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MICROFLORA POPULATION IN MANGROVE SEDIMENTS OF CROSS RIVER ESTUARY

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ABSTRACT

Microflora associated with the sediments of riverine mangrove forests dominated by *Rhizophora*, *Avicennia* and *Nypa* in Cross River (Nigeria) estuary were studied. The sediment samples (1.0g) collected monthly (February to October 2004) from swamps dominated by the three mangrove forest types were separately diluted serially with distilled water for microbial population estimation. Gram reactions were carried out for bacteria while wet mount with Lactophenol in cotton blue reagent was carried out for fungi. Microalgae count was carried out microscopy and counting on a counting chamber. The major microflora identified were algae belonging to the class *Dinophyceae, Bacillaiaophyceae, Rhodophyceae* and *Chlorophceae;* fungi belonging to the class *Ascomycetes* and *Deuteromycetes* and bacteria belonging to the species of *Bacillus, Flavobacterium, Clostridium, Proteus* and *Pseudomonas*, respectively. The result obtained also showed significant difference ($P < 0.01$) in total microbial populations among the mangrove forest types studied with *Avicennia* ranking higher than *Nypa* and *Rhizophora* dominated forests. Significant ($P < 0.05$) seasonal variations in the microflora abundance in the three mangrove forest types were noticed with the dry season having higher microflora abundance than the rainy season. This suggests that the forest types probably influenced the relative microbial population in the mangrove sediments

INTRODUCTION

Nigeria has the largest mangrove forest in Africa and ranks fourth in the world after Indonesia, Brazil and Australia (Spalding *et al.*, 1997). According to Ibianga (1985), the mangrove zone constitutes about 10% of the total forest area in the rainforest belt of Nigeria with Cross River estuary having a mangrove area of about 950km^2 (ENPLAN, 1974). Christensen (1983) reported that *Rhizophora species, Avicennia species, Lagunculania racemosa, Concarpus erectus* and *Nypa fructicans* were the dominant mangrove plants in Nigeria. Day *et al.*(1989) observed that estuaries are widely recognized as one of the most biologically productive ecosystems, whose organic carbon provides energy-rich food source to microorganisms and invertebrates at the base of the food chain.

Microbial populations in the mangrove ecosystem play a vital role in the decay of plant litter, nitrogen fixation, denitrification and nitrification, formation and consumption of trace gases, transformation of metals, sulphate reduction and production of phytohormones Microflora of the mangrove ecosystem support the food chain through the decomposition of mangrove litter. During litter decomposition, the energy-rich large molecules of carbohydrates and proteins are broken down by microorganisms (Mellilo *et al.*,1989).

The decomposition of organic materials from mangrove plants by microorganisms results in protein enriched fragments of detritus. This detritus is the primary energy source in tropical coastal areas and supports commercial fisheries (Woodroffe, 1992; Alongi *et al.*, 1992).

The activities of microorganisms in mangrove sediments have been well documented (Panikov, 1995; Li *et al*, 2001,). The microbial community structure of the mangrove ecosystem varies with variation in the environmental factors governing the sites. The distribution of bacteria in coastal sediments is specifically dependent on oxygen availability, which is in turn dependent on the inter-particulate air spaces. The sediment pH, organic matter and population of bacterial species, especially anaerobes, are among the factors known to affect bacteria existence and distribution in sediments (Panikov, 1999). Boga (1993) reported that sediments are the largest reservoirs for bacteria in the mangrove ecosystem. Heterotrophic aerobic bacteria are among the most numerically abundant group of bacteria in sediments.

Fell and Master (1980) reported that about 23 species of fungi have been isolated at different depths of mangrove mud. These include *Phytosphora, Drechistera, Glaeosporium, Glioclodium, Lulworthia, Nigrospora* and *Phyllostica* species. Gang (1983) observed that the highest number of fungi is isolated from surface layer of mangrove and their number and frequency decrease with increasing soil depth, establishing a direct correlation between fungi population and amount of organic matter.

Few relevant studies have been devoted to soil microbial communities inspite of the invaluable importance attached to them, especially, in Cross River estuary. This study therefore is aimed at determining the microflora associated with the Cross River estuary mangrove sediments.

MATERIALS AND METHODS

This study was carried out in the estuarine mangrove forests of the Great Kwa River in the West bank of the Cross River estuary, which lies within latitudes 04° 30' and 05° 45' North and longitudes 008° 05' and 008° 45' East of the Greenwich Meridian. This area is characterized by a mean annual rainfall of 402.1cm, a temperature in the range of 21°C to 29°C and average relative humidity in the range of 80 – 90% in the morning. The experimental site was observed to exhibit a water level variation due to tidal changes.

Sampling was carried out in three designated stations: *Rhizophora, Avicennia* and *Nypa* dominated areas. Soil samples were collected monthly at low tides from February to October, 2004, covering the dry and wet seasons. Sampling was systematically carried out along a transect at each station. Scrapple sediments were taken at 5 locations along the transect from each station. The wet soil samples were preserved in clean labeled back polythene bags and used for microbial analyses.

MICROFLORA ANALYSIS

The sediment samples (1.0g) were serially diluted

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with distilled water and used for the preparation of pour plates in triplicates for microbial isolation. Nutrient agar and malt extract agar were used as culture media for bacteria and fungi. The plates were later incubated at a temperature of 30°C for 48 hours for bacteria and 3 – 5 days for fungi.

Gram reactions and biochemical tests were carried out for bacteria while wet mount with lactophenol in cotton blue reagent was used for fungi. Microalgal count was carried out in a counting chamber using a microscope. Identification and characterization were carried out according to the method used by Newell and Newell, (1977).

STATISTICAL ANALYSIS

A nested analysis of variance was used to evaluate the different microflora and their abundance in sediments from the different mangrove forest types. Differences in means were separated using Least significant difference (LSD) test.

RESULTS

The distribution of microflora in scrapple sediments from different mangrove forest types (Table1),

Table 1: Microflora of scrapple sediments of different mangrove forest types

Rhizophora dominated forest	*Avicennia* dominated forest	*Nypa* dominated forest
Algae		
(a) Class: *Dinophyceae* *protoperidium crassipes* (Kofoids) Balech *Dinophysis acuta* Ehrenberg (Sxhiller) *Gymnodinium abbreviatum* Kofoid & Swezy	(a) Class: *Dinophyceae* *Gymnodinium abbreviatum* Kofoid & Swezy *Prorocentrum minimum* Schiller *Prorocentrum micans* Ehrenberg *Ceratum furca* Ehrenberg (Schiller)	(a) Class: *Dinophyceae* *Dinophysis acuta* Ehrenberg *Protoperidium crassipes* (Kofoids) Balech
(b) Class: *Bacillariophyceae* *ceratium tripos* Ehrenberg (Sxhiller) *Coscinodiscus wailesil* (Gran and Angst)	(b) Class: *Bacillariophyceae* *Nitzchia pungens* (Grunow) Hasle *Chaetonema laciniosus* schutt *Skeletonema costatum* (Greville) cleve Thalassiosira gravida Cleve	(b) Class: *Chlorophyceae* *Cladophora limicola* Etilers *Vaucheria Sp.* Vauch de Candolle
(c) Class: *Chlorophyceae* *Vaucheria sp.* (Vauch de Candolle)	(c) Class: *Rhodophyceae* *Catenella nipae* Zanardini *Bostrychia radicans* Montagne *Bostrychia binderi* Harvey	(c) Class: *Rhodophyceae* *Bostrychia radicans* (Montagne) *Bostrychia binderi* Harvey *Catenella nipae* Zanardini *Catenella ripens* Zanardini
Fungi		
(a) Class: *Ascomycetes* *Helicascus kanaloanus* Kohlmeyer *Didymosphaeria enalia* Kohlmeyer *Didymosphaeria rhizophorae* Kohlmeyer *Hydronectria tethys* Kohlmeyer & Kohlmeyer	(a) Class: *Ascomycetes* *Didymosphaeria enalia* Kohlmeyer *Didymosphaeria rhizophorae* Kohlmeyer *Liggnincola laevis* Hohnk *Leptoshaeria avicenniae* Kohlmeyer& Kohlmeyer	(a) Class: *Ascomycetes* *Helicascus kanaloanus* Kohlmeyer *Didymosphaeria enalia* Kohlmeyer
(b) Class: *Deuteromyceters* *Cytospora sp.* Kohlmeyer & Kohlmeyer *Phoma sp* Sacc & Roum *Cirrenelia pygmea* Myers & Moore	(b) Class: *Deuteromyceters* *Cirrenelia pygmea* Myers & Moore *Cirrenelia tropicalis* Kohlmyer *Cytospora sp.* Kohlmyer & Kohlmyer	(b) Class: *Deuteromyceters* *Phoma sp* Sacc & Roum *Helicascus kanaloanus* Kohlmeyer *Cytospora sp.* Kohlmeyer & Kohlmeyer *Cirrenelia pygmea* Myers & Moore
Bacteria		
Bacillus sp. *Flavobacterium sp.* *Clostridium sp* *Proteus sp.* *Pseudomonas sp* *Escherichia coli*	*Bacillus sp.* *Flavobacterium sp.* *Clostridium sp.* *Proteus sp.* *Pseudomonas sp.*	*Bacillus sp.* *Flavobacterium sp.* *Clostridium sp.* *Proteus sp.* *Pseudomonas sp.* *Escherichia coli*

revealed that the dominant algae populations identified were *Protoperidum crassipes, Dinophysis aceta, Ceratium tripos, Coscimodiscus wailesii* and *Vaucheria sp* for Rhizophora dominated forest, *Gymmodinium abbreviatum, Prorocentrum minimum, Prorocentum micans, Ceratum furca, Nitzschia pungengs, Skeletonema costatum, Chaetoceros lacimosus, Thalassiosira gravida, Catenala nipae, Bostrychia radicans*, and *B. binderi* for *Avicennia* dominated forest, while Nypa dominated forest had the following algae: *Dinophysis acuta, Protoperidium crassipes, Cladophora limicola, Bostrichia radicans, B. binderi, C. nipae* and *C ripens*. The fungi in sediments from the *Rhizophora* dominated forest were *Helicascus kanaloanus, Didymosphaeria analia, Didymosphaera rhizophorax, Hidonectria tethys, Cytospora sp, Phoma sp* and *Cirrenalia pygmea*. The differences in fungi population in sediments from Rhizophora and Avicennia dominated forest are *Lignicola laevis, Leptosphaena avicenniae* and *Grienalia tropicalis* while sediments from *Nypa* dominated forest had similar fungi population with that of *Rhizophora* dominated mangrove forest.

The bacteria isolated and identified in sediments from the three mangrove forests (Table 2)

Table 2: Data for monthly microbial counts in sediments of different forest types

Bacteria ($x10^5$ $CFUg^{-1}$)									
Months	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPTOCT	
Rhizophora	160	155	140	135	143	185	145	180	183
Avecinnia	190	149	140	110	146	150	126	180	150
Nypa	150	100	110	120	80	128	90	147	119

FUNGI ($x10^5$ $CFUg^{-1}$)									
Months	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPTOCT	
Rhizophora	97	62	54	13	71	86	46	14	95
Avecinnia	61	90	89	48	53	74	34	84	81
Nypa	63	98	85	50	10	28	66	60	102

ALGAE (Cells)									
Months	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPTOCT	
Rhizophora	60	50	75	53	70	76	75	73	77
Avecinnia	102	118	101	100	150	137	117	122	140
Nypa	80	104	136	127	122	104	138	88	75

were identical except *Escherichia coli*, which were not identified in sediments from Avicennia mangrove forest. These bacteria include *Bacillus sp.*, *Flavobacterium sp.*, *Clostridium sp.*, *Proteus sp.*, and *Pseudomonas sp.*

The monthly variations in the mean bacterial count (Fig.1)

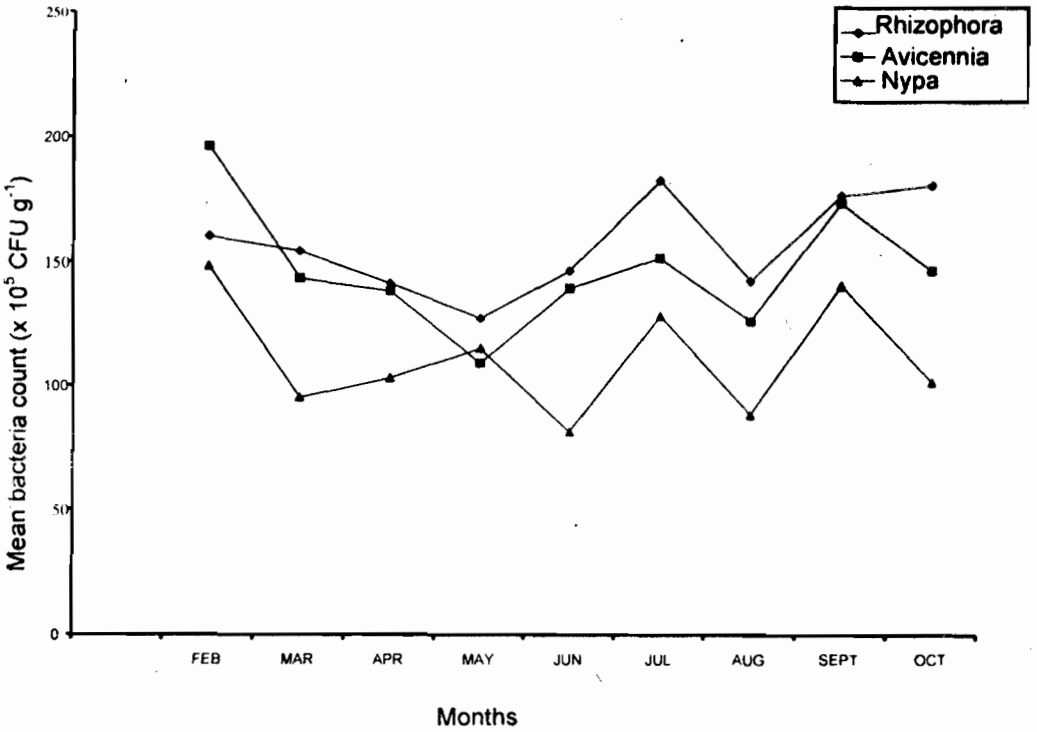

FIG. 1: Monthly variations of mean bacterial count

suggest that sediments from Avicennia dominated forest had the highest bacterial count in February, 2004 (190 x 10^5 CFUg^{-1} (dry season) while the least (110 x 10^5 CFU/s was obtained in May, 2004 (wet season). For sediments from the Rhizophora dominated mangrove, the highest bacterial count was obtained in October, 2004 (183 x 10^5 CFU/g) (wet season) with the least (135 x 10^5 CFUs^{-1}) in May, 2004. The maximum bacterial count was obtained in February, 2004 (150 x 10^5CFUg^{-1}) in sediments from Nypa dominated mangrove forest while the minimum count was in June, 2004 (80 x 10^5 CFUs^{-1}). Generally, sediments from Rhizophora dominated forest had the highest bacterial population followed by those from Avicennia dominated forest.

Rhizophora dominated forest sediments had the highest mean fungal population in February, 2004 while Avicennia and Nypa dominated forests had the highest fungal population in April and October, 2004, respectively. Figure 2

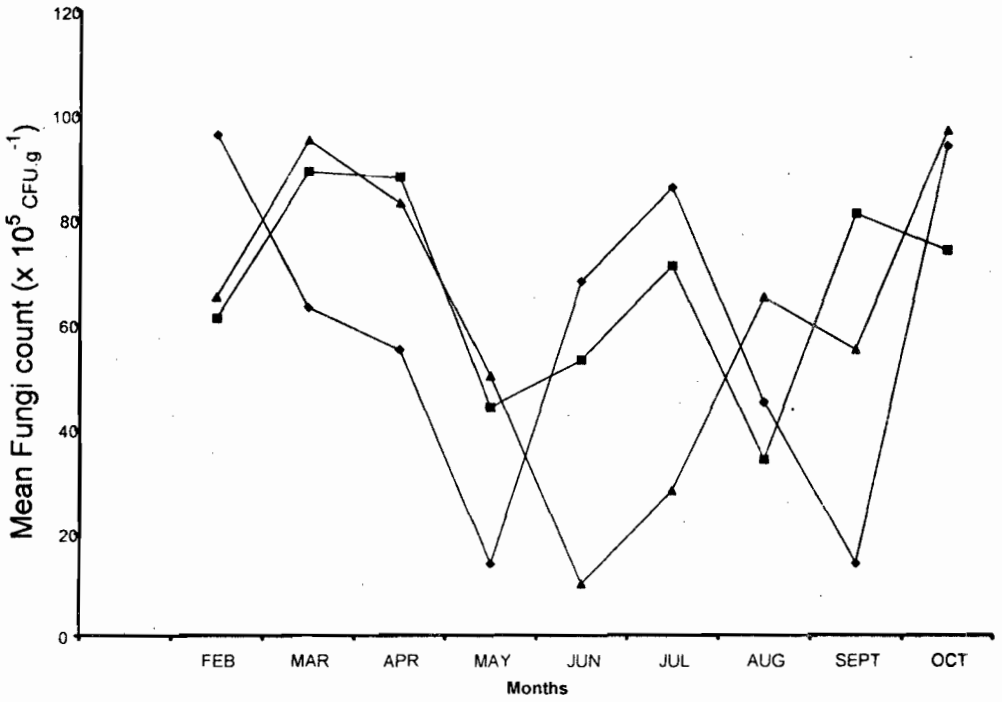

FIG. 2: Monthly variations of mean fungal count

shows that there were significant monthly variations of fungal population in sediments from the three mangrove forests. Avicennia dominated forest recorded the highest microalgal count in June , 2004 while Nypa and Rhizophora dominated forests recorded the highest microalgal count in August and April, 2004, respectively (Figure 3).

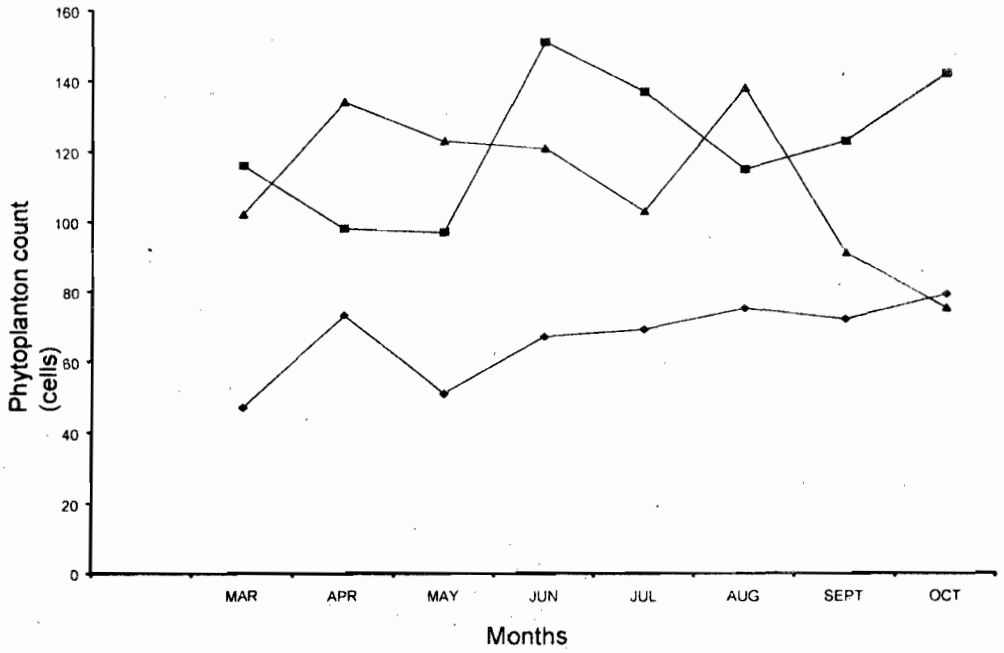

FIG. 3: Monthly variations of microalgal count

The total microbial population in sediments varied significantly ($P<0.00$) among the mangrove forest types studied with *Avicennia* ranking higher than *Nypa* and *Rhizophora* dominated forests. There were also significant ($P < 0.05$) seasonal variations in the abundance of microflora in the sediments from the three mangrove forests, suggesting higher microflora abundance in the dry season than in the rainy season.

DISCUSSION

Microorganisms play important role in detritus base food webs in near shore environment (Fell and Master, 1980). Microbial activities may lead to the enrichment of mangrove soils with nutrients. The various and diverse microbial life forms identified in the sediments studied showed variations in distribution over time. The presence of microbes and their populations in the soil are governed primarily by the amount of organic matter, the plant root activity, soil temperature and moisture, and nutrients availability (Alexander, 1994). The variations in microflora populations observed in these mangrove sediments can be attributed to variations in the amount of organic matter, temperature, moisture , etc.

Microalgal population was found to be higher in sediments from *Avicennia* dominated forest. This might be attributable to the fact that *Avicennia* leaves decompose faster than those of other mangrove species (Wafar *et al*., 1997). This difference in decomposition may be biological since *Avicennia* leaves are thinner and may sink faster than other mangrove leaves and as such are expected to decompose more readily leading to availability of nutrients with the resultant greater microbial abundance.

Fungal (isolates) counts in May, June and September, 2004 were minimal in sediments from the 3 forest types studied. This might possibly be due to a decrease in the rate of drop of senescent leaves or plant debris, which also harbour some fungi. The similarity observed in fungal population in sediments from *Rhizophora* and *Nypa* dominated mangrove forests might be a result of leaf characteristics, which are different from those of *Avicennia* species, especially the salt contents of the leaves (Christensen, 1983; Oyieke, 1996).

The bacterial population in sediments obtained from this study reveals that more bacterial isolates were recorded in the dry season. It is possible that the high temperatures during the dry season probably enhanced microbial activity, leading to increase in microbial biomass (Mackey and Snail, 1996). The existence of either facultative halophytes or tolerant holophilics in a saline environment may also increase bacterial load (Panikov, 1999; Li *et al*., 2001). Among the three mangrove forests studied, sediments from *Nypa* dominated forest had the least bacterial count. This might be due to the recalcitrant nature of the leaves of Nypa palm and plant debris (Panikov, 1995).

CONCLUSION

The analysis of the microflora in mangrove sediments of Kwa river mangrove forest of Cross River State, Nigeria revealed a significant influence of forest type on the relative abundance of microflora population. The appreciation of the microbial population in the mangrove forests, the geographical location notwithstanding, will be a pointer to knowing the level of activities in these forests and may have implications for the management of mangroves.

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