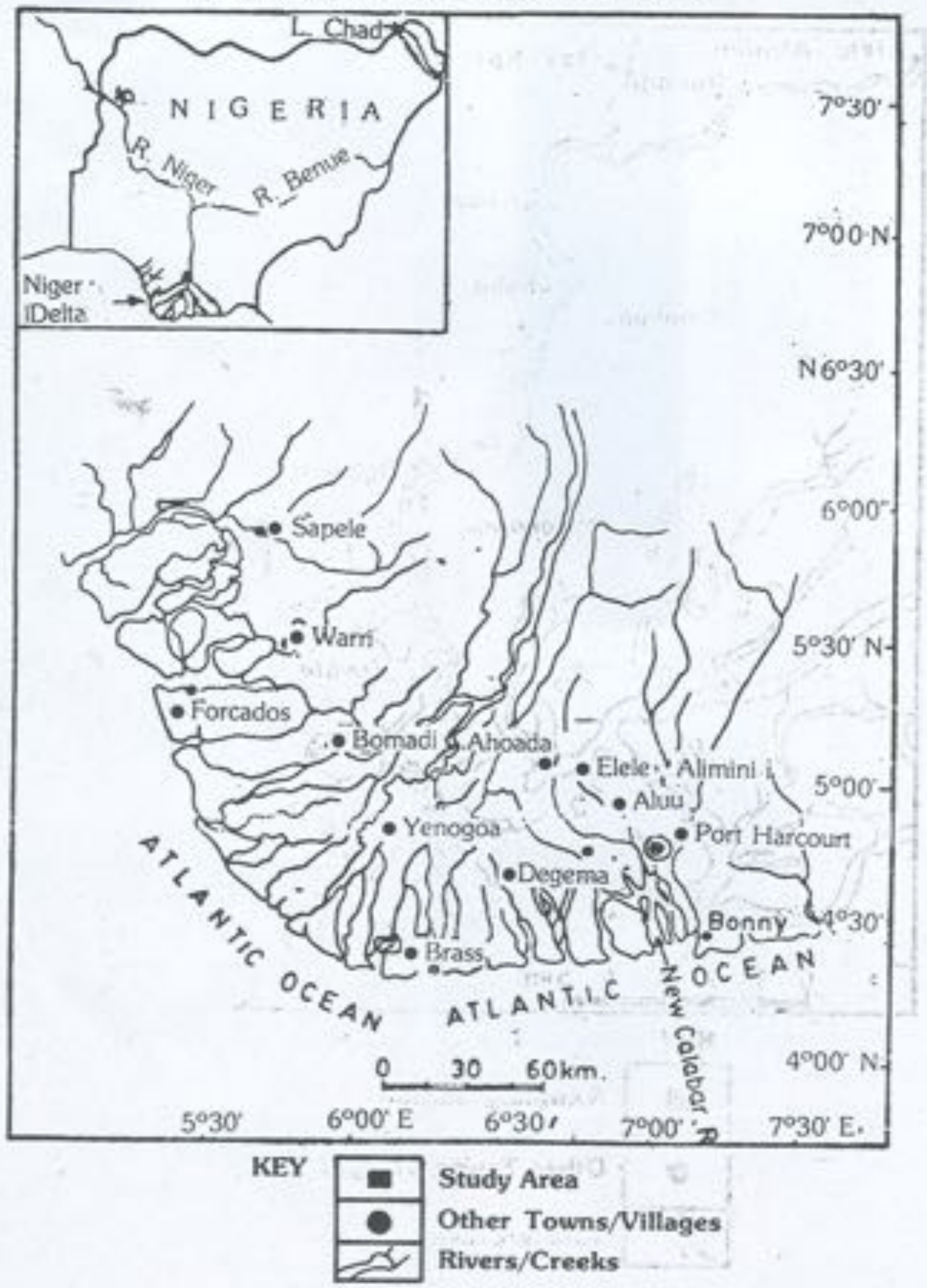

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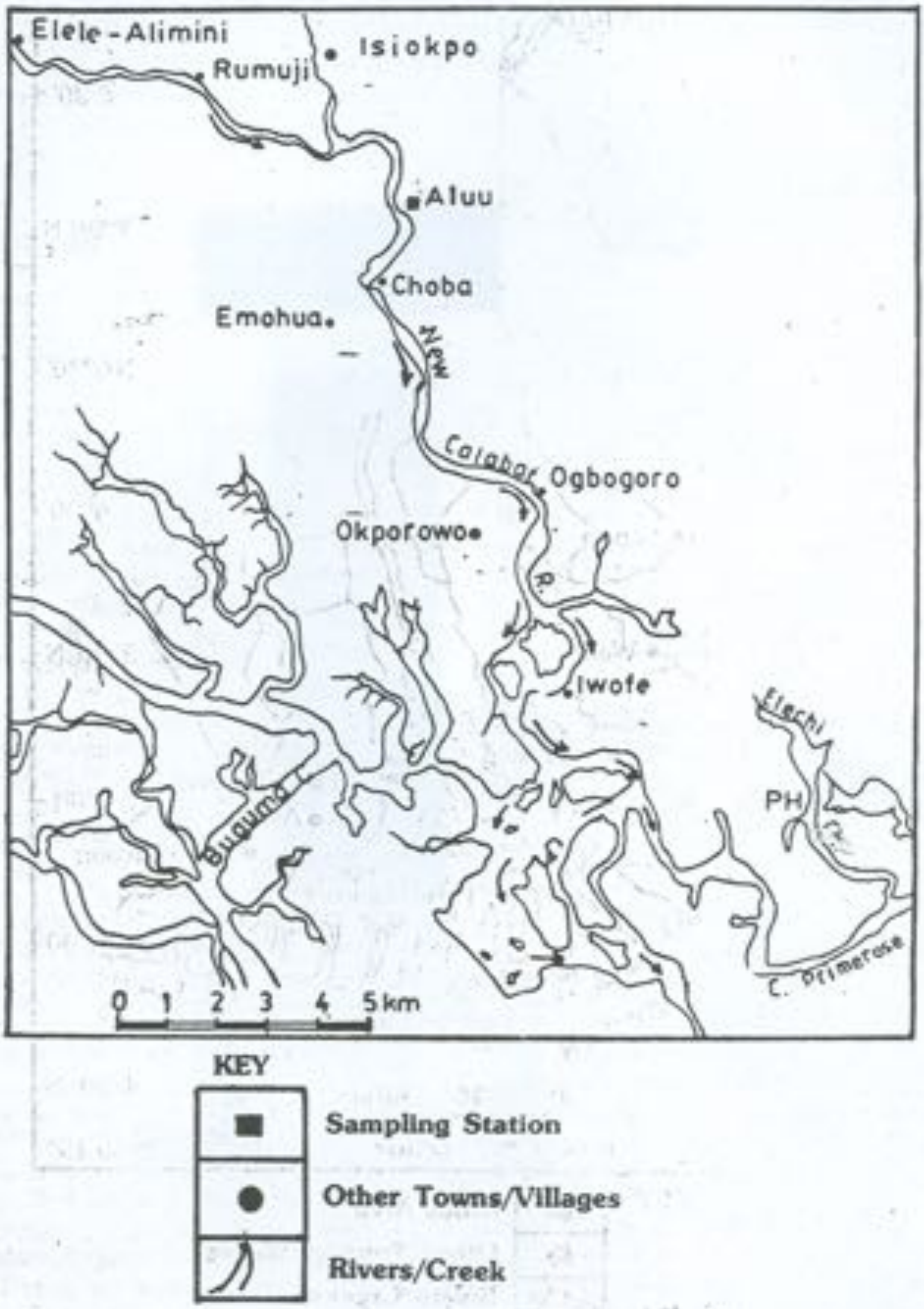
Fig. 2: MAP OF THE NEW CALABAR RIVER SHOWING SAMPLING STATION



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Sample Collection
Four samples were collected from a depth of about 25-30 cm from each sampling station for Microbial assay, two samples were collected for Dissolved Oxygen (D.O) analysis and BOD and one sample was collected for the physico-chemical analyses.

Determination of physicochemical parameters
The following physico-chemical parameters - water temperature, transparency, hydrogen ion concentration, (pH), flow velocity, chloride, sulphate and phosphate, total dissolved solids, total oil and grease, total organic carbon, dissolved oxygen and biological oxygen demand (BOD), Orthophosphate (PO^{3-}), Sulphate (SO_4^{2-}), Total Organic Carbon (TOC), and Oil and Grease were determined according to standard methods of APHA, 1992 which was modified by Obire *et al.* (2008).
The analytical procedures used for the determination of these parameters are modifications from ASTM standards, (1986); APHA, (1992); and AAC (official methods of analyses, Association of Analytical Chemists).
The temperature (°C) reading was taken at each sampling station by dipping a mercury thermometer into each homogenously mixed sample and held for about two minutes. The thermometer was rinsed in buffer solution of pH – 7 before reuse.
The hydrogen ion concentration (pH) of samples from each sampling station was immediately checked on arrival at the laboratory using the pH meter model: JENWAY 3020.
The dissolved oxygen (DO) of the samples was determined using the DO. meter YSI model 51B Oxygen meter.
Conductivity of samples was determined using a digital bench-top conductivity meter model. JENWAY 4010 Conductivity meter.

Total Dissolved Solids (TDS) and conductivity has the following ratio (relationship), 2.2:1, therefore, to determine the TDS, the conductivity value was divided by 2.2 to give the TDS value in mg/l. Total phosphate (T/PO_4) in the samples was determined using the spectrophotometric method. Spectrophotometer model no. UV 160 U, Shimadzu (UV- Visible recording) was used, at 650 nm wavelength.

Microbiological Methods

Fungal Cultivation, Enumeration, Characterization and Identification

Czapek Dox Agar was prepared in accordance with the modification of Czapek solution by Smith (1971) in agar. A 0.1ml aliquot of 10^{-1} dilution was plated onto agar plates and incubated at 27.5°C in an inverted position for 5 days. The discrete colonies that developed were counted and the mean of replicate plates were recorded. The counts were also computed into colony forming units (cfu) of fungi per gram soil. Fungal cultures were observed while still on plates and after wet mount in lacto-phenol on slides under the compound microscope. The observed characteristics were recorded and compared with the established identifications keys of Malloch (1997).

Statistical Analysis.

The analysis of variance (ANOVA) was done according to the method of Steel and Torrie (1980). The mean were later separated using Duncan's Multiple Range Test

RESULTS

The monthly average range of results obtained from measurements and analyses of physico-chemical parameters and fungal counts of samples from the various stations of the New Calabar River are as shown in Table 1.

TABLE 1: Monthly average range of physicochemical parameters and fungal counts of the New Calabar River

STATIONS

Parameter	A	B	C	D	E	F	G
Temp (°C)	24.8-31.4	24.8-31.4	24.8 - 31.4	25-31.35	24.5-31.4	24.4-28.1	23.7-27.6
pH	5.06-5.86	5.07-5.85	5.19 -5.9	5.07-5.87	5.05-8.83	5.11-5.84	4.84-5.31
Transparency	108 – 152	98 - 151	82 – 148	86 - 153	87 – 152	146 – 177	160 - 200
DO	4 -5.7	4.4 – 5.7	3.8 – 5.2	4.1 – 5.6	4.4 – 5.6	2.9 – 5.1	6.8 – 11.9
BOD	26.7 – 31.4	26- 30.5	27.2-32.6	27- 30.4	26.4 – 32.7	19.4-29.5	4.3- 8.6
TOC	24.3-35.1	23.5-34.7	24.7-35.9	25- 34.9	24.8-30.4	17.7-20.1	12.4-14.2
Oil & Grease	0.22-0.81	0.26-0.75	0.19-0.92	0.14-0.85	0.15-0.64	0.16-0.32	ND
Sulphate	8.3- 10.4	8.4- 10.4	8.4- 10.2	6.9- 9.7	7.3- 9.6	7.1- 10.3	6.4- 8.6
Phosphate	0.18-0.23	0.16-0.24	0.18-0.22	0.20-0.24	0.21-0.24	0.27-0.45	0.18-0.31
Fungal count($x10^2$)	2 – 7	2 – 8	2 – 8	2 – 9	2 – 9	8 – 32	7 – 14

Generally, the temperature of the stations ranged from 23.7 to 31.3°C, pH ranged from 4.84 to 5.90, BOD ranged from 4.3 to 32.7mg/l, phosphate ranged from 0.16 to 0.45mg/l, while fungal count ranged from 2 x 10^2 to 32 x 10^2cfu/ml. Statistical analysis of the fungal count revealed the presence of higher significant differences between the stations. The cal F-value was 3.94^{**} against F-tabulated values of 1.95 and 2.60 at 5% and 1% respectively. Total organic carbon (TOC) content, Biological Oxygen Demand and Transparency have highly significant differences between the Zones. The F-Calculated values are 18.3, 42.46 and 19.077 repectively. Water temperature and pH have F-Calculated values of 7.10 and 6.378 respectively.

The identified fungal genera and their isolation frequencies are as shown in figure 1.

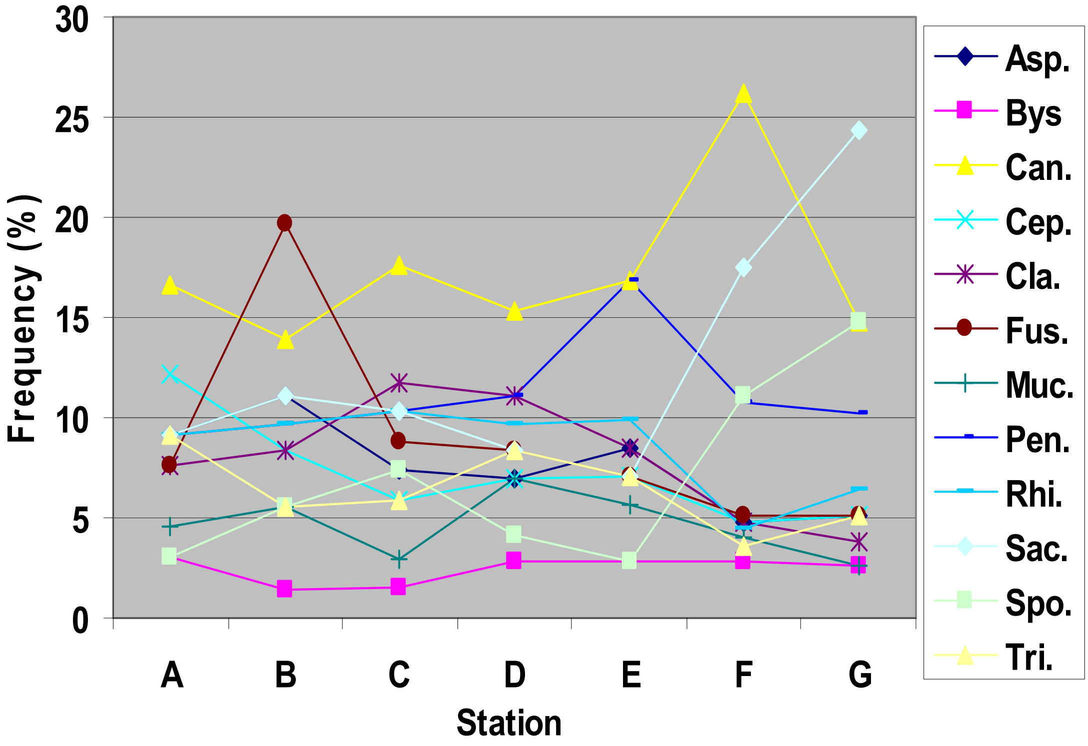

Fig 1: Isolation Frequency of Fungal Genera

Asp = *Aspergillus niger,* Bys. = *Byssochlamys,* Can. = *Candida albicans,* Cep. = *Cephalosporium* spp, Cla = *Cladosporium fulvum, Fus = Fusarium oxysporum,* Muc = *Mucor,* Pen. = *Penicillium chrysogenum,* Rhi. = *Rhizopus,* Sac. = *Saccharomyces,* Spo. = *Sporobolomyces, and* Tri. = *Trichoderma harzianum*.

Identified fungal genera and species were *Aspergillus niger, Byssochlamys, Candida albicans, Cephalosporium, Cladosporium fulvum, Fusarium oxysporum, Mucor, Penicillium chrysogenum, Rhizopus, Saccharomyces cerevisiae, Sporobolomyces and Trichoderma harzianum*. Fungal count was high in Zone II and III than in Zone I, generally up to 10^2cfu/ml. Zone II had the highest frequency of Candida (>25%). The average frequency of *Candida* for the entire zone was 17.29% and was followed by *Fusarium*, 7.37%.

CONCLUSION

This investigation has revealed that, the water temperature of the New Calabar River and its tributary stream ranged from 24.50 ^{o}C to 31.50 ^{o}C, and 22.50 ^{o}C to 28.20 ^{o}C respectively. These temperatures are within the mesophilic range of temperatures i.e. between 20 ^{o}C to 45 ^{o}C. Most fungal species that are involved in environmental biogeochemistry are active within this mesophilic range. Most of the fungal genera isolated are mesophiles. The few thermophilic fungi such as Aspergillus and Mucor can tolerate the mesophilic range of temperature and in-fact, are active within the upper mesophilic range. Erondu and Chinda (1991) recorded an average temperature of 27 ^{o}C $\pm$ 2. ^{o}C. The lower temperature experienced in the forest stream is due the shading effect direct sunlight.

The degree of acidity (pH) of New Calabar River and the forest stream reveals that both waters are extremely acidic. The average pH of the New Calabar River is 5.47 $\pm$ 0.37 while that of Umuihuechi forest stream is 5.33 $\pm$ 0.29. The lower limits for the river was 5.05 in station E (African Regional Aquaculture Fish Farm Sampling Station) and the upper limit was 5.79 in station B (Wilbros Nigeria Limited Sampling Station). This upper limit is a tendency towards neutrality, which is quite high for a black water type draining rain forest. It can be seen as an evidence of the presence of waste materials in the water. For the forest stream the pH of station F (Omuihuechi stream) went as far as 5.84 and was consistently high through most part of the year. This is an indication of low waste assimilation capacity of the stream. The pH of station G upstream of domestic activities on the stream is consistently low and typical of black water type, especially flowing through a matured forest with supply of humic substances.

The acidity of the study area is contributed by sulphuric acid. This is revealed by the result of anion studies during the period, which shows that the sulphate concentration of the river and the stream is high. Gilmour (1992) agreed that sulphuric acid is a major strong acid component of deposition in most industrialized regions of the world and those landmasses downwind of these regions. This is further corroborated by the presence of persistent gas flaring from various oil companies scattered across the Niger Delta. Natural waters with low buffering capacity are most at risk of acidification and its ensuing effects. These effects include changes in biological species composition and densities and changes in biogeochemical cycling of a number of elements (Mitchel, 1992). Low pH was one of the factors associated with general low abundance of Phytoplankton in African black waters including the New Calabar River (RPI, 1985; Adeniyi, 1986; IPS, 1986). It is obvious that the results of fungal count agree with the observed changes in pH value. Hence the fungal populations suffered reduction when the pH was high and increased when pH was low. Fungal sulphate reduction in sediments is capable of generating alkalinity through the reduction of sulphate to sulphide and permanent storage of reduced sulphur in sediments (Mitchel, 1992). Though analysis showed slightly higher sulphate concentration in stations A and B, it is possibly due to absence of dilution with floodwater associated with dry seasons, inflow of blood and animal dung contaminated wastewater from abattoir, the use of this portion for latrine and other human activities taking place within Wilbros and Horizon fibers. Also this portion receives more brackish water compared to other stations as marine water contains more sulphates, which may increase acidity.

It is important to recognize the fact that fish reproduction can be reduced at pH values just above 6. This is significant as the economy of the fishermen in the Choba-Aluu axis depend on fish reproduction.

This investigation reveals that the sulphate ion concentration of the New Calabar River ranged from 7.1mgl^{-1} to 10.4mgl^{-1} with an average of 9.09mgl^{-1}. Generally the range of sulphate ion concentration was not much. Normally SO_4^{2-} is seen as a conservative ion since its natural availability is always more than its demand by living organisms. Hence its concentration did not fluctuate appreciably during the study. At these levels sulphate availability cannot be a limiting factor to productivity in this water. The value of 10.30mgl^{-1} obtained for station F is associated with domestic activities and aesthetic uses of the water, which resulted in introduction of domestic cleaning agents into the water.

The phosphate values obtained in this investigation were generally low. They are however within the range expected of black waters. The concentration for New Calabar River is 0.21mgl^{-1}. The maximum value of 0.24mgl^{-1} was lower than 0.3mgl^{-1} obtained by Odokuma and Okpokwasili (1993). High concentrations recorded in stations D (Rural Development Centre Sampling Station) and E (African Regional Aquaculture Fish Farm Sampling Station) is due to gradual input of phosphates from fish pond fertilizers as reported by Brian (1993), and inputs from tributaries during rainstorm (Antweiler,1995). The forest stream recorded an average concentration of 0.37mgl^{-1} phosphates in station F. This is responsible for the proliferation of fungi shown by the increase in fungal count and the lower dissolved oxygen tension observed at station F. The levels of phosphate are moderately high. The stable level of this ion is an indication that the waste dump and more importantly faeces from human, Cow dung and blood from the abattoir serve as sources of chronic input of waste containing phosphates into the River. Ramesh *et al.,* 1997 oserved that water quality gradually deteriorates monthly due to the effect of organic and metabolic wastes in the farm.

Total Dissolved Solid values were observed to increase moderately from June to September due to increased rainfall bringing in floodwaters. The average TDS is 115.2mgl^{-1} for stations along the new Calabar River and 141.2mgl^{-1} for the Forest stream stations.

The low TDS of the River contributed to the high transparency recorded in the investigation (an average transparency 132.9cm Secchi disc). This is within the range of 1.3-2.3m recommended by Whitton (1975) for classification of River types as black water. The olive brown colour of the water is due mainly to supply of humic substances from podsol soil (Whitton, 1975) and leaf litters undergoing degradation on forest floor, which is flooded during high tide, and dry during low tide. The New Calabar River can be categorized as Black Water River (Brackish water with organic matter) based on its colour, transparency; low concentration of inorganic ions, rich dissolved humic organic matter and high acidity. The chromophoric organic matter data were not collected. This would have helped in comparing with the parameters obtained by Bracchini *et al.* (2006) who worked on the role of chromorphore dissolved organic matter released and its relation to aquatic ecosystems optical properties.

Dissolved oxygen (DO) for the New Calabar River ranged from 3.8mgl^{-1} to 5.7mgl^{-1}. That of the forest stream was 2.9mgl^{-1} to 11.9mgl^{-1}. During the study period the DO content of New Calabar River did not get below 3.0mgl^{-1}. The concentration of dissolved oxygen is a major limiting factor to the mineralization of both carbonaceous and nitrogenous waste materials in a river considering the oxygen-sag curve for a river (Echenfelder, 1980). Hence the oxygen concentration at any given time would depend on the velocity of flow of the water in the bi-

direction. For the New Calabar River the flow is bi-directional. Previous data on flow velocity is not available though Dangana 1985 noted fast ebbing rate of the New Calabar–Bonny system. The high velocity (0.213m/s ± 0.015) resulting there from creates the required turbulent diffusion coefficient that results in mixing of dissolved and fine particulate substances (Berthlin, 1991) that creates oxygen availability for biochemical processes in the river. Hence the dissolved oxygen level did not get below 3.0mg/L in spite of relatively high BOD through most part of the year. The DO can be driven also by the presence of phytoplankton. Loiselle *et al.* (2007) reported that the presence of chromorphoric dissolved organic matter may represent an algal grown limitation which is as a result of competition for light.

Most of the isolated fungal genera contain species that are potential pathogens or opportunistic pathogens. The main hazardous species belong to *Aspergillus, Penicillium, Cladosporium, Mucor,* and *Fusarium.* Various strains of these families of molds have been implicated in being causative agents in asthma, hypersensitivity pneumonitis and pulmonary mycosis. The mean total fungal counts were up to x10^2cfu/ml. Candida was predominantly high in all the stations with an average of 17.29%. This high occurrence of *candida* is of considerable concern as the genera can cause candidiasis, endocarditis, septicemia, protracted urinary tract infections, kidney and lung infections, esophagitis and other soft tissues infections.

Fusarium, with a mean isolation frequency of 7.37% has species, which are common plant pathogens and causative agents of superficial and systemic infections in humans (Mayayo, 1999). The presence of these organisms is a clear indication of the need to constantly monitor the water quality of the New Calabar River.

The isolation frequencies of *Saccharomyces* was very high in stations F and G, and is likely associated with palm wine tapping activities along this stretch where raffia palm form the predominant tree in the swamp.

Results showed a high frequency of mould (72.38%) against yeast (27.62%) along the New Calabar River. Along the stream about 46.23% of enumerated fungi were moulds, while 53.77% were yeasts.

Statistical analysis of the physicochemical constituents and of fungal count revealed the presence of higher significant differences between the stations. This is an indication that the activities taking place at specific points (stations) along the river course have effect on the water quality and biological endpoint which is the fungal population.

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