

Inferring Taxonomic Relationships among *Rhizophora* Species in Nigeria Using Leaf Morphometrics and Pollen Information

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Abstract

Leaf and pollen samples of *Rhizophora* individuals and the associated soil and water physico-chemical parameters were conducted to generate taxonomic relationships. Samples were collected across Niger Delta region between 2013-2016. Although, application of multiple nuclear genes to the molecular phylogeny, population genetics and hybrid identification has been used. However, there are no sufficient molecular markers to address these topics. Three hundred and sixty four (364) *Rhizophora* samples were obtained in all. Leaf length, leaf width, stipule length, petiole length, number of lateral nerves, space between lateral nerves, distance between internodes, distance of mid rib away from symmetry and number of foliages per twig was used to obtain morphological relationships. Standard laboratory methods were adopted. Morphologically, the result established five statistically significant differences in *Rhizophora* Operational Taxonomic Units (OTUs) with leaf length, stipule length, number of lateral nerves and distance between lateral nerves as characters that delimited one *Rhizophora* unit from the other. The study also proved specific character that separated each OTU and locations occupied by each. The result of the pollen analysis was used as complimentary evidence to morphology. The apertural morphoforms was tricolporate, while differences exist in exine patterns and pollen shapes. The application of ANOVA and Principal Component Analysis showed OTUs 1 and 2 as significantly different. Although, OTUs 3, 4 and 5 showed statistical difference among themselves, cluster analysis revealed close affinities. The influence of soil and water physico chemical parameters further confirmed the taxonomic distinctness of OTUs 1 and 2 and similarities among OTUS 3, 4 and 5. The finding is in contrast to the widely held notion that only three putative *Rhizophora* species exist in Nigeria. Genetic research into these five OTUs is recommended.

Keywords: Physico-chemical; Niger Delta; *Rhizophora*; Subtypes; Mangrove; Unical

Introduction

Mangroves as excellent candidates of productivity had long been established. They offer various ecosystem services such as shoreline stabilization [1,2], habitat, nursery and breeding ground for many fish species and other fauna [1,3-8], wood for fuel wood, timber, poles, boats [4,9-13]. Mangroves also aids in the establishment of restrictive impounds that offer protection for maturing offspring, filtering and assimilating pollutants from upland run-off and stabilization of bottom sediments [14] among other products. The common characteristics they all possess are tolerance to salt and brackish waters. It confers an excellent sense of place, aesthetic grandeur and serenity value to the inhabitants. They have been shown as excellent candidates for carbon capture and sequestration [15]. Mangrove habitat is found along the coastlines of Nigeria. It straddles such states as Lagos, Ondo, Delta, Bayelsa, Rivers, Akwa Ibom and Cross River. Rudolf et al. [16] placed Nigeria mangrove habitat as the eight largest in the world. There are five indicator genera of the mangrove environment in Nigeria. *Rhizophora*, *Avicinnia*, *Laguncularia*, *Conocarpus* and lately but regrettably, the invasive *Nypa*. Of them all, *Rhizophora* is the embodiment of the mangrove environment in Nigeria. Classed in the family *Rhizophoraceae*, its root system, height and hanging roots make it easily distinguishable. *Rhizophora* could be highly structured due to various barriers to gene flow, such as land masses, directions of ocean currents, as well as historical vicariant events. The pore space it creates in the soil makes it an excellent keystone engineering species that houses the hermit crabs and lobsters. In turn, the presence of these invertebrates is attraction for varieties of *Mona* and *Cercopithecus* taxa. The inevitable role of *Rhizophora* in shoreline protection is better appreciated where and when the coastlines are inadvertently cleared of it. Coastline embankment costing millions of dollars has been spent in such instances. The efficiency and life cycle of

such artificial embankments is incomparable to the natural *Rhizophora* species.

The genus is composed of 364 species as found in the Niger Delta region. The ability of *Rhizophora* to perform a task is not found in the genus; rather it is seen in the species level. Hence species is the only tangible unit of life. Previous phylogenetic analysis have demonstrated that the genus *Rhizophora* have viviparous propagules, which makes them have potential for long-distance dispersal [17]. It is therefore intuitive to suggest that the ecological niche of one *Rhizophora* species just like other genera (for instance *Irvingia gabonensis* versus *Irvingia wombolu*, *Vernonia colarata* versus *Vernonia amygdalina*) would differ albeit how little, from the other.

In this study, an operational taxonomic unit (OTU) was used to classify groups of closely related individuals. Sequences can be clustered according to their similarity to one another, and operational taxonomy units are defined based on the similarity threshold (usually 97% similarity) as set by this study. Each of the clusters in this work intends to represent a taxonomic unit of a *Rhizophora* species or genus depending on the sequence similarity threshold.

More so, the alarming rate of mangrove conversion in the country calls for urgent and species specific studies. The size of the Nigeria

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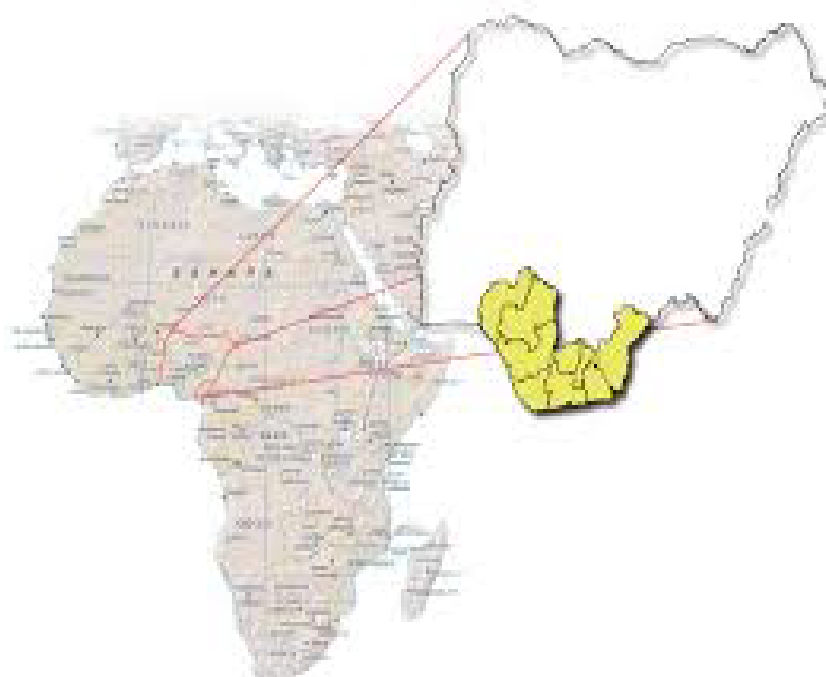
mangrove was 997,700 ha prior to 2000 as against the current size of 240,400 ha in 2015 [16]. Worst still, the high rate of speciation in the tropics makes frequent species characterization inevitable. Also, the number of *Rhizophora* species in the delta has been a subject of controversy [18]. Literature is scanty on the influences of physico chemical parameters on mangrove types and distribution in the Niger Delta. It is in light of this study aims at determining the taxonomic relationships of the various *Rhizophora* species across Niger delta using leaf morphology and pollen characters. The use of soil and surface water physico chemical parameters was also employed to determine

influences of environmental factors in the mangrove types and distribution.

Methodology

Study area

The Niger delta sedimentary basin is home to the largest mangroves in Africa [16]. The topography of the area is generally low lying as seen in the altitude in Table 1. The tide dominated delta experiences a flooding and ebbing regimes approximately every 12 hours interval (six hours each). The height of the tide ranges from about 0.5 m to



Map of Africa showing the study area

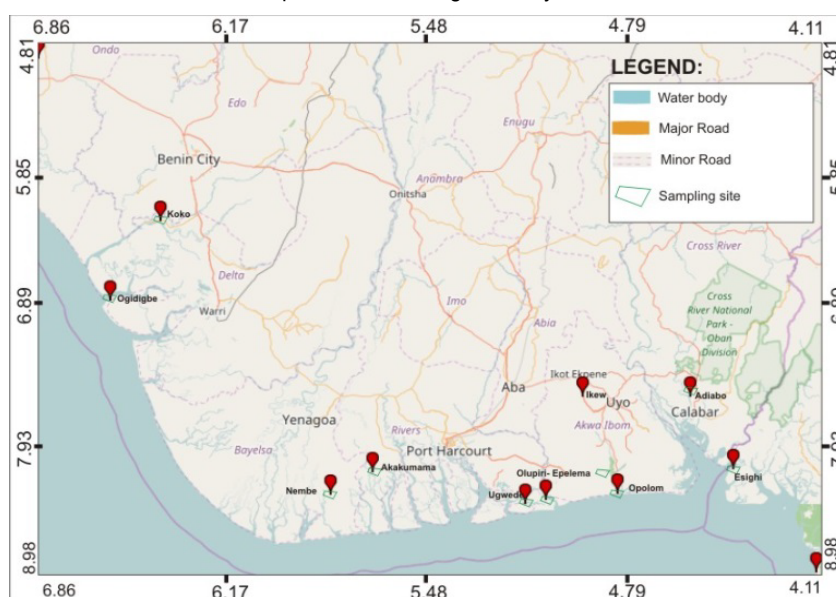


Figure 1: The map of study Area.

about 4 m [19]. The delta is dominated by five indicator flora genera - *Rhizophora*, *Avicinnia*, *Laguncularia*, *Nypa* and *Conocarpus* (Figure 1).

The climatic data obtained from Nigeria Meteorological Agency shows the mean value of temperature in °C to be very high in the month of January February and March and minimum in April to September, which finally rises again in November and December. The highest relative humidity was experience in the month of June to September and was low at around the month of November to May. The highest

rainfall was seen to be experience in July from the result gotten in the study area. While wind speed was experience most between the periods of January to May [19] (Figure 2).

Methodology

To achieve the aim of the study, five groups of data were identified to be generated, and include all the factors related wholly or partially to the growth and distribution of *Rhizophora* on the Delta. The first group included natural factors (geology, topography, and geomorphology),

S/N	Sample Number	Location	State	Coordinates (UTM 32 in degrees, minutes and seconds)		Height above Mean Sea Level (m)	Year of Collection
				N	E		
1	KOK 1-34	Koko	Delta	05° 58' 33" - 5° 59' 03"	005° 23' 119" - 005° 24' 09"	12	2013
2	OGD 35-69	Ogidigben		05° 23' 47" - 05° 24' 29"	005° 39' 42" - 005° 41' 13"	10	2013
3	AKA 70-104	Akakumama	Bayelsa	04° 36' 29" - 04° 37' 16"	006° 10' 35" - 006° 11' 18"	4	2013
4	NEM 105-144	Nembe		04° 37' 55" - 04° 38' 29"	006° 14' 46" - 006° 15' 28"	3	2013
5	OPM145-179	Olupiri- Epelema	Rivers	04° 43' 35" - 04° 44' 48"	007° 18' 49" - 007° 19' 27"	13	2014
6	UGD180-214	Ugwede		04° 40' 05" - 04° 41' 33"	007° 22' 16" - 007° 23' 30"	10	2014
7	IKW215-249	Ikwe	Akwa Ibom	04° 32' 08.6" - 04° 33' 02.6"	007° 54' 24.0" - 007° 54' 56.7"	16	2015
8	UNK250-294	Opolom		04° 32' 37" - 04° 33' 09"	007° 55' 5" - 007° 55' 34"	8	2015
9	AUB 295-329	Adiabo Ukanabio	Cross River	05° 02' 59" - 05° 02' 33"	008° 16' 36" - 008° 17' 07"	10	2016
10	ESG 330-364	Esighi		004° 54' 20" - 004° 55' 43"	008° 26' 43" - 008° 27' 16"	5	2016

Table 1: Sampling Details for the study.

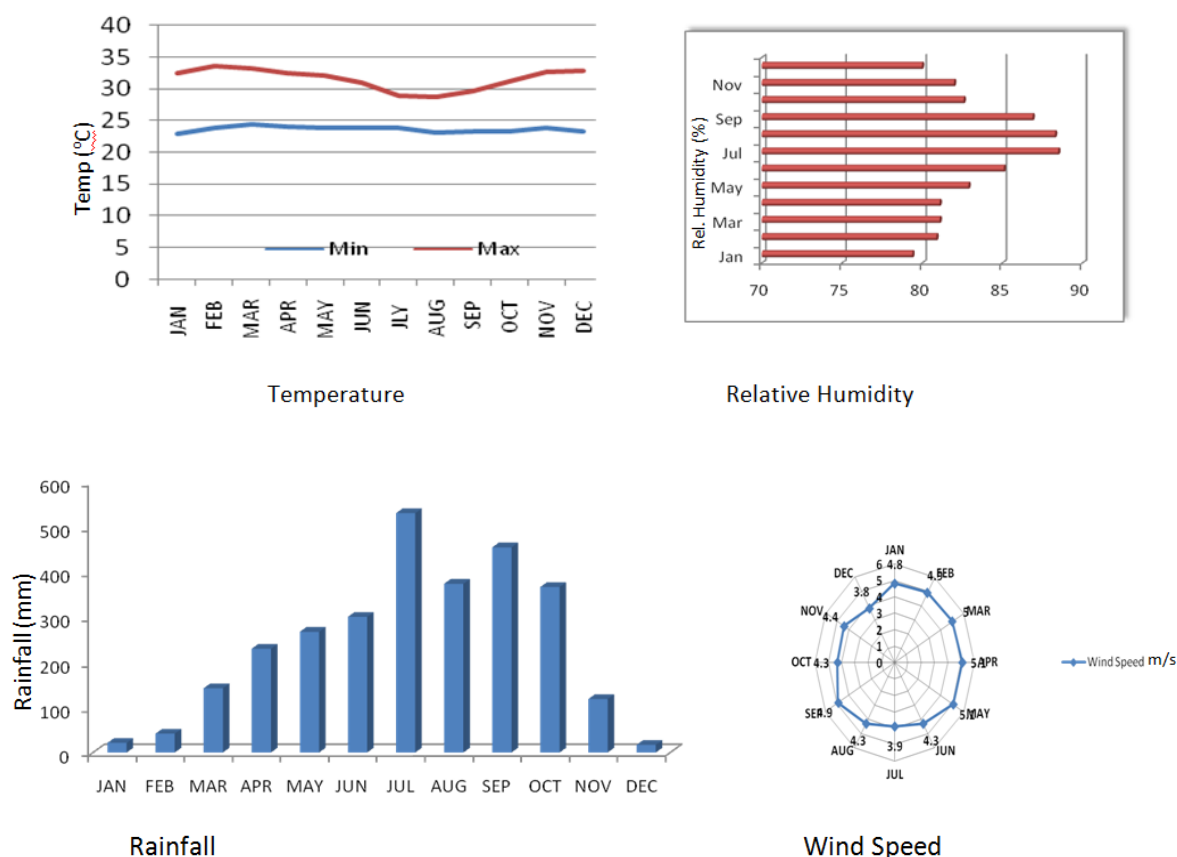


Figure 2: Climatic data from 1985-2015 over Niger Delta Region (NIMET, 2017).

climatic factors (temperature, humidity, wind speed, precipitation) and the tidal range. Meteorological data of thirty years (1985-2015) obtained from Nigeria Meteorological Agency (NIMET) was used to calculate the monthly averages.

The second group includes the quantitative study of the leaf morphological characteristics of the *Rhizophora* species. Ten sites were selected including the full extent of the area occupied by the *Rhizophora* species. Site selection was made every 75m. The main reason for site choice was the physical observation of variations in the characteristics of the *Rhizophora* and the need to find an explanation for these differences by analyzing samples of water and soil. This technique is identical to that employed for the Conservation of the Red Sea and Gulf of Aden [20], as a part of the rapid assessment of Coastal Environments for Agricultural Research (CEAR). In this study, squares of 10 m × 10 m are marked with wooden frames, ropes and tape. The operation is repeated 3 times. The length and diameter of the rod are measured at a height of 30 cm from the soil. Five twigs containing pollen of *Rhizophora* found in 2 × 2 m² surfaces were harvested.

The third group includes the analysis of water and soil for mangrove species at each site. Non composite Soil samples were taken using a graduated plastic tube of 50 cm at three depths: 10 cm, 20 cm, and 30 cm [21]. In the laboratory, samples are dried and passed through a 2 mm sieve. Physical analysis of the soil allows determining the texture in each sample, using a texture triangle and to identify the proportions of clay, sand, and silt. We also recorded certain physical properties of the soil including colour, structure, Electric conductivity (EC), and (pH). For chemical analyses, we measured a number of dissolved salts (TDS), Total Organic Matter, ion availability, primary macronutrients (Nitrogen, Phosphorus, Potassium) secondary macro elements (Calcium, Magnesium) and micro nutrients. We also measured the sodium adsorption ratio (SAR) and the amount of organic matter in the soil. All analyses were consistent with the criteria and methods accredited by the International Centre for Agricultural Research in the Dry lands [22].

The fourth group of the work was devoted to identify members of *Rhizophora* genus based on morphological and pollen information.

The fifth group concerned itself with applying the physico-chemical parameters to each *Rhizophora* unit to determine if and why statistical differences existed among them.

Five twigs containing leaves and pollen from each of the 364 *Rhizophora* individuals were collected in ten permanent plots spread across five Niger Delta States (Figure 1) over a four year period (2013-2016) as shown in Table 1.

Leaf morphology: Measurements of leaf length, leaf width, stipules length, petiole length, length of lateral nerves, distance between lateral nerves, distance between inter nodes, distance of mid rib away from symmetry and number of foliages per twig were recorded for each of the 364 individuals obtained. Measurements were conducted using line ruler and thread. The use of hand lens was used for number and space between lateral nerves. The morphological readings were compiled on recording sheets for each sample. Mean and standard error figures were entered into an Excel spread sheet and the raw data coded to allow analysis using Unistat 4.0 for Windows. Analysis of variance (ANOVA) was carried out for to determine level of significance. Newman Keuls Multiple Comparison Test (NKMCT) was used to determine significant differences between taxonomic units and sites of soil collection.

Cluster Analysis and PCA ordination was also conducted to

determine relationships. Principal component analysis (PCA) is a statistical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (or sometimes, principal modes of variation). The number of principal components is less than or equal to the smaller of the number of original variables or the number of observations. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components. The resulting vectors are an uncorrelated orthogonal basis set.

Pollen: At each location, five samples were collected each at 1- 75 m, 76-150 m, and 151- 225 m, 226-300 m, 301-375 m, 376-450 m and 451-525 m from the shoreline to the inland. No *Rhizophora* species was observed beyond the 525 m distance except in Nembe where it was observed at 604 m (hence an extra five samples were collected between 526-604 m).

Pollen collection & storage: Collected pollen samples were labelled and stored in vials/sample bottles containing glacial acetic acid (GAA) for preservation prior to laboratory analysis as prescribed by Erdtman [23].

Pollen sample preparation: The widely accepted method of pollen analysis by Erdtman 1960 as adopted by Albert and Ekine [24] was used. The obtained anthers were crushed with a glass rod, and the debris removed with a needle to release the pollen grains. Glacial acetic acid (GAA) was used to transfer the crushed anthers into plastic test tubes and centrifuged for about 15 min at 5,000 revolution per minute (RPM) at room temperature. The centrifuged samples were decanted. The residues were washed, centrifuged, decanted and rinsed with distilled water three times. Samples were acetolysed per Erdtman method: The acetolysed mixture (9 part acetic anhydride and 1 part sulphuric acid) was added to the samples, and water bathed at 84°C for 10 min. The heated samples were centrifuged and washed with distilled water three times, each decanted to remove the acetolysed mixture. The residues were transferred into sterile vials. Glycerine jelly was added to the prepared samples at a ratio of 50 part sample: 50 part glycerine.

Mounting and photomicrography: The prepared samples were pipette into a clean glass slides, covered with slid and sealed using a transparent nail hardener. The prepared pollen samples were properly examined under light microscope (AmScope microscope with X100 magnification). Photograph of the prepared pollen samples were taken with the aid of AmScope MA1000 camera with an in-built micrometer for measurement. Permanent slides of the prepared pollen samples were deposited in the Department of Botany, University of Calabar-Calabar, Nigeria. Various quality assurance protocols as outlined in Erdtman method were followed.

Soil and water collection and analysis

Soil samples were collected in triplicates as earlier described. This was in agreement with the method of CEAR. Same numbers of samples of standing water were collected at each of the earlier mentioned distance from the shore. Quality assurance protocols adopted for the work is shown in Table 2.

Results

Result of *Rhizophora* morphometrics

When the result of each parameter for the 364 individuals was recorded, samples having same mean value and/or differences of less than 0.1cm (0.001mm) per parameter was grouped together as constituting an OUT (Table 3). The mean values richness were similar among the species, such as leaf length, leaf width, stipules length, petiole

length, Distance between lateral nerves, length of inter node, number of foliage per twig, Number of lateral nerves, Distance of mid rib from line symmetry with averaged 7.8, 4.9, 7.42, 2.04, 0.01, 3.9, 9, 51, 0.06 in OTU 1 respectively, although the range of the estimates among populations was much broader as shown in Tables 4-6.

In Adiabo Ukanabio, individuals that constituted OTUs 1, 2, 3, 4 and 5 were observed in 1-87 m, 83-124 m, 106-271 m, 235-379 m and 344 – 488 m respectively. In Ikwe, the individuals that comprised each

Soil					
Parameter	Sample Quantity	Container	Preservative	Holding Time	Container Pre-treatment
General Appearance, Colour, Odour, Depth,	Observation recorded on site in a note book using relevant charts.	-	-	-	-
Metals (Mn, Fe, Cu, Zn, Pb, Ni, Cd, Cr, Ca, Mg, K, Na,)	1kg	Plastic	Ice below 0°C	6 months	Rinsed with HNO ₃
Physico-chemical (%TOC, pH, Particle size,)	1kg	Plastic	Cool below 0°C	28 days	Rinsed with distilled water
Water Samples					
Parameter	Minimum Sample Volume	Container	Preservative	Holding Time	Container Pre-Treatment
salinity, Temperature, pH	<i>In-situ</i> Measurements	-	-	-	-
Metals (Mn, Fe, Cu, Zn, Pb, Ni, Cd, Cr, Ca, Mg, K, Na)	1.0l	Plastic	Add 2ml conc. HNO ₃ & cool, 4°C ± 2°C	6months	Rinsed with HNO ₃

Table 2: Methods of storage and Preservation of Samples.

Parameters for Water Analysis	Methods	Detection Limits
Temperature (°C)	APHA 2110B	-
pH	APHA 4500H*B	-
Salinity (mg/l)	APHA 2520B	0.01
Nitrate (mg/l)	EPA 352.1	0.02
Phosphate (mg/l)	APHA4500-P D	0.002
Ammonium (mg/l)	APHA 4500-NH ₃	0.02
Calcium (mg/l)	APHA 3111B/ASTM D3561	0.1
Magnesium (mg/l)	APHA 3111B/ASTM D3561	0.1
Potassium (mg/l)	APHA 3111B/ASTM D3561	0.1
Sodium (mg/l)	APHA 3111B/ASTM D3561	0.1
Lead (mg/l)	APHA 3111B	0.20
Total Iron (mg/l)	APHA 3111B	0.05
Copper (mg/l)	APHA 3111B	0.05
Zinc (mg/l)	APHA 3111B	0.05
Manganese (mg/l)	APHA 3111B	0.10
Cadmium (mg/l)	APHA 3111B	0.02
Total Chromium (mg/l)	APHA 3111B	0.10
Parameters for Soil Analysis		
pH (H ₂ O)	ASTM D4972	-
TOC/TOM (mg/kg)	BS 1377	-
Salinity (mg/kg)	APHA 2510B	-
Nitrate (mg/kg)	EPA 352.1	0.02
Phosphate (mg/kg)	APHA 4500-P D/CAEM	0.002
PSD (mg/kg)	ASTM D422	-
Calcium (mg/kg)	APHA 3111D	0.1
Magnesium (mg/kg)	APHA 3111B/ASTM D3561	0.1
Potassium (mg/kg)	APHA 3111B/ASTM D3561	0.1
Sodium (mg/kg)	APHA 3111B/ASTM D3561	0.1
Zinc (mg/kg)	ASTM D5198/APHA 3111B	0.05
Lead (mg/kg)	ASTM D3111B /D5198	0.20
Total Iron (mg/kg)	APHA 3111B/ASTM D5198	0.05
Copper (mg/kg)	APHA 3111B/ASTM D5198	0.05
Cadmium (mg/kg)	APHA 3111D/ASTM D5198	0.02
Total Chromium (mg/kg)	APHA 3111B/ASTM D5198	0.10

Table 3: Laboratory analytical methods.

	Leaf length	Leaf width	Stipules length	Petiole length	Distance between lateral nerves	Length of inter node	Number of foliage per twig	Number of lateral nerves	Distance of mid rib from line of symmetry
OTU 1	7.8 ± 0.03	4.9 ± 0.12	7.42 ± 0.02	2.04 ± 0.4	0.01 ± 0.01	3.9 ± 0.01	9 ± 0.02	51 ± 0.02	0.06 ± 0.04
OTU 2	12.7 ± 0.17	5.8 ± 0.16	7.59 ± 0.02	2.05 ± 0.16	0.07 ± 0.23	4.2 ± 0.24	8 ± 0.07	39 ± 0.02	0.08 ± 0.21
OTU 3	11.0 ± 0.02	5.1 ± 0.17	7.78 ± 0.16	2.04 ± 0.18	0.12 ± 0.02	4.0 ± 0.23	9 ± 0.08	26 ± 0.02	0.04 ± 0.24
OTU 4	13.01 ± 0.17	5.6 ± 0.16	7.90 ± 0.23	2.05 ± 0.15	0.14 ± 0.02	4.3 ± 0.16	10 ± 0.16	28 ± 0.23	0.02 ± 0.22
OTU 5	11.7 ± 0.07	5.5 ± 0.05	7.80 ± 0.02	2.05 ± 0.16	0.16 ± 0.33	4.3 ± 0.02	9 ± 0.41	30 ± 0.23	0.06 ± 0.26

Table 4: Morphometrics showing five distinct groups.

Taxonomic Units	Distance in metres from shorelines for each OTU				
Communities	OTU 1	OTU 2	OTU 3	OTU 4	OTU 5
Adiabo Ukanabio	1-87	83-124	106-271	235-379	344-488
Ikwe	1-90	87-139	120-296	240-390	350-511
Koko,	1-91	88-132	116-274	264-372	359-499
Ugwede	1-93	90-132	124-265	243-378	357-504
Esighi	1-94	89-133	123-274	254-385	364-507
Akakumama	1-98	97-140	139-270	264-380	369-514
Olupiri-Epelema	1-101	96-145	126-262	244-373	363-508
Nembe	1-105	100-147 485-604	136-287	263-378	375-489
Ogidigben	1-107	102-146	136-280	259-398	384-518
Opolom	1-105	106-146	140-272	266-388	360-525

Table 5: Occurrence of OTU from shoreline to edge of terrestrial environment per sampling community.

OTU	Pollen Morphology	Surface Pattern	Equatorial Size			Polar Size			Equatorial Shape	Polar Shape	Grain Arrangement
			Min	Max	Mean	Min	Max	Mean			
1	Tricolporate	Reticulate	17.2	19.3	16.4	14.7	16.2	15.5	Sub prolate	Circular	Monad
2	Tricolporate	Baculate	32.9	38.6	34.86	28.1	31.6	29.15	Prolate	Triangular (convex)	Monad
3	Tricolporate	Striate, Rugulate	23.5	27.5	25.0	21.0	25.0	24.0	Oblate	Triangular	Monad
4	Tricolporate	Gemmate	27.2	34.3	30.2	22.8	27.8	24.6	Oblate	Circular	Monad
5	Tricolporate	Reticulate	18.6	20.2	15.4	26.4	29.2	27.9	Prolate	Trilobate	Monad

Table 6: Results of Pollen characters measurement shown in Plates 1-5.

of the 5 OTUs were observed in the interface between 1-90 m, 87-139 m, 120-296 m, 240-390 m and 350-511 m respectively.

Further results on the individuals that made up each OTU for each sampled site is provided in Table 5.

Morphometrics

The result of the morphological analysis revealed spatial areas where only one *Rhizophora* candidate occurred and also areas where two *Rhizophora* units co-occur. The study does not reveal any area where more than two *Rhizophora* units occur. In Adiabo Ukanabio, for instance, while OTU 1 was observed as the sole *Rhizophora* candidate between distance 1-82 m, OTU 1 and OTU 2 occurred as mixed units between 83-87 m, OTU 2 and OTU 3 as mixed candidates between 106m to 124m and OTU 3 and OTU 4 co-occurred between 235-271 m. OTU 4 and OTU 5 were found as mixed units between 344m to 379m (Tables 6-8). Table 9 provides information on the spatial areas where unitary occurrence of *Rhizophora* were observed and areas of mixed occurrence.

Irrespective of sampling location, monotypic occurrence of a *Rhizophora* member occurred between distance 1-82 m for OTU 1, 88-105 m for OTU 2, 125-234 m for OTU 3, 263-343 m for OTU 4 and 373-488 m for OTU 5. The mixed occurrence zone for OTU 1 and 2 was observed at about 4.4 m (between 82.6-87 m) as against OTU 2 and OTU 3 that had an overlap area of about 11.8 m. OTUs 3 and 4 had an overlap of 21.8 m while OTUs 4 and 5 had a 19.4 m joint area of occurrence. At

0.05 confidence level, the occurrence of OTUs 2 and 3, 3 and 4 and 4 and 5 was statistically significant ($p < 0.001$). Ebigwai and Akomaye [25] reported mixed *Rhizophora* occurrence in the Niger Delta. This implied that at those specified distances away from the shoreline, species identification may be challenging [26]. However, when the means of the leaf length, leaf width, petiole length, stipules length, distance between lateral nerves, distance between the internodes, the number of foliage leaves per twig, number of lateral nerves and distance of midrib away from centre of symmetry was subjected to Analysis of Variance, (ANOVA) and Newman Keuls Multiple Comparison Test at 0.05 confidence level, some of them proved statistically significant. Analysis of variance (ANOVA) result based on 9 quantitative parameters of *Rhizophora* species (Table 10).

Results of pollen analysis

Results of the pollen samples is presented according to the five boundaries established by morphological study. These shapes are shown in plates 1-5 and Table 2. Table 3 provides the analytical methods employed and the detection limits of the equipment. Results of the laboratory measurements of the pollen characters shown in Plates 1-5 is provided in Table 6.

Result for soil physico-chemical parameters

The prevailing temperature of the various depths had a range of 27.65-27.8 °C. High rainfall and strong tidal influences and decrease evaporation rate tend to influence the low temperature regime.

Parameters	1-87	88-124	125-262	263-372	373-488
Depth (cm)	15	15	15	15	15
Colour	brown	Dark	grey	Dark	Dark
Temperature	27.80 ± 4.5	27.72 ± 3.7	27.78 ± 2.6	27.65 ± 3.1	27.71 ± 2.8
PH	4.87 ± 1.2	4.85 ± 1.4	5.40 ± 1.2	5.15 ± 1.3	5.18 ± 1.7
Salinity (ppm)	38.9 ± 1.6	41.2 ± 1.3	57.6 ± 1.4	58.4 ± 1.5	55.2 ± 1.6
Moisture Content (%)	23.61 ± 1.5	23.52 ± 1.6	16.73 ± 1.7	19.72 ± 4.3	13.68 ± 3.8
Clay (%)	7.40 ± 2.6	5.31 ± 1.4	5.74 ± 2.0	6.42 ± 2.1	3.13 ± 1.1
Silt (%)	16.95 ± 3.5	26.42 ± 2.6	16.84 ± 3.2	15.66 ± 2.4	15.10 ± 5.3
Sand (%)	77.2 ± 2.6	68.28 ± 7.6	77.46 ± 8.4	78.03 ± 6.6	81.81 ± 8.3
Nitrogen	3.32 ± 1.1	3.34 ± 0.7	3.37 ± 1.1	3.40 ± 1.3	3.38 ± 0.9
Ext. Nitrate (mg/kg)	2.01 ± 0.5	2.00 ± 0.4	1.97 ± 0.6	1.98 ± 0.2	2.01 ± 0.6
Nitrite	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0
Ammonium	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	31.60 ± 0.0
Ext. Sulphate (mg/kg)	64.37 ± 6.4	69.66 ± 7.1	67.39 ± 5.7	67.29 ± 6.4	67.28 ± 7.9
Ext. Sulphides (mg/kg)	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0	0.02 ± 0.0
Ext. Phosphate (mg/kg)	3.99 ± 0.7	3.91 ± 1.0	4.10 ± 1.2	4.01 ± 1.3	3.98 ± 1.4
TOC	2.36 ± 0.8	3.43 ± 1.1	3.58 ± 1.3	4.19 ± 0.9	5.05 ± 1.4
CEC	20.12 ± 3.5	22.83 ± 2.5	23.02 ± 3.2	25.88 ± 3.7	28.94 ± 3.2
Magnesium	2112.92 ± 11.4	2405.43 ± 18.6	2035.15 ± 15.4	3533.89 ± 18.7	2112.09 ± 21.3
Sodium	7210.60 ± 21.0	8048.81 ± 24.3	6488.16 ± 16.6	6822.24 ± 17.9	5801.33 ± 19.2
Potassium	7931.33 ± 23.1	10835.11 ± 16.8	7951.82 ± 26.4	8954.42 ± 21.5	8400.61 ± 28.8
Calcium	2195.90 ± 15.3	1891.21 ± 19.4	1322.79 ± 20.8	1598.52 ± 22.3	1680.81 ± 18.3
Total Chromium (mg/kg)	21.10 ± 2.8	19.35 ± 3.1	20.29 ± 2.8	19.65 ± 4.3	19.99 ± 8.3
Total Iron (mg/kg)	2196 ± 4.1	2192 ± 9.7	2198 ± 12.1	2199 ± 18.2	2197 ± 13.8
Copper (mg/kg)	6.14 ± 1.7	5.98 ± 1.3	4.23 ± 0.9	6.11 ± 1.6	5.67 ± 1.2
Lead (mg/kg)	11.01 ± 2.7	11.21 ± 1.6	10.94 ± 1.3	11.37 ± 1.7	10.36 ± 1.5
Zinc (mg/kg)	25.61 ± 3.7	36.06 ± 4.2	23.05 ± 7.1	21.68 ± 3.3	36.31 ± 4.5
Manganese (mg/kg)	187.60 ± 11.2	188.71 ± 14.3	181.48 ± 17.2	183.18 ± 10.3	185.34 ± 13.7
Selenium (mg/kg)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± v	0.1 ± 0.0
Molybdenum (mg/kg)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cobalt (mg/kg)	3.00 ± 0.3	3.02 ± 0.2	3.00 ± 0.1	3.00 ± 0.2	3.00 ± 0.2

Table 7: Mean and Standard Error for soil parameters.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Mean
pH	7.00 ±	6.96 ±	6.94 ±	6.72 ±	6.32 ±	6.96 ±	7.04 ±	6.86 ±	6.85
Temperature (°C)	28.72 ±	28.22 ±	30.24 ±	29.52 ±	28.58 ±	28.82 ±	28.41 ±	28.39 ±	28.86
Salinity (g/L)	3.92 ±	3.93 ±	3.93 ±	3.93 ±	8.4 ±	3.2 ±	2.02 ±	3.77 ±	4.14
Nitrate (mg/L)	0.3 ±	0.29 ±	0.29 ±	0.34 ±	0.23 ±	0.3 ±	0.3 ±	0.24 ±	0.29
Sulphate (mg/L)	324.1 ±	325.13 ±	300.16 ±	315.62 ±	351.86 ±	398.99 ±	572.06 ±	380.33 ±	371.03
Phosphate (mg/L)	0.87 ±	0.95 ±	0.96 ±	0.7 ±	1.87 ±	1.13 ±	1.07 ±	0.78 ±	1.04
Magnesium (mg/L)	136.17 ±	126.5 ±	125.47 ±	133.11 ±	100.52 ±	97.34 ±	96.15 ±	131.02 ±	118.29
Potassium (mg/L)	28.03 ±	33.31 ±	67.85 ±	29.42 ±	29.61 ±	25.47 ±	89.66 ±	28.47 ±	41.48
Sodium (mg/L)	997.99 ±	943.73 ±	961.59 ±	1110.78 ±	955.25 ±	1027.32 ±	272.14 ±	937.6 ±	900.80
Calcium (mg/L)	35.42 ±	42.5 ±	31.34 ±	40.38 ±	35.53 ±	33.69 ±	53.1 ±	37.92 ±	38.74
Chromium mg/L)	0.08 ±	0.07 ±	0.07 ±	0.06 ±	0.11 ±	0.13 ±	0.05 ±	0.05 ±	0.08
Manganese (mg/L)	0.09 ±	8.81 ±	0.11 ±	0.09 ±	0.09 ±	0.08 ±	0.13 ±	0.1 ±	1.19
Lead (mg/L)	0.13 ±	0.13 ±	0.09 ±	0.11 ±	0.1 ±	0.12 ±	0.09 ±	0.13 ±	0.11
Zinc (mg/L)	0.06 ±	0.09 ±	0.06 ±	0.06 ±	0.15 ±	0.06 ±	0.07 ±	0.06 ±	0.08
Copper (mg/L)	0.05 ±	0.07 ±	0.72 ±	0.05 ±	0.05 ±	0.05 ±	0.10 ±	0.05 ±	0.14
Total Iron (mg/L)	1.04 ±	0.97 ±	0.92 ±	1.04 ±	1.29 ±	0.98 ±	0.95 ±	1.14 ±	1.04
Cobalt (mg/L)	0.02 ±	0.03 ±	0.06 ±	0.08 ±	0.09 ±	0.02 ±	0.02 ±	0.02 ±	0.04
Ammonium	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.03 ±	0.02 ±	0.02 ±	0.02 ±	0.02
Sulphide	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.02 ±	0.02
Cadmium	0.01 ±	0.01 ±	0.02 ±	0.03 ±	0.04 ±	0.03 ±	0.02 ±	0.01 ±	0.02
Molybdenum	0.1 ±	0.1 ±	0.01 ±	0.1 ±	0.1 ±	0.19 ±	0.1 ±	0.1 ±	0.1
Chloride	53.52 ±	75.76 ±	50.8 ±	23.9 ±	39.56 ±	49.6 ±	34.81 ±	39.84 ±	45.97

Table 8: Mean and Standard Error for surface water parameters across sampling sites.

OTU	Distance (m) away from shorelines									
	Adiabo Ukanabio		Ikwe		Koko		Ugwede		Esighi	
	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed
1	1-82	83-87	1 -86	87-90	1-87	88-91	1 -89	90 -93	1-88	89-94
2	88-105	106 - 124	91-119	120- 139	92-115	116- 132	94 -123	124- 132	95-122	123-133
3	125-234	235 - 271	140- 239	240- 296	133- 263	264- 274	133- 242	243- 265	134-253	254-274
4	272-343	344-379	297- 349	350- 390	275- 358	359- 372	266- 356	357- 378	275-363	364-385
5	380- 488		391- 488		373- 499		379- 504		386-507	
OTU	Akakumama		Olupiri-Epelema		Nembe		Ogidigben		Opolom	
	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed	Unitary	Mixed
1	1-96	97-98	1-95	96-101	1-99	100-105	1-101	102-107	1-105	
2	99-138	139-140	102-125	126-145	106-135	136-147	108-135	136-146	106-139	140-146
3	141-263	264-270	146-243	244-262	148-262	263-287	147-258	259-280	147-265	266-272
4	271-368	369-380	263-362	363-373	288-374	375-378	281-383	384-398	273-359	360-388
5	381-514		374-508		490-604		399-578		389-525	

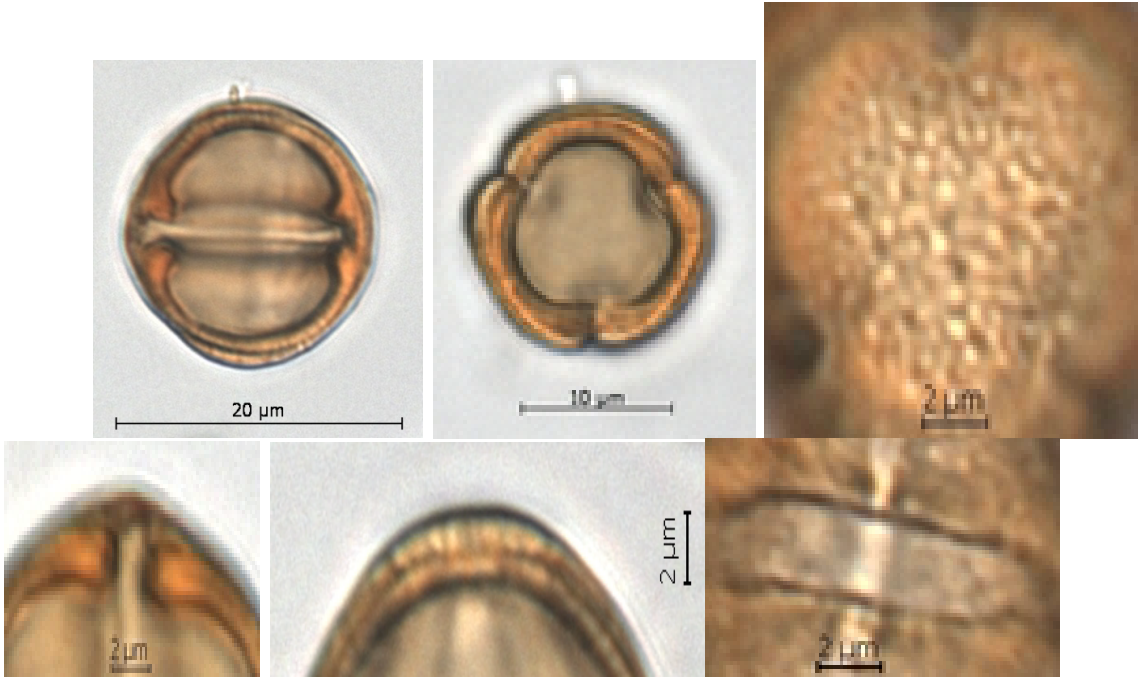
Unit of measurements = Metres

Table 9: Areas of unitary and mixed occurrence of *Rhizophora* units.

Leaf Parameters	P value	F value	Error Term	Newman- Keuls Multiple Test
*Leaf Length	< 0.001	297.25	0.04	<u>1, 3, 5, 4, 2</u>
Leaf Width	0.005	9.04	0.05	<u>4, 5, 2, 3, 1,</u>
*Stipule Length	<0.001	55.92	0.47	<u>1, 2, 3, 5, 4</u>
Petiole Length	1.000	0.00	0.19	<u>1, 3, 2, 5, 4</u>
*Distance between lateral Nerves	<0.001	38	0.00	<u>1, 3, 2, 5, 4</u>
*No of lateral nerves	<0.001	83.96	3.72	<u>1, 2, 5, 4, 3</u>
Internodes Distance	0.401	1.15	0.27	<u>2, 1, 3, 4, 5</u>
No of foliages per Twig	0.007	7.80	0.19	<u>2, 1, 3, 4, 5</u>
Distance of mid rib away from centre of symmetry	0.204	1.90	0.00	<u>4, 3, 1, 5, 2</u>

*P < 0.05 implies statistical significance, Newman Keuls Multiple Test - numbers joined together by a single line are not significant difference.

Table 10: Analysis of variance (ANOVA) result based on 9 quantitative parameters of *Rhizophora* species.



Equatorial, polar, surface ornamentation, special features, wall, aperture

Equatorial, polar, surface ornamentation, special features, wall, aperture

Plate 1: Pollen characters for OTU 1.

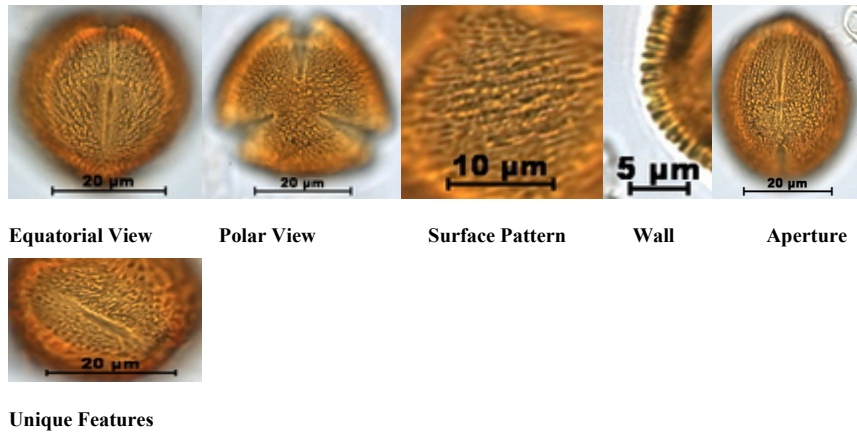


Plate 2: Pollen Characters of OTU 2.

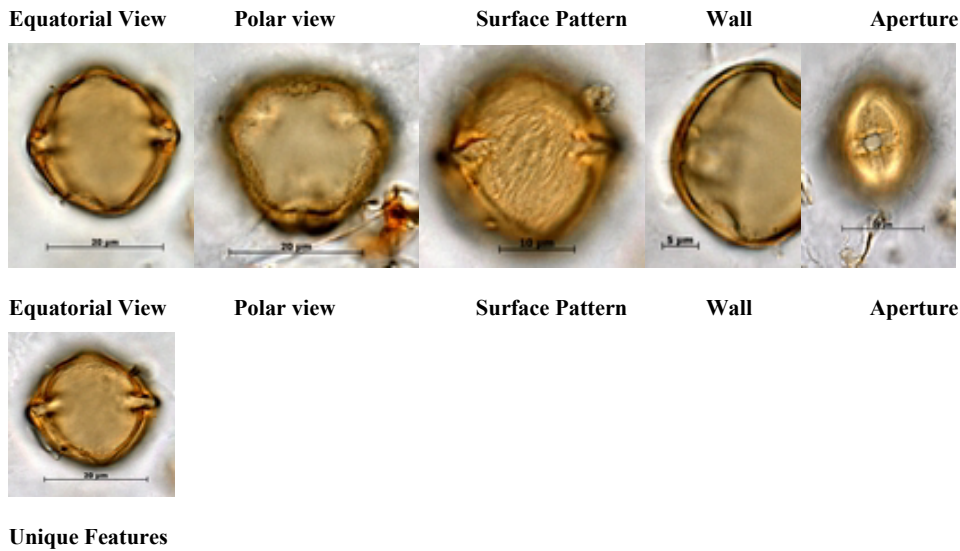


Plate 3: Pollen Characters of OTU 3.

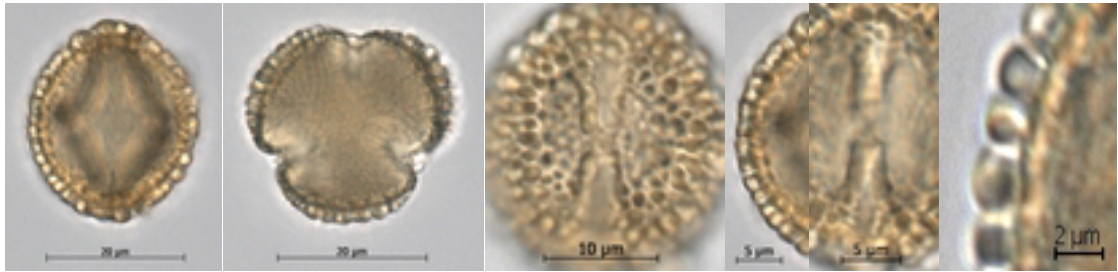


Plate 4: Pollen Characters of OTU 4.

Statistically, there was no significant difference in the soil temperature among the various distances under investigations. The pH of the soils as shown in Table 7, ranged from 4.78 to 5.40 with a mean of 5.07. The mean pH (6.85) of the water samples (Table 8) was less acidic when compared to that of the soil. Decrease in salt and abundance of the ions

of sodium, magnesium, and calcium (compare the mean values of these salts in Tables 7 and 8) possibly by leaching action may be the causal factor. The least mean soil pH value occurred within the mangrove-terrestrial boundary interface while the highest value was observed at the 151 - 225m range. The prevailing mean pH value for each OTU was analyzed as 4.87, 5.26, 5.16, 5.18 and 5.08 for OTUs 1,2,3,4 and

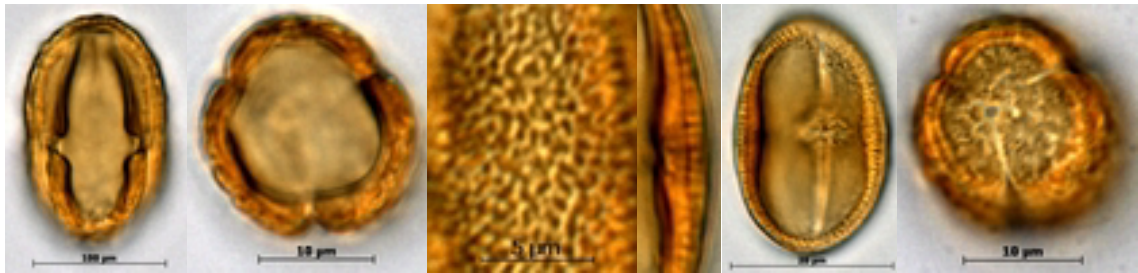


Plate 5: Pollen characters of OTU 5.

5 respectively. The result proved statistically significant ($p < 0.001$) in general but specifically for OTU 1, OTU 2 and OTU 5. The mean pH for OTUs 3 and 4 were not statistically significant. The values recorded in the study agree with those obtained by Essien and John [27] and Oyem and Oyem [28] for soils in the Niger Delta. The results for soil analysis are presented as mean and standard error for each distance from the shoreline as shown in Table 7.

Result for water physico-chemical parameters

The results for water analysis are presented as mean and standard error for each water body obtained from areas colonized by each of the five *Rhizophora* OTUs (Tables 8 and 9).

Discussion

The result showed that leaf length, stipule length, distance between lateral nerves and number of lateral nerves proved statistically significant at the 0.05 % confidence limits. This implied that these four leaf characters could be employed in the identification of some *Rhizophora* species. This is in contrast to Nyannanyo [29] who posited that morphological characters cannot be used in delimiting *Rhizophora* taxon. The application of leaf length alone resulted in the identification of only three OTUs. This was so since leaf length could not delimit OTU 1 from 3 and OTU 2 from OTU 4. However, it was clear that application of leaf length alone was efficient in delimiting OTU 5. On further re-examination, it was observed that application of leaf length on an average of 32.5 leaves from an individual *Rhizophora* plant proved successful in separating OTU 5 from others. As could be observed in Table 10, variations in stipule length proved useful in separating OTU 4 from others. The mean stipule length of 7.90 cm over 54 replicates was confirmed on further re-examination as sufficient in delimiting OTU 4. The number of lateral nerves as shown in the Newman Keuls Multiple Comparison Test proved useful in separating OTU 1 and OTU 2 from the other OTUs. The average number of lateral nerves in OTU 1 was 51 as against 39 in OTU 2. The measurement of lateral nerves involving 27 leaf samples from a single *Rhizophora* individual was evaluated as the minimum number required for successful split of OTU 1 and OTU 2. No one of the characters employed was able to separate OTU 3. Nonetheless, since 4 of the 5 OTUs were delimited successfully, the remainder is OTU 5. However, two indirect elimination techniques were applied to delimit OTU 3 using four leaf characters that were statistically significant. First, the use of stipule length followed by leaf length identified OTU 3. Second, the use of distance between lateral nerves followed by the use of number of lateral nerves. The use of Principal Component Analysis (PCA) in Tables 11-13 strongly asserts results of the morphological relationships among the five OTUs. The information contained in Tables 11-13 was used to produce a Principal Component Analysis scatter plot shown in Figure 3.

Principal Component	Eigen values	% Variance
1	109.113	97.638
2	2.254	2.0168
3	0.375	0.3352
4	0.0112	0.010

Table 11: Principal component analysis.

OTU	Axis 1	Axis 2	Axis 3	Axis 4
1	16.533	-1.1317	0.21593	-0.0049735
2	3.9607	2.2385	-0.50952	-0.039655
3	-8.6614	-1.5219	-0.5094	-0.094301
4	-7.0144	0.6085	0.95393	-0.041813
5	-4.8178	-0.1933	-0.15094	0.18074

Table 12: Principal component analysis scores.

The scatter plot re asserts the taxonomic distinctness of OTU 1 and OTU 2. This is exemplified by their distances. Leaf length was observed as the dominant character state influencing their distinctness. OTU 3, 4 and 5 on the other hand were observed closely packed together. Although, shown to be distinct morphologically and statistically, OTU 4 and OTU 5 exhibited close affinities.

Pollen

As evident across plate 1-5 and Table 2, the basic pollen type in the genus is Tricolporate. However four different surface patterns were observed. They are reticulate, baculate, strait regulate and germate. The equatorial shape on the other hand ranged from sub prolate to prolate to oblate as against triangular, circular and trilobate for the polar shape. The grain arrangement across the samples was uniform, Monad. The pollen dimensions showed a polar size range of 15.5 µm in pollen shapes 1 to 29.15 µm in pollen shapes 2. Similar trend was observed in the equatorial size. However, the polar to equatorial ratio (0.81) was smallest in pollen shapes 4 and largest (0.99) in pollen shapes 5. To ensure data integrity by reducing redundancy, the result of data normalization for the application of cluster analysis is presented.

As could be seen in Table 14, the transformed values ranged from negativity in the P/E ratio to positivity in the other three parameters. Similarity and distance among the five operational taxonomic units was obtained using Euclidean index. The result is as shown in Table 15.

Principal Component Analysis to depict the relationship of the five OTUs is shown in Figure 4.

As shown in Table 15 and exemplified in Figure 3, the summed distance between OTU 1 and the other OTUs was 1.3807, as against 0.8245, 0.6356, 0.6559 and 0.6570 for OTUS 2, 3, 4 and 5 respectively. The percentage dissimilarity among the OTUs is shown in Table 16.

	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9
leaf length	-0.1458	0.9445	0.1699	-0.2009	0.003146	0.1322	0	0	0
leaf width	-0.01367	0.2241	0.01117	0.5484	-0.0438	-0.7957	0.0293	0.03911	0.1062
stipules length	-0.01751	0.005687	0.08711	0.1764	0.9431	0.07381	0.01897	0.2414	-0.08652
petiole length	-0.0001804	0.002943	0.001958	0.02211	-0.2661	0.01619	0.02119	0.8611	-0.4319
Distance between lateral nerves	-0.005384	0.001886	0.009853	0.1987	-0.08049	0.2718	-0.07791	0.3924	0.8485
length of inter node	-0.01011	0.07603	0.07835	0.7626	-0.1592	0.5078	-0.08392	-0.2109	-0.2677
number of foliage per twig	-0.02515	-0.1808	0.9766	-0.04806	-0.07147	-0.06249	0.03137	-0.01127	0.02149
Number of lateral nerves	0.9887	0.1387	0.05248	-0.01134	0.01266	0.01478	-0.0009646	0.00468	0.002226
Distance of mid rib from line of symmetry	0.001221	0.005642	-0.02547	0.06158	-0.02857	0.08804	0.9921	-0.01084	0.05105

Table 13: Principal component analysis loading.

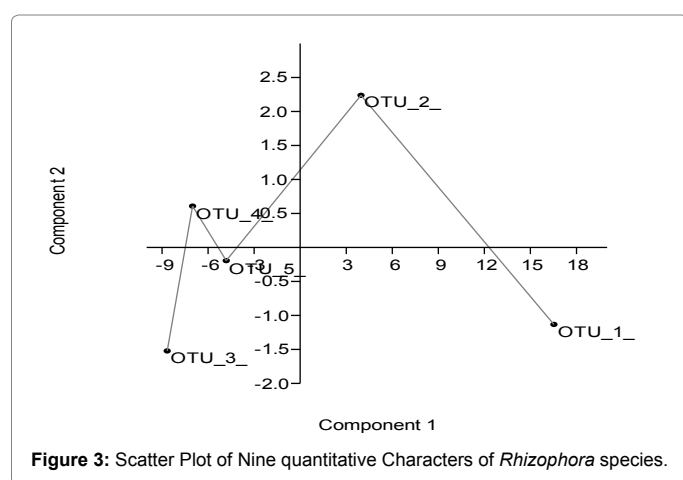


Figure 3: Scatter Plot of Nine quantitative Characters of *Rhizophora* species.

Species/Pollen character	1	2	3	4	5
Polar Diameter	1.19	1.46	1.38	1.39	1.45
Equatorial Diameter	1.21	1.54	1.40	1.48	1.45
P/E	-0.02	-0.08	-0.02	-0.09	-0.01
Number of Aperture	0.48	0.48	0.48	0.48	0.48

Table 14: Data standardization for cluster analysis.

Taxonomic Units	OTU 1	OTU 2	OTU 3	OTU 4	OTU 5
OTU 1	0	0.43052	0.26382	0.33963	0.34666
OTU 2	0.43052	0	0.17703	0.097803	0.11924
OTU 3	0.26382	0.17703	0	0.11088	0.083857
OTU 4	0.33963	0.097803	0.11088	0	0.10753
OTU 5	0.34666	0.11924	0.083857	0.10753	0

Table 15: Euclidean index of Similarity and Distances among Five OTUs.

OTUs	% Dissimilarity
1 & 2	55.62
1 & 3	74.51
1 & 4	72.48
1 & 5	72.37
2 & 3	18.89
2 & 4	16.86
2 & 5	16.75
3 & 4	2.03
3 & 5	2.14
4 & 5	0.11

Table 16: Percentage Dissimilarity Index among *Rhizophora* OTUs.

Statistical WINKS SDA 6 revealed a $p < 0.001$ at 0.05 confidence interval. The analysis further revealed;

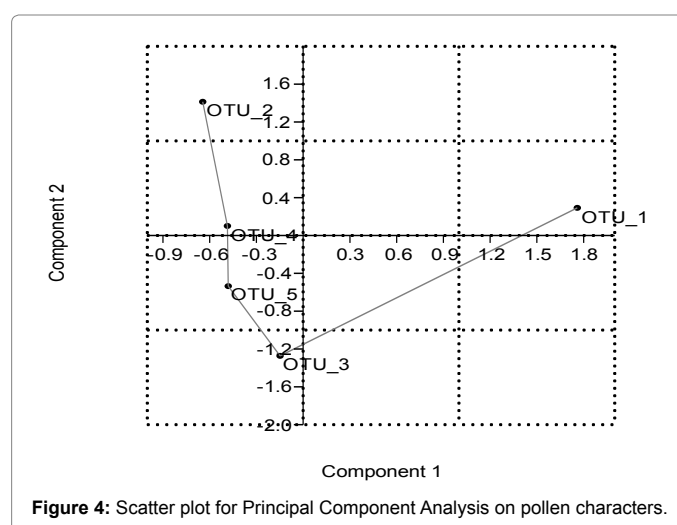


Figure 4: Scatter plot for Principal Component Analysis on pollen characters.

- Significant differences between OTUs 1 and 2, OTUs 3 and 4, OTUs 3 and 5 and OTUs 4 and 5.
- No significant difference between OTUs 1 and 3, OTUs 1 and 4 and OTUs 1 and 5.
- No significant difference between OTUs 2 and 3, OTUs 2 and 4 and OTUs 2 and 5.

These findings are graphically shown in Figure 5 (Graphical illustrations of taxonomic relationships among studied OTUs).

Based on this study, four possible deductions could be made. Subject to other taxonomic lines of evidence, OTU 1 may represent a distinct species so do OTU 2. OTU 3, 4 and 5 though distinct from each other could represent subtypes of either OTU 1 or OTU 2. This suggestion was further strengthened by result of the cluster analysis shown in Figure 5 (Cluster Analysis using Past Software (version 2)) (Figure 6).

Mangrove species just like other flora are known to colonize soils with specific physico chemical attributes [30]. In the Nigeria Niger Delta, edaphic factors influenced by tidal regimes had long been identified as causal factors of species type, speciation or mimicry [31]. Rajakaruna and Boyd [32] listed pH, organic matter, texture, structure, depth, ion availability, micro nutrients and macro nutrients as edaphic factors affecting plant growth. FAO, 2007 listed soil salinity as a dominant parameter in the mangroves [33]. Soil physico chemical parameters were conducted to determine the influences of each OTUs with reference to soil type preference. It was generally observed that depth 1-87m (zone of monotypic occurrence of OTU 1 was 1-83m) was

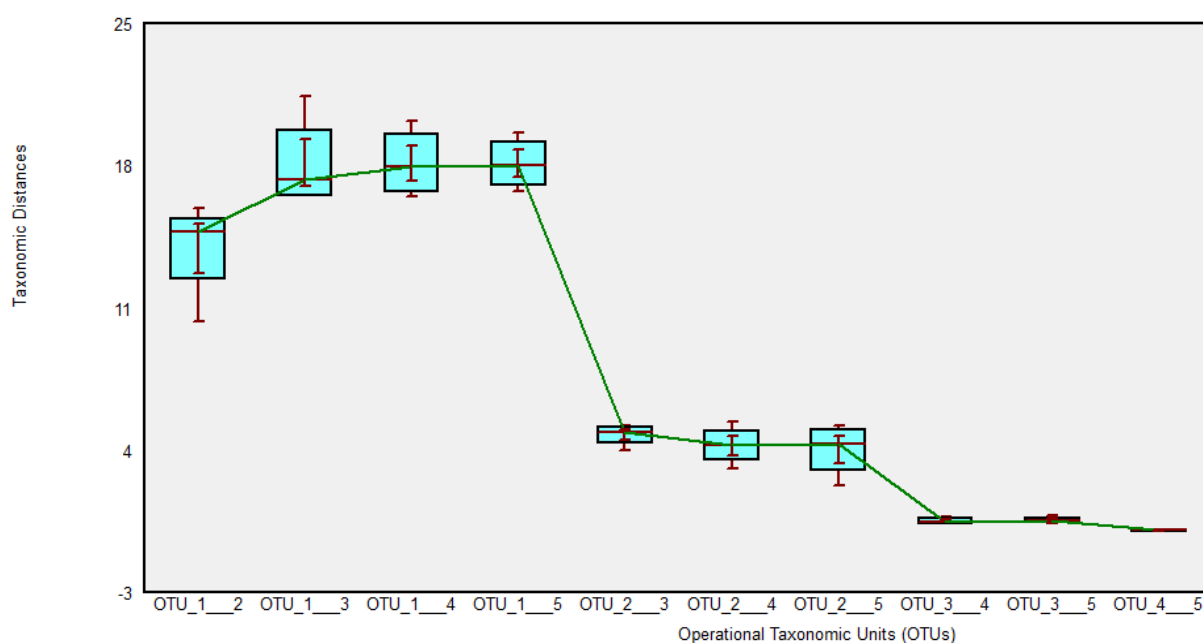


Figure 5: Graphical Illustration of taxonomic relationships among the studied OTUs.

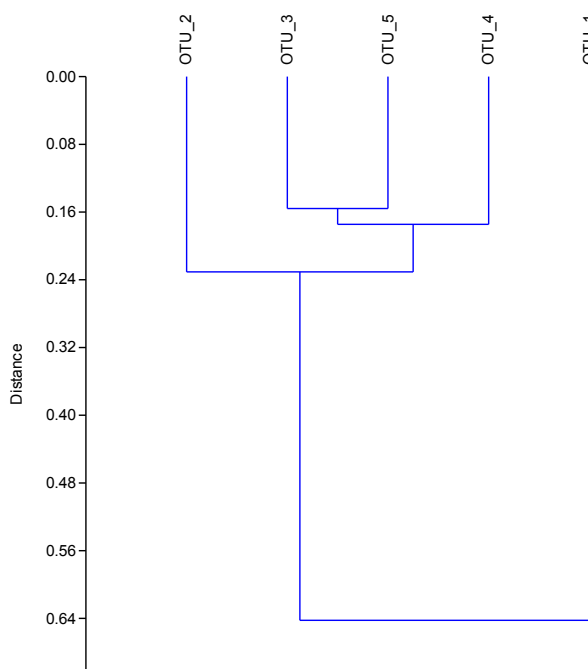


Figure 6: Cluster Analysis using past Software version 2.

a perennially flooded forest as against 88-124m that floods on the onset of the slightest tidal regime. Depth 125m to about 262m floods after about 1.30 min from the flooding episode as against depth 263-372m that flood after about 2hrs into each flooding exercise. Depth 373m - 488m floods only after about 2.45 hours (maximum flooding period

and do so for about 30 min only).

Conductivity as a measure of the degree of salinity, showed an increase in the salinity of mangrove water relative to that of seawater, confirming the ability of mangrove to tolerate high salt levels [34]. The trend of increasing salinity content from the high intertidal zones occupied by OTU 1 and to some extent, OTU 2 to mid-stream occupied by OTU 2 and 3 to the low intertidal zone or terrestrial border occupied by OTUs 4 and 5. This trend was also observed in soil salinity levels. This salinity was observed to be closely linked to the tide. Tidal waters reach the first site, located closer to the sea (area mainly occupied by OTU1) and continuously for a longer period, allowing leaching of salts made possible by soil permeability. For areas in the middle of the intertidal zone, the mean soil salinity value recorded was about 49.4 ppm. This area corresponds to soil colonized by OTUs 2 and 3. The low intertidal zone (site 4 and 5 that is areas closest to the continental shelf were characterized by stagnant water for a short time) had a mean soil pH of about 56.8ppm. Two factors affecting soil salinity are likely to be at play. First is the limiting influence of the tidal action and second is the nature and properties of the soil [35]. The existence of a statistical significant difference ($p < 0.001$) in salinity levels between areas occupied by OTU 1, OTU 2 and OTU 3,4 and 5 indicated a defining role played by salinity gradients in *Rhizophora* types. Each of the five OTUs showed peculiar preference for moisture contents as evident in the Newman Keuls multiple comparison test (NKMCT) that separated each.

Soil texture

The textural nature of the soil varied from high sand content through silt to clay. The clay content in soils colonized by OTUs 2, 3 and 4 were not statistically different indicating similar clay preferences for their distribution. However, the clay content need for OTU 1 was significant difference from all other so also was that of OTU 5. OTUs 1, 2 and 3 showed unique silt content preferences as shown by the NKMT as against OTUs 4 and 5 that were similar. OTUs 1, 3 and 4 showed a no

significant difference in sand content as against OTUs 1 and 5. Similar trend in the sand-silt-clay proportion was reported by Ihem, *et al.*, 2014 [36].

Total organic carbon and ion availability

Organic matter in observed in the study soils ranges from plant parts (roots, leaves, stems), decomposing faunal remains to humus, which is partly decomposed plant material that was amorphous. As evident in the results, a direct relation existed between the amount of TOC present and cation exchange concentrations per distance. For instance, depth 5 (88-124m) had the highest TOC value and CEC value while depth 1 had the least for both parameters. This may be attributable to the erosive, sedimentation and depositional patterns of the environment. As expected, the availability of certain salts (often Na, Mg, or Ca salts) that affects plant growth and distribution was abundant. The sodium adsorption ratio (SAR) was found to be higher in areas towards the terrestrial environment than do towards the shorelines. This may be attributed to the duration and extent of the area to stagnant water or perennial flooding episode a suggestion also made by Aishawafi *et al.* [37]. The differences in TOC and CEC concentrations with respect to zones colonized by each OTU showed as statistical significant difference for all. The significant differences between ions of sodium and potassium across all the points on one hand and that of calcium and magnesium on the other hands are owed partly to the trend witnessed in the clay portion of the study. Salts with lone valence electrons have been shown to aid clay dispersal as against those with two valencies that aids clay flocculation [37]. OTUs 1, 2 and 5 were clearly separated on the basis of sodium adsorption ratio.

Macronutrients

Total nitrogen, nitrates, nitrites, ammonium, phosphates, sulphates, sulphides and potassium availability to each soil colonized by each OTU was measured. The result showed no statistical differences in nutrient availability to the various OTUs. Hence differences in the pollen and morphology are not due to differences in nutritional content, quality and compositions.

Micronutrients

The roles played by iron, cobalt, chromium, copper, manganese, selenium, zinc and molybdenum in soils occupied by each OTU were evaluated. There were significant differences among all the OTUs for in chromium, lead and zinc. Iron and manganese showed significant differences in soils colonized by OTUs 1, 2 and 5 while there were significant differences in soils that were colonized by OTUs 1 and 4. The possibility of heavy metal presence from oil activities may be responsible for significant differences shown in some of these soils.

Conclusion

The study established five *Rhizophora* OTUs in the Niger Delta. Their occurrence was in direct relation to tidal influences. The use of leaf morphology in *Rhizophora* species identification was also revealed. The effect of soil and water physico chemical parameters as causal factors in *Rhizophora* species distribution was also determined.

Recommendation

Samples and the permanent plots established for this study should be further examined for genetic, anatomical, and phytochemical analysis. Such studies may reveal more interesting findings.

Acknowledgement

My sincere appreciation to the community leaders for granting social license

and permanent plots to conduct the research. My appreciation also goes to my post graduate students and the Department of Botany of the University of Calabar for allowing me access to equipment and materials.

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