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Bacterial Profiles and Physico-chemical Parameters of Water Samples from Different Sites of the New Calabar River, Nigeria

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Abstract

Water samples were collected from three sites (Ogbakiri, Choba and Iwofe) of the New Calabar River and were evaluated for microbiological and physico-chemical characteristics. Bacterial isolates consisting mainly of gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Acinetobacter* spp, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella* spp., *Vibrio* sp. and *Nocardia asteroides*) were isolated from the samples. The highest total coliform counts $\log_{10} 2.9$ were encountered in water samples from Iwofe but the lowest counts $\log_{10} 1.9$ occurred in the water samples from Ogbakiri. Ecological parameters varied among the sites with the highest pH value of 7.87 occurring at Iwofe and lowest of 6.12 at Choba. Maximum salinity level of 15‰ was observed at Iwofe and lowest at Choba. In contrast, maximum dissolved oxygen of 10.1ppm was observed at Choba and the minimum at Iwofe. The different bacterial profiles are probably attributable to the anthropogenic and industrial activities of the sites.

Keywords: anthropogenic activities, bacterial profiles, ecological parameters, industrial activities, water samples

1. Introduction

The New Calabar River is located in Rivers State, Nigeria and is a major source of fish, shrimps and crabs. It lies on the eastern arm of the Niger Delta (RPI, 1985). At the source (Choba), the water is fresh and acidic but brackish at the mouth (Iwofe). Choba is in the upstream part of the river where the water is fresh and tidal while Ogbakiri and Iwofe are down streams (Figure 1).

The climate of the area is characterized by dry season being November to March and the rainy season from April to October with monthly average rainfall of 254mm (Department of Meteorological Services Report, Rivers State, 2000). The area is characterized by mangrove ecosystems which are intertidal wetlands and periodically flooded by incoming and outgoing tides. The salinity fluctuates and this is dependent on the balance between tidal flushing and freshwater inputs (Corredor & Morell, 1994).

Human activities including defaecation, washing, recreation (swimming/bathing and fishing) and waste disposal are prevalent especially where human settlements exist along the river banks. Similarly, several industries are located along the river banks. The implications are numerous since most communities living on the river banks use the shores as dumpsites for their wastes, which are washed into the ecosystems during rains. Such occurrences have been reported elsewhere (Leff, Leff, & Lemke, 1998; Jiang, Noble, & Chu, 2001). Population growth results in conversion of open land into non-permeable surfaces which increase the rate of run-off and can negatively impact water quality (Jiang et al., 2001).

In the light of the prevalence of several domestic and industrial activities along the New Calabar River, this study was therefore undertaken to evaluate the microbial profiles and the physico-chemical parameters of different sites of the River.

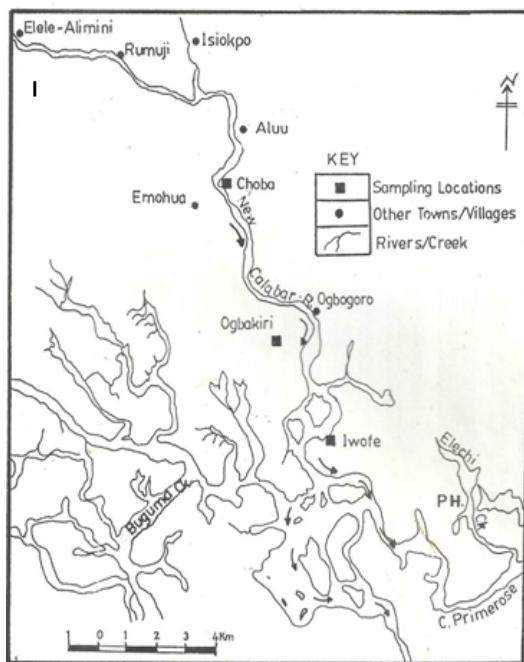


Figure 1. Map of the New Calabar River showing the sampling locations

2. Materials and Methods

2.1 Collection of Water Samples

Water samples (301 ml) were collected from three sites along the New Calabar River. The three sites were Ogbakiri, Iwofe and Choba. All samples were transported to the laboratory and analysed within 1-2 hours of collection.

2.2 Microbiological Analysis of the Water Samples

2.2.1 Analysis for Total Heterotrophic Counts

The water samples were serially diluted and spread-plated in duplicate on tripticase soy Agar (Oxoid) and incubated at 37°C for 24 hours (Anon., 1985) to determine the total heterotrophic counts. Representative discrete colonies were examined culturally, Gram stained and subjected to morphological and biochemical characterizations and identified (Harrigan & McCance, 1976).

2.2.2 Analysis for Total Coliforms

Enumeration of the Total Coliforms was carried out using pre-poured Eosin Methylene Blue Agar (EMBA). Aliquots (0.1 ml) were spread-plated on duplicate plates of EMBA and incubated at 37°C for 18-24 hours. Colonies having 2-3 mm diameter, exhibiting greenish metallic sheen with dark centres; large pinkish mucoid colonies; presence of typical colonies were enumerated as total coliform counts.

2.2.3 Determination of Physico-chemical Parameters

a. pH

The pH values of the surface water was measured in the field by immersing a digital pH meter (HANNA instruments, Portugal) which had been calibrated with buffer solutions of pH 4 and 7 into the water body (river) (Benka-Coker & Ohimain, 1995).

b. Temperature

The temperature of the waterbody was measured in-situ using the designated probe on the digital (dual) pH/temperature meter indicated above.

c. Salinity

The salinity of the water samples was measured with a hand refractometer (Atago Co. Ltd., Japan).

d. Dissolved Oxygen (DO)

The dissolved oxygen was measured in-situ using a digital DO meter (Oxyguard Handy MKII, Catvis, the Netherlands) and the obtained values were recorded in mg/l.

2.2.4 Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) and significant differences of the means determined at $p < 0.05$.

3. Results

The microbial composition of water samples from different sites of the New Calabar River is presented in Table 1. The greatest diversity consisting of eighteen bacterial flora occurred in Choba samples while fewer types were obtained from Ogbakiri and Iwofe both of which did not differ from each other (Table 1). The fungi isolated from the water samples were *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium* spp. and *Penicillium* spp. (Table 1).

Table 1. Occurrence and spatial distribution of micro-organisms isolated from different locations (habitat of shrimps) of the new Calabar River

Bacterial Flora	Locations		
	Ogbakiri	Iwofe	Choba
<i>Acinetobacter</i> sp.	+	+	+
<i>Aeromonas</i> sp.	-	-	+
<i>Alcaligenes</i> sp	-	-	+
<i>Arthrobacter</i> sp.	+	+	+
<i>Bacillus</i> sp.	-	-	+
<i>Enterobacter aerogenes</i>	+	+	+
<i>Escherichia coli</i>	+	+	+
<i>Klebsiella</i> spp.	+	+	+
<i>Micrococcus luteus</i>	+	+	+
<i>Moraxella</i> sp.	+	+	+
<i>Norcardia asterioides</i>	-	-	+
<i>Proteus vulgaris</i>	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+
<i>Pseudomonas fluorescens</i>	+	+	+
<i>Staphylococcus aureus</i>	+	+	+
<i>Staphylococcus epidermidis</i>	+	+	+
<i>Streptococcus faecalis</i>	+	+	+
<i>Vibrio</i> sp.	+	+	+
Mycoflora			
<i>Aspergillus flavus</i>	+	+	+
<i>Aspergillus niger</i>	+	+	+
<i>Fusarium</i> sp.	+	+	+
<i>Penicillium</i> sp.	+	+	+
<i>Rhizopus stolonifer</i>	+	+	+

Note. + = isolated consistently - = Not isolated

Total heterotrophic counts (THCs) of the samples from Iwofe were consistently the highest while the lowest counts occurred in Ogbakiri (Figure 2). Initially (January to February), the counts were high (except in February for Ogbakiri samples) but showed slight decrease until May and thereafter increased by approximately 1-2 log cfu/ml^{-1} (Figure 2). The fungal counts showed similar trends to those of the THCs (Figure 3) but they were much lower than those of the heterotrophic counts.

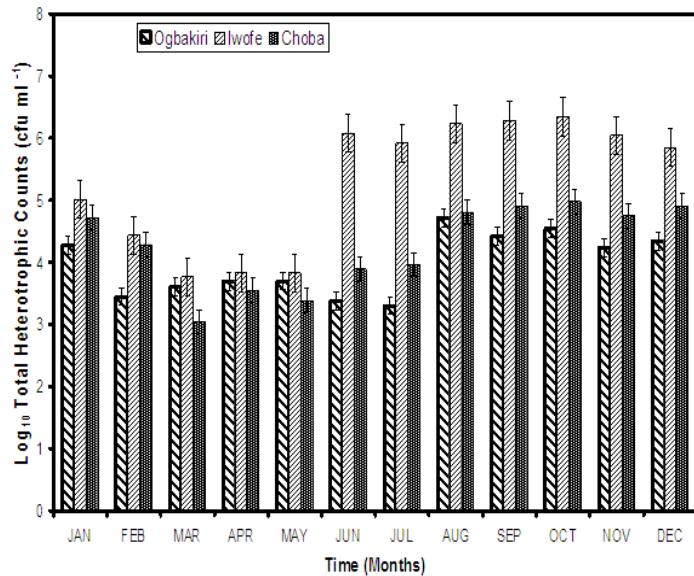


Figure 2. Monthly variations in total heterotrophic count (cfu ml^{-1}) of water samples from the three different sites of the New Calabar River studied. Values represent the mean of six determinations.
Bars indicate standard errors.

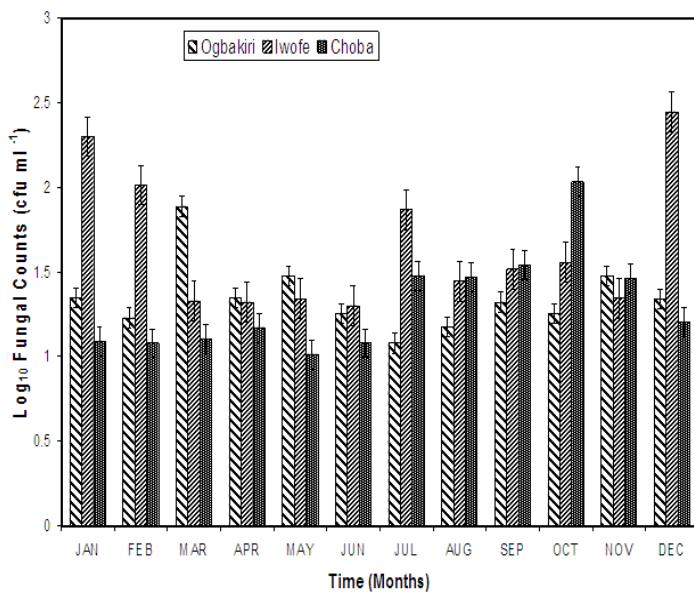


Figure 3. Monthly variations in Fungal counts of water samples from the three different sites of the New Calabar River studied. Values represent the mean of six determinations.

The total coliform counts of water samples from the different sites are illustrated in Figure 4. The highest total coliform counts were encountered most of the time in water samples from Iwofe but the lowest counts occurred most frequently in the water samples from Ogbakiri (Figure 4).

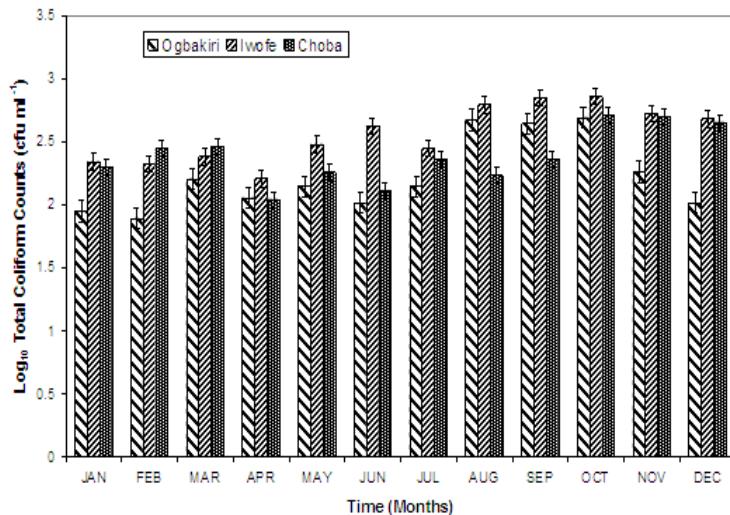


Figure 4. Monthly variations in total coliform counts of water samples from the three different sites of the New Calabar River studied. Values represent the mean of six determinations.

3.1 Changes in the Physico-Chemical Parameters

a. pH

The pH values obtained from the different sites are shown in Figure 5. The highest occurred in June at Iwofe while the lowest value was observed at Choba.

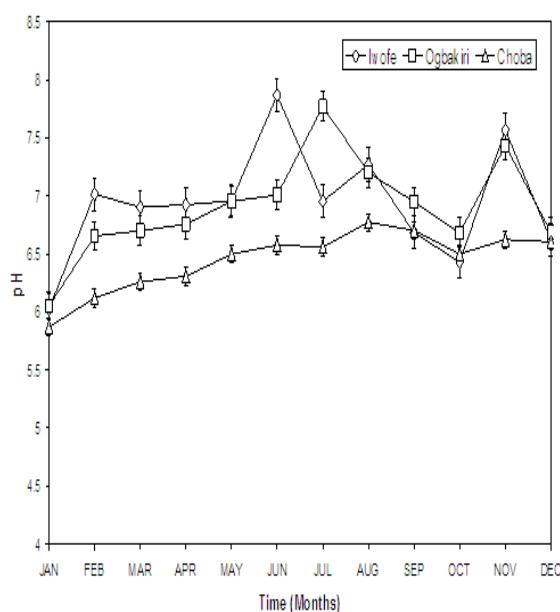


Figure 5. Monthly variations in pH of water samples from the three sites of the New Calabar River studied. Values represent the mean of six determinations. Bars indicate standard errors.

b. Temperature

Temperature variations from the different sites are shown in Figure 6. The values ranged from 26.1°C to 29.1°C with the highest occurring in January (Figure 6).

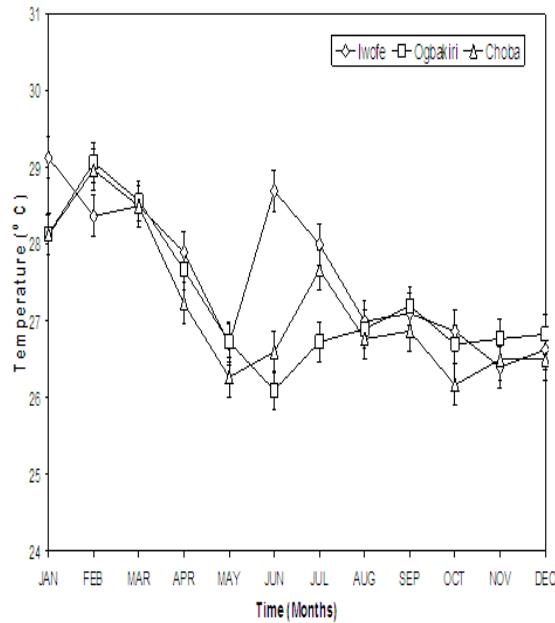


Figure 6. Monthly variations in Temperature (°C) of water samples from the three sites of the New Calabar River studied. Values represent the mean of six determinations. Bars indicate standard errors.

c. Salinity

Variations in the salinity levels are illustrated in Figure 7. The highest salinity level was observed at Iwofe during the dry season. In contrast, the salinity values at Choba were consistently low throughout the year (sometimes undetectable and below 1.0‰) (Figure 7).

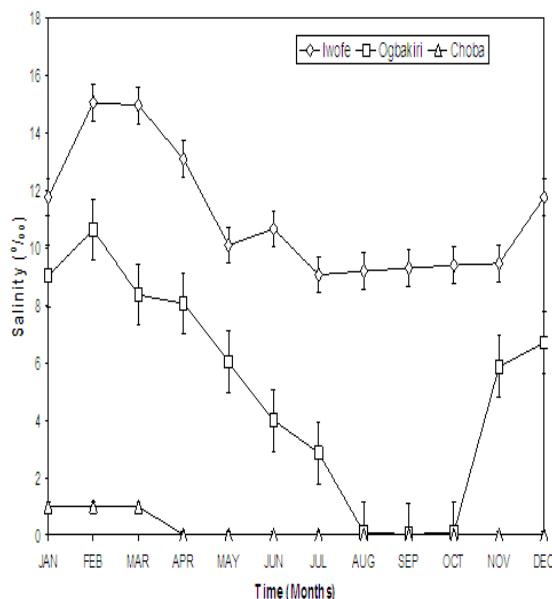


Figure 7. Monthly variations in Salinity of water samples from the three sites of the New Calabar River studied. Values represent the mean of six determinations. Bars indicate standard errors.

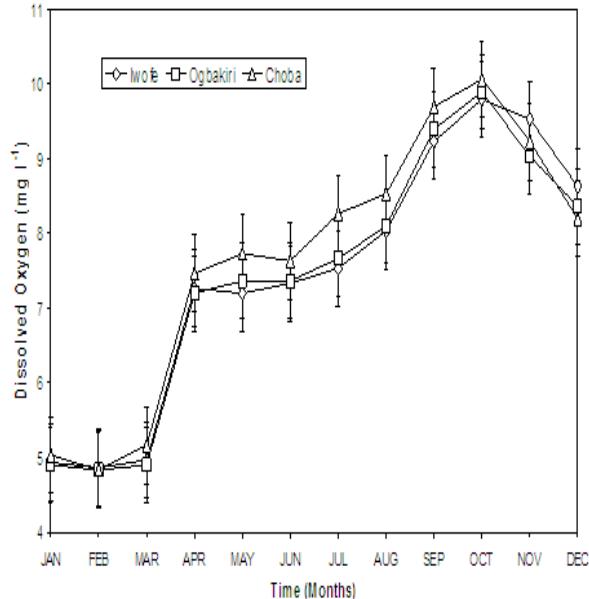


Figure 8. Monthly variations in Dissolved Oxygen of water samples from the three sites of the New Calabar River studied. Values represent the mean of six determinations.

d. DO

Figure 8 illustrates the DO values from the different sites in the course of the investigation. They were not significantly different ($p < 0.05$) but were slightly higher at Choba.

4. Discussion

The results of this work indicate that the water samples from the different sites of the New Calabar River showed variations in the total heterotrophic counts. This may have been influenced by anthropogenic activities such as defaecation, recreation and presence of industries sited along the New Calabar River. For example, the water body is used by residents for daily chores. In addition, different industries discharge their effluents into the river. Therefore, these findings confirm the observations by other workers (Atlas & Bartha 1983; Benka-Coker & Ohimain, 1995; Anon, 2000; Efiuvwevwere & Ezeama, 2004) that high heterotrophic bacterial counts were associated with water discharge, manure run-off coupled with high density farming and greater human activities. The THCs from Iwofe were highest; this may be due to the fact that Iwofe is the most industrialized.

The seasonal changes observed in the various microbial groups could be related to the influence of the physico-chemical properties in combination with human activities. Earlier findings have shown slightly elevated levels of faecal coliforms from river waters during the colder months which were attributed to the effects of high salinity (Shibata, Solo-Gabrielle, Fleming, & Elmira, 2004) turbidity, pH, low temperature and sunlight exposure (Anon, 2000; Craig, Fallowfield, & Cromar, 2002; Whitman, Shively, & Byappanahalli, 2003) on microbial survival.

The Gram-negative bacterial flora isolated from the water samples accounted for 61% of the microbial composition. This may be due to the presence of high human wastes in the aquatic environment. This corroborates the observation by other workers that Gram-negative bacteria predominate where there is abundance of human wastes (Vanderzant, Cobbs, & Thomson, 1973; Peele, Singleton, & Colwell, 1981; Sharmila, Abraham, & Sundararagi, 1996; Leff et al., 1998; Anon, 2000).

The ecological parameters play a major role in the microbial profile of any aquatic environment. The pH values

ranging from 5.87 to 7.87 observed in the present work favour the growth and survival of many types of microorganisms (Jay, 1996; Efiuvwevwere & Ezeama, 2004). The apparent fluctuation in the pH of water samples from Iwofe especially as from June reflects the effect of mixing with the marine waters since Iwofe is located downstream. The temperature also varied from 26.1°C to 29.1°C, a range which is favourable mainly for the growth of mesophilic organisms. This is probably why mesophiles were the dominant group encountered in this study. In addition, tropical waters tend to harbour high population of mesophiles (Evison & James, 1973; Jay, 1996; Solo-Gabrielle, 2000; Desmarais, Solo-Gabrielle, 2002).

The seasonal variations in salinity of the New Calabar River are probably related to rainfall regime and other factors. The occurrence of maximum salinity values during the dry season coincides with a period of low rainfall (Figure 7). On the contrary, low salinity was associated with rainy season suggesting dilution effect (Dublin-Green, 1990; Shibata et al., 2004).

The observed higher dissolved oxygen content during the rainy season is consistent with the finding that low temperatures and high current velocities favour solubility of oxygen in water (Lamb, 1985; Odokuma & Okpokwasili, 1997). Similarly, high temperatures lead to decrease in dissolved oxygen content as evidenced from October (dry season) (Figure 8).

5. Conclusion

The New Calabar River is used throughout the year for recreation (fishing and bathing). The high numbers of Gram-negative bacterial flora isolated in this work may therefore pose a significant risk of exposure to faecal contamination. The occurrence of pathogens from the three sites of the New Calabar River is of great public health significance.

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