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The diversity and abundance of littoral microflora in *Chicoco*-mud along the lower reaches of the New – Calabar River, Niger-Delta, Nigeria

[1]Karibi N.O Bob-manuel, [2]Dimabo O. Amachree

[1]Department of Animal and Environmental Biology, Rivers State University, Nkpolu-Oruworukwo , Port-Harcourt , Rivers State , Nigeria ;
[2]Nigerian Maritime Administration and Safety Agency (NIMASA) , Marine Environment Management Department , Eastern Zone , 7[B] Azikiwe Street , Port-Harcourt , Rivers State , Nigeria.

Abstract: **The diversity and abundance of littoral microflora in C*hicoco*-mud along the lower reaches of the New-Calabar River, Niger-Delta, Nigeria, was undertaken from June to October 2008 covering wet and dry seasons. Five stations were randomly chosen while littoral benthic samples were collected at Extreme Low Neap or Spring Tides. Microfloral results indicate the presence of the Raphidophyceae (3 – 5%), Pennatea (69 – 76%) and Centricae (20 – 28%) Bacillariophyceae. Seasonal variations were minimal , however , in wet season , average Margalef's Species Richness Index (d) varied from 2.070 – 2.196, Shannon-Weiner Diversity Index (H) varied from 1.016 – 1.084 bit/Individual (Ind.) and Equitability (E) from 0.947 – 0.958 while in dry season 'd' varied from 2.007 – 2.194 , 'H' from 1.047 – 1.135 and 'E' from 0.943 – 0.954 bits/Ind.; Nevertheless , Hutcheson *t* test for 'H' between Stations indicated 'significant' and 'not-significant' differences at $P < 0.05$ level. The most abundant and only species amongst the Raphidophyceae was the 'red tide causing' and toxic *Chattonella antiqua* (Y.Itada.) .C. Ono , while for the Centricae it was *Coscinodiscus eccentricus* Ehrenb. and *Stephanodiscus niagarae* Ehrenb. and for the Pennatea – *Nitzschia sigmoidea* (Nitzsch.) W. Smith , *Navicula anglic* Ralfs , *Surirella fastuosa* (Ehrenb.) Ehrenb. and *Tropidoneis lepidoptera* (Greg.) Cleve. . *Chicoco*-mud 'stations' and 'monthly' physicochemical data indicated that Sand, Silt, Clay, Fibre, Wet conductivity, TOC , pH ,Avail. P., and NO^-_3 – N were significantly different and affected the diversity and abundance of microflora , thus the continuous use of *Chicoco*-mud for land reclamation should be discouraged.**

Keywords: **Benthic, *Chicoco*-mud, Diversity, Littoral, Microflora, New-Calabar River, Niger-Delta.**

I. INTRODUCTION

The *Chicoco* mud, is the local name given to a darkish brown to pitch-black organic marine mud that superficially covers over 90% of the saline tidal flat (or mangrove swamp) zone or about 40% of the entire Niger Delta region of Southern Nigeria(Omotosho,2008). *Chicoco* mud can best be described as soft grey organic fibrous clays which resembles wood and is usually located at or near the earth's surface and was described as a brittle brown plant material containing a great deal of water and a low percentage of carbon (Teme , 2002;Bernstein *et al.*, 1996). *Chicoco* mud is scientifically known as peat and it occurs as top soil in this zone, however, the choice of *Chicoco*-mud rather than peat was drawn from the fact that there is no universally accepted terminology to describe this environment, which reflects traditional differences in understanding and comprehension among specialist (IPS, 1984; Joosten and Clarke, 2002; Häkan and Jeglum, 2006). It occupies precisely the intertidal or littoral flat of the mangrove swamp and is used as substitute material for landfill/reclamation by local inhabitants of the mangrove ecosystem.

Chicoco mud formation commences during senescence of the plant, when certain nutrients are recycled while others are lost by rain water leaching [McCarthy *et. al.*,1989) and occurs over a long time period by natural silting processes that comprised decayed/decaying fossils, mangrove particles, planktons, and other microorganisms that exist in life or dead states. The parent materials of mangrove soils in a tidal marsh are alluvial materials in the sedimentary environment, (Hseu and Chen, 1999). According to (Leh, 2002), there is a great need to sustainable manage the mangrove forest resources now than ever , this is because , the direct and indirect benefits derivable from the mangrove ecosystem are highly threatened and Imevbore *et al.*, 1997 reported that the mangrove floor is important to an innumerable macro flora and fauna , and so ultimately to the whole food chain leading to man .

In Nigeria, Niger Delta, there is a dearth of information about microfloral or microalgal assemblage in *Chicoco* mud with the exceptions of some foreign studies (Flensburg , 1965;Yung *et. al*., 1986 ; Hingley , 1993) , nevertheless, most other studies focused on fungi and microorganisms (Dickinson ,1983 ; Zhang & Andrews, 1993 ; Wheatley *et. al*., 1996), thus**,** knowledge of the benthic microflora in the *Chicoco* mud becomes highly relevant in trying to understand its ecological significance within the context of identification , diversity and abundance using the littoral fringes of the New-Calabar River as a case study.

II. MATERIALS AND METHODS

A. The study area:

This study area is located within three communities of the Asari-Toru Local Government Area (ASALGA) in the Kalabari Kingdom, Rivers State. Fig. 1 shows the map of the study area which cuts across Buguma (BGM) through Ido (ID) to Abalama (ABL) communities and consisted of five Stations *viz* BGM 1 , ID 2 , ID 3 , ABL 4 and ABL 5 . ASALGA lies within the transition zone of the Niger Delta region and is bounded by Latitude 4^{o}40’N to 4^{o}50’N of the Equator and Longitude 6^{o}50’E to 6^{0}48’E of the Greenwich Meridian. The New-Calabar River is the major tidal river that flows through this Local Government Area , it is a tidal river having a highly productive mangrove community These areas were chosen because they had huge deposit of pristine mud flats, *Chicoco* and mangrove vegetation. *Rhizophora racemosa, R. mangle, Avicenna africana* were the dominant species of the mangrove vegetation. Sampling within this study area was done monthly for five months, covering June to October 2008.

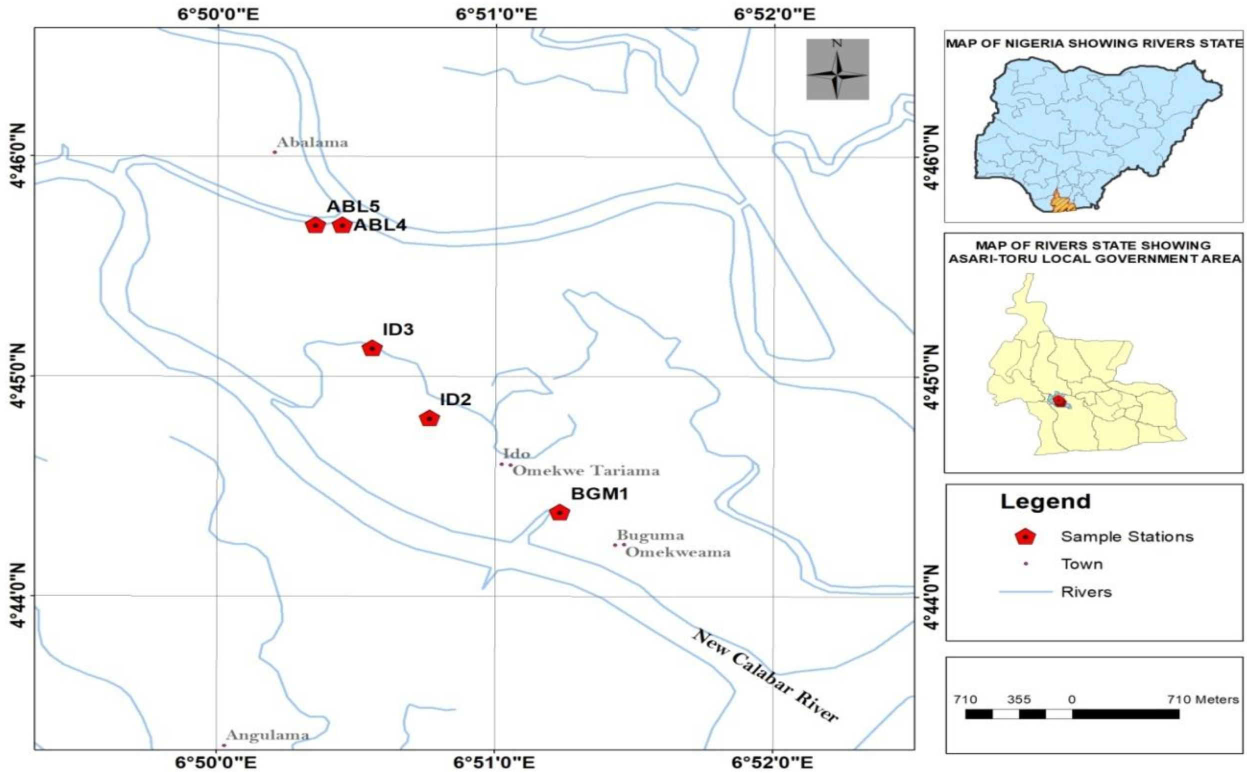

Fig 1: Map of study area showing locations where samples were collected

B. Chicoco mud sampling and analysis:

The *Chicoco* mud was sampled with the aid of a 0.0625 m^2 Quadrat which was used to mark the mud surface and a Border spade with a solid forged head size of 0.0322m^2 was used to excavate cubes measuring 0.014 m^3 at two different random points of a particular Station during Extreme Low Tide (ELT), all samples had two replicates. These cubes were put in labeled black cellophane bags before proceeding to the laboratory for *Chicoco* mud physicochemistry and benthic microfloral analysis. One kilogram of the sampled cube was weighed, put in a plastic bowl and five liters of tap water was added, this mixture was gently washed thoroughly, swirled, and decanted several times through a 0.05mm sieve so as to remove the plant fibres, while the sediment in solution was allowed to settle before decanting the clear water and collecting the sediment. This sediment sample was put in a polyethylene bag and air dried.

C. Benthic Microfloral Refining

Hundred grams of the sampled cube (surface to 10 cm depth) was weighed and put in a bowl , 100 ml of water was added to it to form a slurry and specific gravity was measured in order to ensure a consistent composition , this mixture was made up to 500mls and initially poured into two sieves (1mm and 0.5mm mesh sizes) so as to remove the fibers before finally pouring into six sieves of different sizes (200 µm , 177 , 200 , 63 , 38 , 20 µm) placed vertically in a serial order , refined with tap water controlled by a power hose to a clear consistency , the clean sediment retained in the 20 µm sieve size was removed from the rack and transferred into a 500ml beaker (Matsuoka and Fukuyo, 2000 , 2003) , 100ml of water was added , swirled and decanted into a 20ml vial container and preserved with neutral formalin (3% against the volume of the sample) . To this final refined sample, 0.05 ml of brilliant cresol green was added and one drop (0.01ml) was sub-sampled onto a glass slide, covered with a cover slip and examined with a binocular microscope (APHA,1998) at x100 magnification ,further examination at high power objective was also done to adequately identify the species.

D. Chicoco-mud Textural Analysis

One kilogram of the mud was weighed and put inside a plastic bowl and five liters of tap water was added. The mud was thoroughly washed and decanted using a 0.05 µm sieve so as to separate the fibers from the sediments. The wet fibers were weighed while the textural analysis of the sediment was carried out by adopting the method of Bouyoucos, 1951.

E. Sediment Physicochemical Analysis

The following analysis was carried out: Sediment pH, textural class, total organic carbon, available phosphorus, nitrates and sediment conductivity. Sediment pH was determined following the method described by Bates (1954) , textural class followed the method described by Bouyoucos , 1951 , total organic carbon followed the method of Walkley and Black , 1934 , available phosphorus was determined by the method of Bray and Kurt (1945) , nitrate followed the Brucine-Sulphate method (APHA , 1998 – Section 419 D) and wet sediment conductivity was carried out using a *pH* meter.

F. Biometry

Analysis of variance (ANOVA) was carried out on the data and the means of the data were presented along with ± SD (Standard Deviation) and separated using the Duncan Multiple Range Test (DMRT). Diversity indices adopted for this study were Margalef's Species Richness Index – d , Shannon-Wiener diversity Index-H (Shannon and Weaver , 1964) and Evenness Index-E (Pielou ,1977), while Hutcheson (1970) was used in calculating *T* so as *to* test for significant differences between sample H. The Coefficient of Variability (CV) was determined for the respective variables (Ogbeibu,2005).

III. RESULTS

TABLE 1: MEAN MONTHLY VARIATION (± SD & DMRT) OF PHYSICO-CHEMICAL DATA FOR CHICOCO MUD

S/No.	Variables	June	July	August	September	October	CV%
1.	Sand (%)	20.80±11.19a	11.00±5.96d	13.40±13.49c	15.00±8.86b	9.40±4.17e	31.0
2.	Silt (%)	5.60±3.38e	6.60±4.93d	22.60±35.90a	8.20±6.85b	9.00±4.29c	133.6
3.	Clay (%)	73.60±13.17d	82.40±10.67b	64.00±34.19e	76.80±12.27c	82.60±6.50a	20.2
4.	Fiber (g/kg)	215.86±44.81c	178.06±27.36e	189.52±53.82d	224.56±22.83b	240.48±30.46a	24.4
5.	Conductivity(µS/cm)	1186.60±144.0e	1449.40±193.2d	1517.80±447.3c	1842.60±405.2a	1635.00±347.9b	31.6
6.	TOC (mg/kg)	4.10±0.64e	5.13±0.85c	4.91±0.60d	5.24±0.35b	5.58±0.89a	22.2

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7.	pH	4.57±0.32e	4.90±0.41c	4.92±0.90a	4.88±0.33d	4.92±0.39b	5.73
8.	Avail. P (mg/kg)	5.83±2.70e	13.18±5.06b	10.26±2.76d	13.07±5.69c	14.72±2.39a	61.4
9.	NO_3^- - N (mg/kg)	9.81±4.20e	11.73±2.87d	14.07±4.35b	15.14±7.24a	11.93±1.59c	33.4

TABLE 2: MEAN STATIONS VARIATION (± SD & DMRT) OF PHYSICO-CHEMICAL DATA FOR CHICOCO MUD

S/No.	Variables	BGM 1	ID 2	ID 3	ABL 4	ABL 5	CV%
1.	Sand (%)	10.00±6.21d	14.60±14.14b	21.00±7.83e	21.00±9.20a	14.00±7.23c	29.7
2.	Silt (%)	8.20±6.26c	6.40±2.13d	22.40±6.10a	9.60±6.20b	4.40±2.67e	139
3.	Clay (%)	81.80±11.88a	79.00±14.68c	34.42±36.10e	69.40±9.39d	81.60±8.37b	58.1
4.	Fiber (g/kg)	220.98±54.26b	224.72±40.04a	218.80±25.15c	166.56±39.88e	217.42±24.69d	23.1
5.	Conductivity(µS/cm)	1740.20±285.09a	1521.20±351.05c	1508.00±425.60d	1334.80±114.96e	1527.20±512.97b	18.8
6.	TOC(mg/kg)	4.52±0.45e	5.02±0.82c	4.88±0.68d	5.22±1.13b	5.33±0.83a	12.7
7.	pH	4.83±0.49c	4.80±0.26e	4.81±0.35d	4.84±0.23b	4.92±0.40a	1.96
8.	Avail. P (mg/kg)	12.86±6.93b	12.33±5.82c	9.20±3.53e	13.43±3.91a	9.24±2.00d	35.7
9.	NO_3^- – N (mg/kg)	12.64±7.09b	12.43±5.26c	11.80±4.28d	14.02±3.40a	11.79±2.67e	14.5

H. Physicochemical parameters

Wet Conductivity

Fig. 2 shows the monthly and station conductivity variations which indicated that monthly conductivity levels increased consistently during the study period, with values ranging between 1186.60±144.02 in June and 1842.60 ± 405.16 µS/cm in September and decreased in October while Stations conductivity variations ranged from 1334.40±114.96 in Station ABL4 to 1740.20 ± 285.09 in Station BGM1. The CV for monthly and stations conductivity data was 31.6 and 18.6 %.

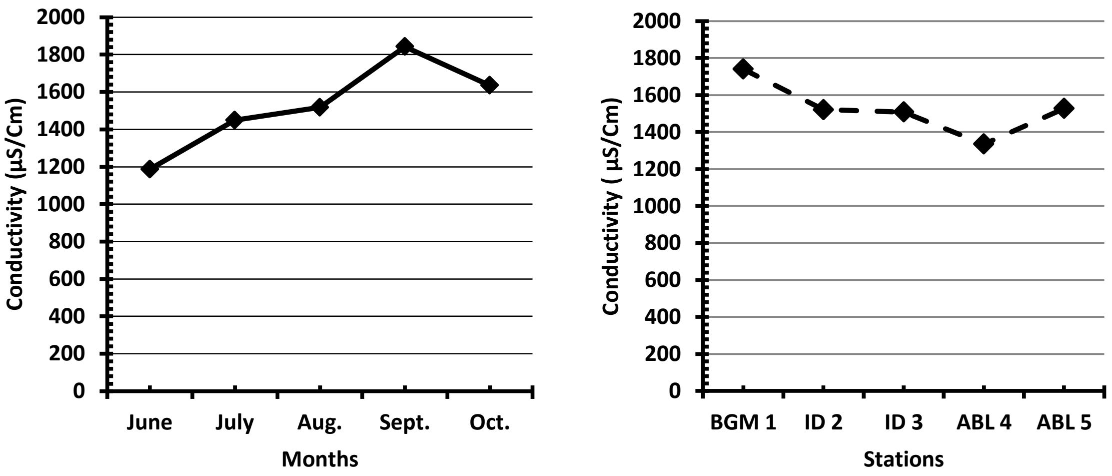

Fig. 2: Monthly and Stations variations of Conductivity levels of *Chicoco* mud

Sediment Textural Analysis:

Fig. 3 shows the mean monthly and station variations of the sediment textural analysis of *Chicoco* mud which indicates that Sand ranged from 9.40±4.17 in October to 20.80±11.19 percent in June representing the highest value while for Stations it was highest in Station ABL 4 at 21.00± 9.20 and lowest in Station BGM1 [10.00 ± 621] and ID3 [10.00 ± 7.38]. The highest value of Silt was recorded in August (22.60±35.90) and the lowest value was in June (5.60±3.38), however, Station value recorded for Silt was 22.40 ± 36.10 in Station ID3 representing the highest value and the lowest value was recorded in Station ABL5. Clay content in the mud was highest in October (82.60±0.50) and lowest in August (64.00±34.19), while in Station ID3 it ranged from 67.60 ± 34.42 to 81.80 ± l11.88 percent in Station BGM1. Nevertheless, the fibre content decreased from 215.86±44.81 in June to 178.06±27.36 g/kg in July and gradually increased through the months and peaked in October (240.48±30.46) while variations in the fibre content was different with respect to the Stations and was highest in Station ID2 (224.72 ± 40.04] and lowest in Station ABL4 [166.56 ± 39.88]. Generally, the monthly Coefficient of Variability (CV) for Sand, Silt, Clay and Fibre was 31, 133.6, 20.2, and 24.4% while that for Stations was 29.7, 139, 58.1 and 23.1 % respectively.

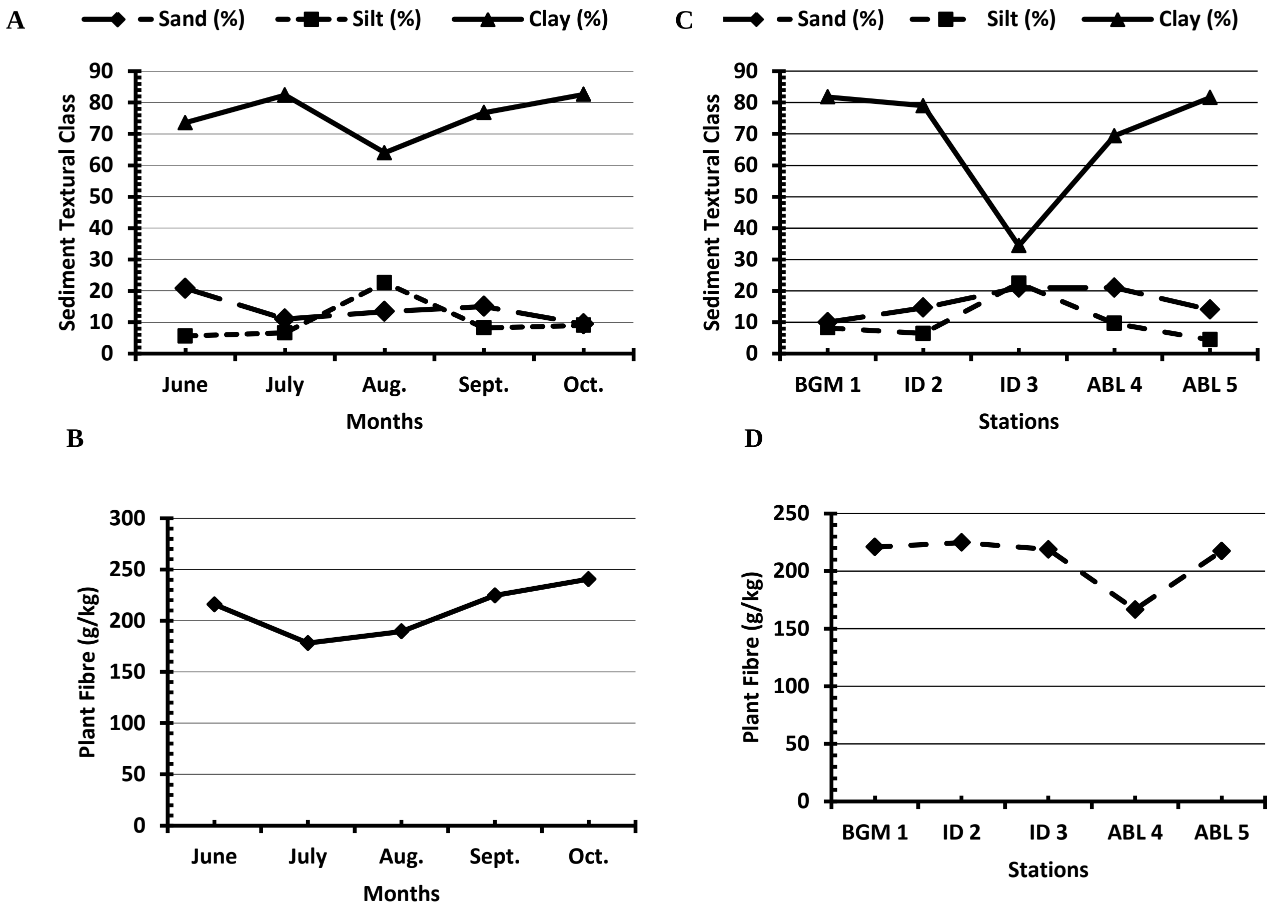

Fig. 3: Mean monthly (A & B) and stations (C & D) variation of textural analysis of *Chicoco* mud

Total Organic Carbon (TOC)

Fig. 4 shows the monthly and stations variations of Total Organic Concentration (TOC) of *Chicoco* mud, which indicated that the TOC level was highest in October and lowest in June while Station ABL5 [5.33 ± 0.83) had the highest value and Station BGM1 the lowest[4.52 ± 0.45]. The CV for monthly and stations TOC data was 22.2 and 12.7 %.

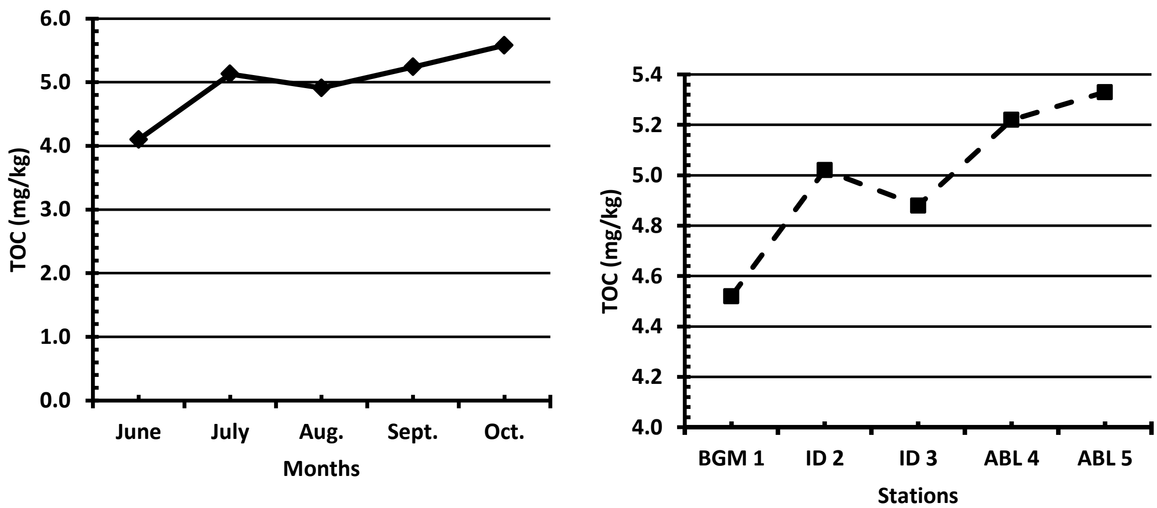

Fig. 4: Monthly and Stations variations of Total Organic Concentration (TOC) of *Chicoco* mud.

pH

Fig. 5 is indicative of the pH values recorded during the study period. Station 5 had the highest level [4.92 ± 0.40] and station 2 had the least level [4. 80±0.26]. The pH values recorded during the study period within the study sites showed station 5 had the highest level [4.92 ± 0.40] and station 2 had the least level [4. 80± 0.26]. The CV for monthly and stations pH data was 5.73 and 1.96 %.

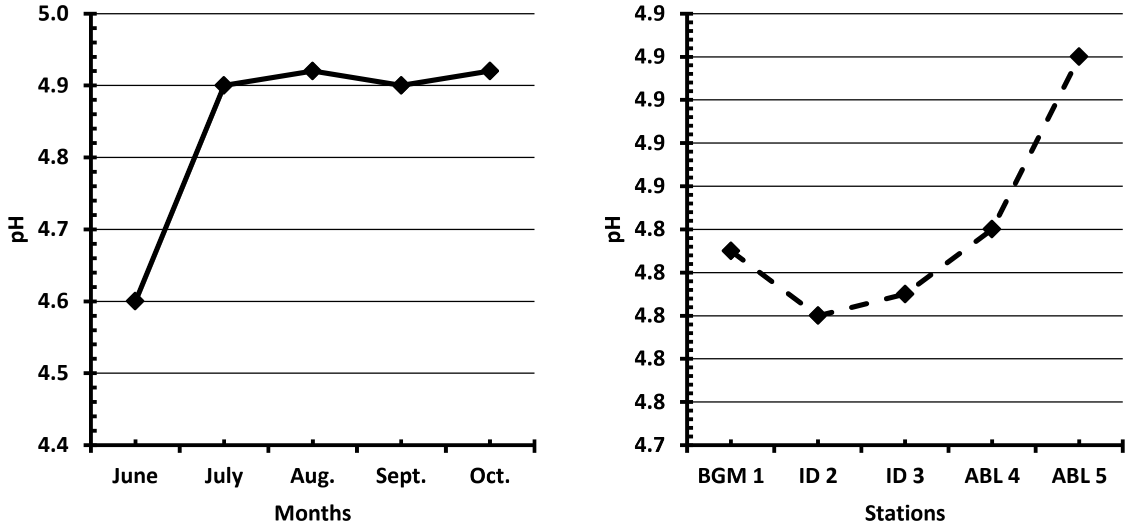

Fig. 5: Monthly and Stations variation in pH (Hydrogen Ion Concentration) of *Chicoco* mud.

Avail. P and Nitrate (NO_3 - N)

Fig. 6 shows the variation in nutrients, PO4 value during the period of study was highest in the month of October (14.72 ±2.39) and lowest in June (5.83±2.70). The NO_3 values during the study period increased through the months and peaked in September L 1.4 ± 7.24). Thereafter the level dropped to 11.93 ± 159 in October. The lowest value was recorded in June (9.81±4.20). The phosphates level recorded during the study period showed decreasing values between station 1 [12.86 ± 6.93] and station 3 [9.20 ± 3.53] being the least value. The level increased again to 13.43 ± 3.91 in station 4 representing the highest level within the study sites. The nitrate values recorded during the study period ranged from 11.79 ± 2.67 in station 5 to 12.64 ± 7.09 in station 1 being the highest value. The CV for monthly Avail. P and Nitrate (NO_3 - N) data was 61.4, and 35.7 % while for stations it was 33.4 and 14.5 %.

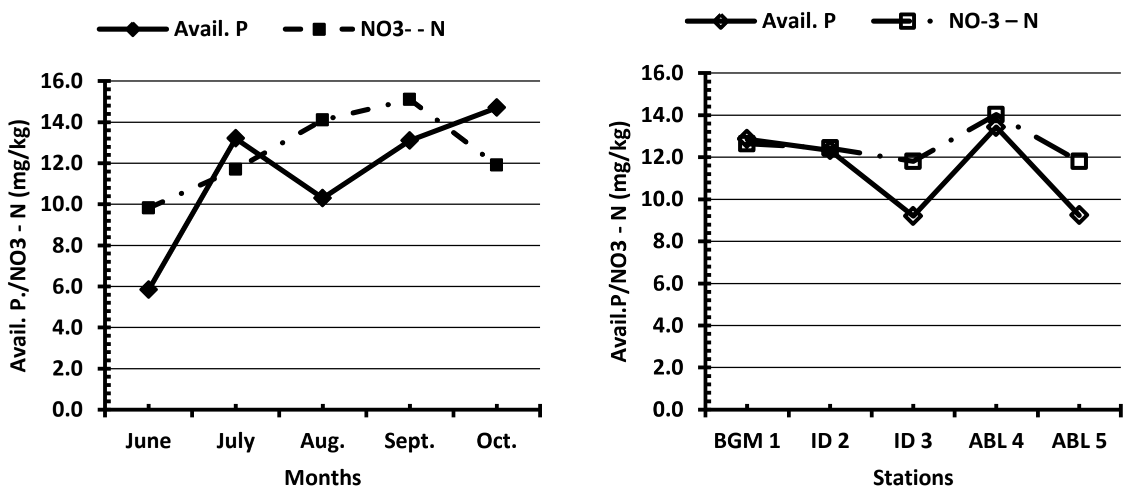

Fig. 6: Monthly and Stations variation in nutrients (Avail. P. and NO_3 – N) of *Chicoco* mud.

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I. MICROFLORA DATA

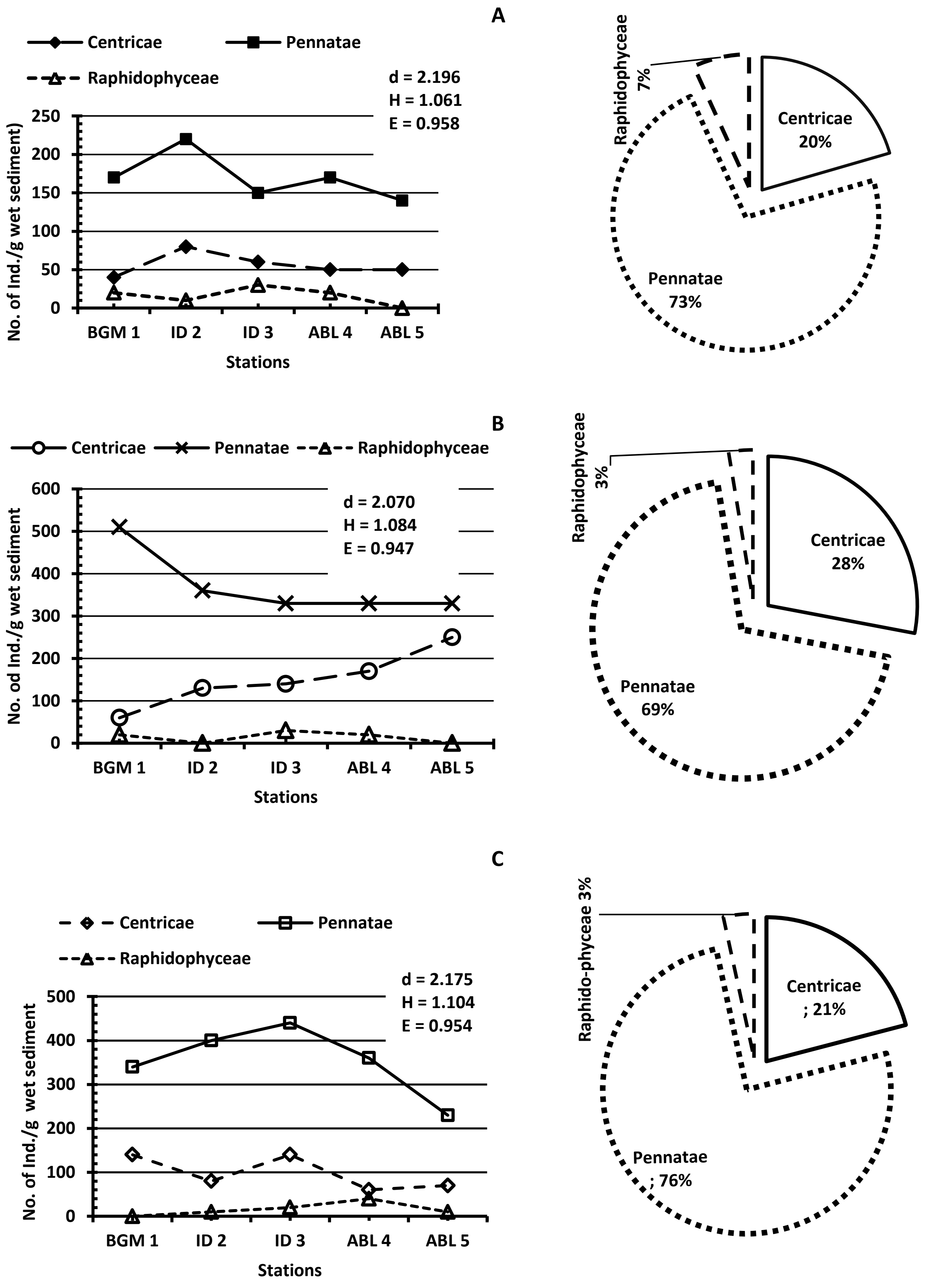

Fig 7: The microflora variation : A. June, B. July and C. August ; Inset is the mean Margalef's Species Richness (d) ,Shannon-Wiener Diversity (H) and Evenness Index (E) for Stations.

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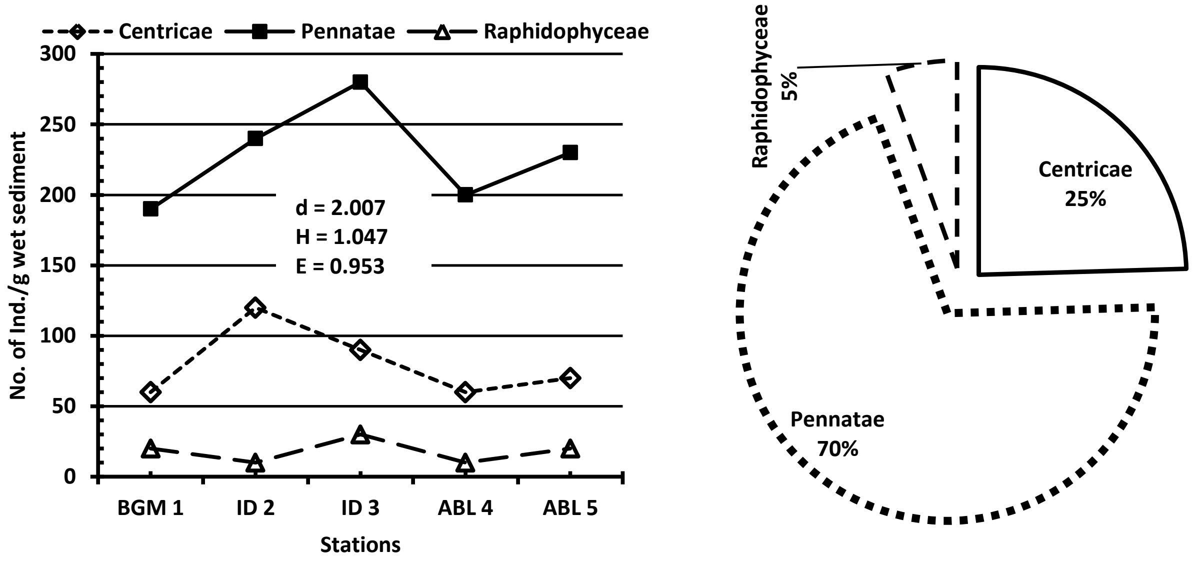
D



E

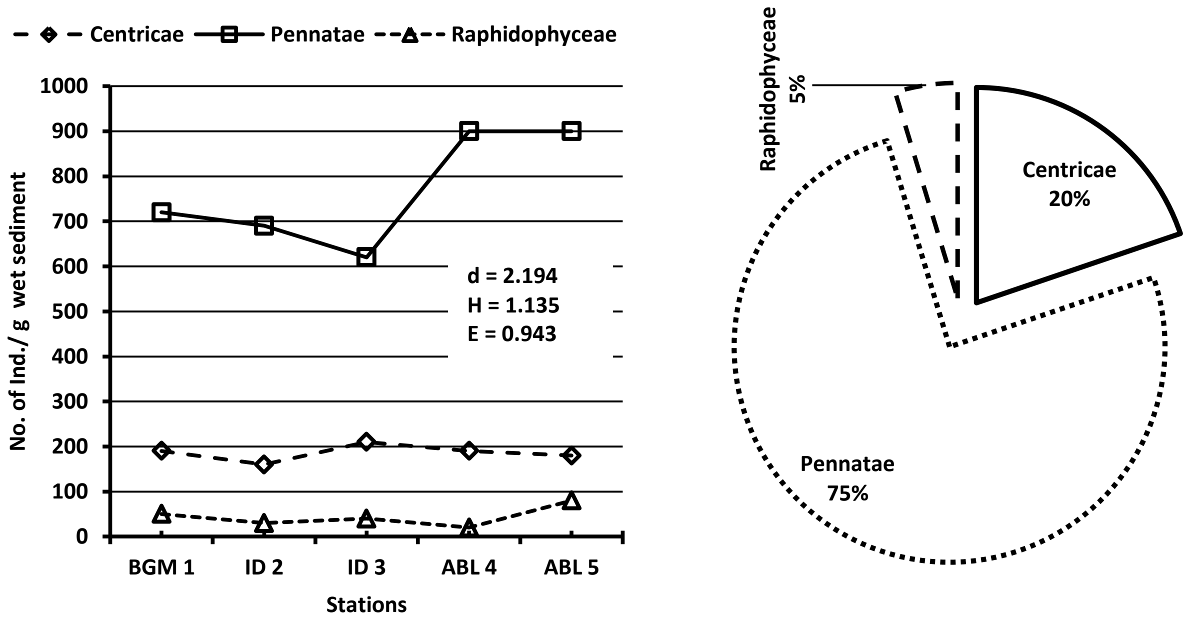


Fig 8: The microflora variation: D. September and E. October. ; Inset is the mean Margalef's Species Richness (d), Shannon-Wiener Diversity (H) and Evenness Index (E) for Stations.

IV. DISCUSSION

The *Chicoco* mud is used as substitute material landfill/reclamation by local inhabitants of the mangrove ecosystem in the Niger Delta. This usage often causes incompatibility in the ecosystem and upset the balance, harmony and interdependence of the whole environment (Odiette, 1999). Leh, 2002, reported that, there is great needs to sustainable manage the mangrove forest resources now than ever, since the benefits derivable from the mangrove ecosystem are highly threatened. A second issue regarding this phenomenon is that the *Chicoco* mud serves as sediment on which mangrove trees grow. Other concern highlights the fact that the *Chicoco* mud contains sediments that serve as food for some resident species and the mangrove floor is important to innumerable smaller flora and fauna, and so ultimately to the whole food chain leading to man (NDES, 1997). The various flora and fauna constitute a community and the component members relocate as the substrate is distorted. Nevertheless, the micro flora has been completely ignored or overlooked despite the fact that they form the basis of the entire system and their disruption may delay ecological activity and result perhaps to ecological inbalance.

Two taxa of microflora present in the *Chicoco*-mud were Raphidophyceae and Bacillariophyceae (Centric and Pennates). The Raphidophyceae was composed of *Chattonella antique* while the Centricae Bacillariophyceae was composed of *Amphora ovalis var. pediculus* , *Bacillaria sp*., *Cosinodiscus excentricus* and *Stephanodiscus niagra*e , and the Centricae Baccillariophyceae was composed of *Caloneis sp*., *Gyrosigma sp*., *Pinnularia sp*., *Navicula anglica* , *N. vulpine* , *Nitzschia sigmoidea* , others include *Stauroneis sp*., *Surirella fatuosa* , *S. ovate* , *Synedra sp*., and *Tropidoneis lepidoptera.* These microflora, particularly, the Bacillariophyceae, represent the primary producers of the environment, however, the presence of *Chattonella antique* posits danger for the environment, since it is considered a toxic algae and its destructive effects on fish are well known (references) other toxic species was *Nitzschia sigmoidea,* nevertheless, the high abundance of diatoms (Bacillariphyceae) may have prevented the spread of these toxic species. Yung et. al., (1986) found 252 taxa (including Cyanobacteria) in samples from 31 bogs in a stretch from Manitoba to Newfoundland, which was composed of desmids (*Actinotaenium cucurbita, Cylindrocytis brebissonii, Netrium digitus*) ,diatoms (*Spirogyra* , *Mougeotus* , *Binuclearia* , *Pinnularia vividis*) and cyanobacteria (*Anabaena* , *Nostoc* , *Calothrix* , *Microchaete* , *Stigonema*). These differences in taxa number may be due to differences in physicochemical variables.

The diversity and abundance in the population density of the species varied within the period of the study, thus , Stations' variations indicated that the average Margalef's Species Richness Index ('d') was generally high but was highest during the raining season and lowest during the dry season , while Evenness or Equitability Index ('E') which is usually constrained between 0 and 1.0 showed species were evenly abundant in all stations, however , the Shannon-Weiner Diversity Index ('H') did not show any considerable seasonal variation, nevertheless, they were little seasonal variations amongst the stations .

The significant differences in species diversity index as provided by Hutchinson (1970) indicated that diversities between stations varied, for instance, in wet season there was no significance difference between the diversities of some stations while in other stations significant differences occurred , similar trend was observed with the dry season data. These variations may have been caused by the effects of the physicochemical variables, for instance , the sediment texture of *Chicoco*-mud was made up of sand , silt , clay and plant fibre, and significantly affected their diversity and abundance as evident from the DMRT values , however , the stability of these physicochemical variables were different considering their Coefficient of Variability (CV) values , which confirmed that fibre was a less variable parameter and hence more stable in the intertidal environment than Clay , Sand and Silt and had specific significant effects, similarly ,monthly variations indicated that fibre and clay components were the most stable unlike Sand and Silt. Thus, the presence of fibre and clay may play a significant role in the diversity and abundance of littoral microflora.

Monthly and station values for Conductivity, Total Organic Carbon (TOC), pH, Available Phosphorus (Avail. P.,) and Nitrate-Nitrogen (NO^-_3 – N) indicate that pH, was the least variable parameter and the most stable followed by TOC, NO^-_3 – N, and Avail. P., high CV values for NO^-_3 – N and Avail. P is consistent with their characteristic nature (absorption and release) in the environment of being regularly absorbed and released by organisms in the environment. Similarly, the mean stations' 'DMRT' values showed that their effects were significant on the diversity and abundance of littoral microflora except in some stations. Some of these physicochemical variables differ in the way they affect the diversity and abundance of littoral microflora by either acting as a limiting or regulatory factor, however, Eltringham (1971) posited the importance of distinguishing between limiting and regulatory factors, thus, the pH and TOC concentration which were less variable parameters and generally stable in the environment could be considered as a regulatory factor because they controlled the size of the population of the microflora without destruction while NO^-_3 – N, and Avail. P. is the limiting factors because they may slow or prevent the population of microflora from spreading, nevertheless, a regulatory factor can under certain circumstances be a limiting factor and vice versa.

The acidic nature of the *Chicoco* mud observed in this study was consistent with other studies (Dickinson, 1983; Zhang and Andrews, 1993; Wheatley *et. al.,* 1996), but the levels of electrical conductivity were different due to the varying localized conditions. Nevertheless, Joosten and Clarke (2002) posited that variations in pH, could be linked also to electrical conductivity, calcium content, and base richness , which may affect the availability of plant nutrients ,although

, the effects of calcium content and base richness were not investigated , the other two parameters were capable of reducing nutrients to the microflora thus reducing their population. However, for microorganisms a key factor is the nature of the peat, specifically its botanical composition and its quality (i.e. the amount of easily decomposed organic matter). Infact, the peat type and amount of quality organic matter are undoubtedly the most important factors influencing rates of decomposition, biomass growth, and diversity (Wheatley *et. al.*, 1996). This may have also contributed to the low abundance of microflora in this study.

V. CONCLUSION

This study has proved the presence of littoral microflora in *Chicoco* mud which was composed of two taxa namely Raphidophyceae and Bacillariophyceae , with the Pennates been more abundant than the Centrics coupled with the presence of toxic species capable of depleting our fisheries , thus , this makes our *Chicoco* mud a sensitive habitat that should be conserved for future ecological studies and monitoring so as to better understand this environment for sustainable management rather than its use as a landfill or fuel. The species diversity was high but was either significant or not significant while the abundance of littoral microflora was low which may have been caused by variations in physicochemical parameters and the clay-fibre complex.

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