West African Journal of Fisheries and Aquatic Sciences, 4, 1-11 (2024)
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Research Article

Phytoplankton Assemblage in a Tropical Harbour: Microscopy versus Chemotaxonomy

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ABSTRACT

Investigations into phytoplankton community structures in Nigerian waters have always been carried out using conventional microscopy. Microscopic methods are not sufficient because very small-sized phytoplankton species are easily missed out from sample processing and examinations. At present, there is a dearth of literature on pigment-derived phytoplankton assemblage in Nigerian waters. Phytoplankton diversity was investigated for twelve months from May 2015 to April 2016 using both microscopic and chemotaxonomic methods. Scanning electron microscopic (SEM) images of phytoplankton species were taken with a JEOL JSM 5310 using between 12 and 15 kV accelerating voltage. Chemotaxonomic assessment of phytoplankton was done using a C8 reverse-phase High-Performance Liquid Chromatography (HPLC) pigment-labeling technique. A total of 96 phytoplankton species belonging to five classes were recorded from microscopic observations. Bacillariophyceae (diatoms) comprised 72 species, Chlorophyceae (green algae) had 2 species, Cyanophyceae (blue-green algae) was represented by 11 species, Dictyochophyceae (silicoflagellates) had 1 species and Dinophyceae (dinoflagellates) comprised 10 species. The results from the HPLC pigment-labeling technique validated microscopic observations for diatoms, dinoflagellates, blue-green and green algae. However, there were pigment-detections of coccolithophores and cryptophytes by HPLC which were not seen under the microscopes. From this study, *Achnanthes eureka*, *Hyalosynedra laevigata*, *Mastogloia cuneata*, *Mastogloia emarginata*, *Navicula formenterae*, and *Palmerina hardmaniana* are new records of diatoms in Nigerian waters. This study documented a pioneering effort at chemotaxonomic assessment in Nigeria, leading to the identification and quantification of nineteen phytopigments.

Keywords: Chemotaxonomy, Harbour, Microscopy, Phytopigments, Phytoplankton
Article History: Received 17 February 2024; Accepted 27 April 2024; Published 15 May 2024

INTRODUCTION

As a result of the key roles phytoplankton play in the biogeochemistry of the earth, alongside the predominant position they occupy in several ecological processes such as climate regulation, food webs, and fossil fuel formation, the scientific interest in the comprehension of their assemblage structure is surging (Di Cicco *et al*., 2017). Assessment of phytoplankton communities involves one or a combination of morphology-based microscopic studies (Nwankwo, 1996; Gomi *et al*., 2007; Onyema, 2008; Not *et al*., 2012), flow cytometric cell counting (DiTullio *et al*., 2003; Smith *et al*., 2007), genetic/molecular characterizations (Suzuki *et al*., 2005; Medlin *et al*., 2006) and chemotaxonomy, that is, groupings based on presence of pigment markers (Vidussi *et al*., 2001; Delizo *et al*., 2007). Of all the methodologies involved in phytoplankton community structure assessments, microscopy is considered to be the most authoritative (Not *et al*., 2012). Although microscopy is relatively inexpensive, sample processing is time-consuming, requires great skill and expertise and is often subjective (Goela *et al*., 2014).

Owing to some of the difficulties and limitations of microscopic identification techniques, microalgal studies are increasingly exploring the use of other complementary tools. Chemotaxonomy based on pigment markers has greatly improved the monitoring efficiency for phytoplankton assemblages, which include nano- and pico-phytoplankton that are easily

missed by microscopic methods (Palomares-Garcia *et al*., 2006; Delizo *et al*., 2007; Goela *et al*., 2014).

In Africa, a report exists which describes the phytoplankton assemblages of Lake Tanganyika, Eastern Africa based on pigment markers (Descy *et al*., 2005). This technique, however, is yet to be adopted for algal studies in Nigeria. It was thus, necessary to study the phytoplankton spectrum of the Lagos harbour with regards to a robust approach in the determination of phytoplankton taxonomic groups using a combination of morphology-based microscopic and pigment-based chemotaxonomic methods.

MATERIALS AND METHODS

Description of the Study Area

The Lagos harbour is the only direct opening for the nine marginal southwestern lagoons to the sea in Nigeria. It falls within the rainforest belt characterized by well-marked wet (May – October) and dry (November – April) seasons (Onyema, 2009; Chukwu, 2011; Edokpayi, 2017). Twelve stations in three ecologically distinct zones (harbour channel, adjoining creeks, and the sea adjacent to the harbour) were selected (Figure 1). There are four stations within the horizontal gradient of the harbour and these are the Lower Lagos harbour (former sewage disposal site at Iddo), Upper Lagos harbour - Defence jetty, Tarkwa bay and the mouth of the Commodore channel, where the open sea meets the harbour. Four other stations are the creeks adjoining the Lagos harbour; they are Ijora, Five cowries, Badagry and Lighthouse creeks. The other four stations are in the sea adjacent to the harbour after the beacon - they are Lighthouse beach 1 and 2; and the Great wall of Lagos 1 and 2.

Collection of Plankton Samples

The phytoplankton samples were collected using standard plankton net of mesh size 50µm. The plankton net was towed horizontally from a motorized boat at low speed (< 4 knots) for 5 minutes and the filtered plankton were emptied into well-labelled plastic container with a screw cap. The plankton samples were preserved with 4 % formalin and transferred to the laboratory for further analysis as described by Julius and Theriot (2010).

Microscopic Analysis of Phytoplankton

Light Microscopy (LM)

Fixed phytoplankton samples were allowed to settle in the laboratory and the supernatant was decanted until reasonable concentrations were obtained. The samples were investigated using a Leica DMLB microscope with 100x full oil immersion optics and 1.35 numerical aperture equipped with a Nikon Coolpix 995 CCD digital camera (3.3-megapixel resolution). Phytoplankton abundance was in cells/filaments/trichomes per liter of seawater using a modified enumeration method described by Perry (2003). Photomicrographs of phytoplankton were taken in the Phytoplankton laboratory of the Academy of Natural Sciences, Drexel, Philadelphia, USA. Confirmation of species identification was done using relevant texts (Nwankwo, 2004; Garcia and Odebretch, 2008; Al-kandari *et al*., 2009; Alvarez-Blanco and Blanco, 2014).

Scanning Electron Microscopy (SEM)

A portion of each of the air-dried samples on the coverslips (as in Light Microscopy above) was cut and mounted to aluminum stubs using adhesive carbon tapes. The aluminum stubs were sputter-coated with argon. All observations were done in the Image Room of St. Cloud State University, Minnesota, United States of America, with a JEOL JSM 5310 using between 12 and 15 kV accelerating voltage.

HPLC Pigments Analysis

Natural seawater (2.5 L) was filtered from 0 - 1 meter depth at each station. Filtration was done using Sartorius Glass-Fiber Filters (GF/F) of 47 mm, with a low vacuum pump. After filtration, filters were folded into halves vertically, dipped into liquid nitrogen and shipped on dry ice to the laboratory for analyses. In the laboratory (DHI laboratory, Denmark), filters were immediately placed in a - 80 °C freezer until the time of analyses. Filters were transferred to vials with 3 mL 95 % acetone and internal standard (vitamin E). Samples were mixed in a vortex mixer, sonicated on ice, extracted at 4 °C for 20 hours and mixed again.

The samples were then filtered through a 0.2 µm Teflon syringe filter into HPLC vials and placed together with DHI mixed pigments in the cooling rack of the HPLC. Buffer and samples were injected on HPLC (Shimadzu LC – 10A HPLC system with LC solution software) in the ratio 5:2 using a pre-treatment program and mixing in the loop before injection. The limit of quantitation is 0.002 µg/L. The laboratory procedure adopted is the HPL method described by Van Heukelem and Thomas (2005). The uncertainty of the method is < 1.0 %. HPLC-separated pigment peaks were routinely identified by comparison of retention time (t_R) values with those of standards. Quantification of pigment concentrations (µg/L) was as follows:

$$\tilde{C} = \hat{A}_{pi} R_{pi} \quad (1)$$

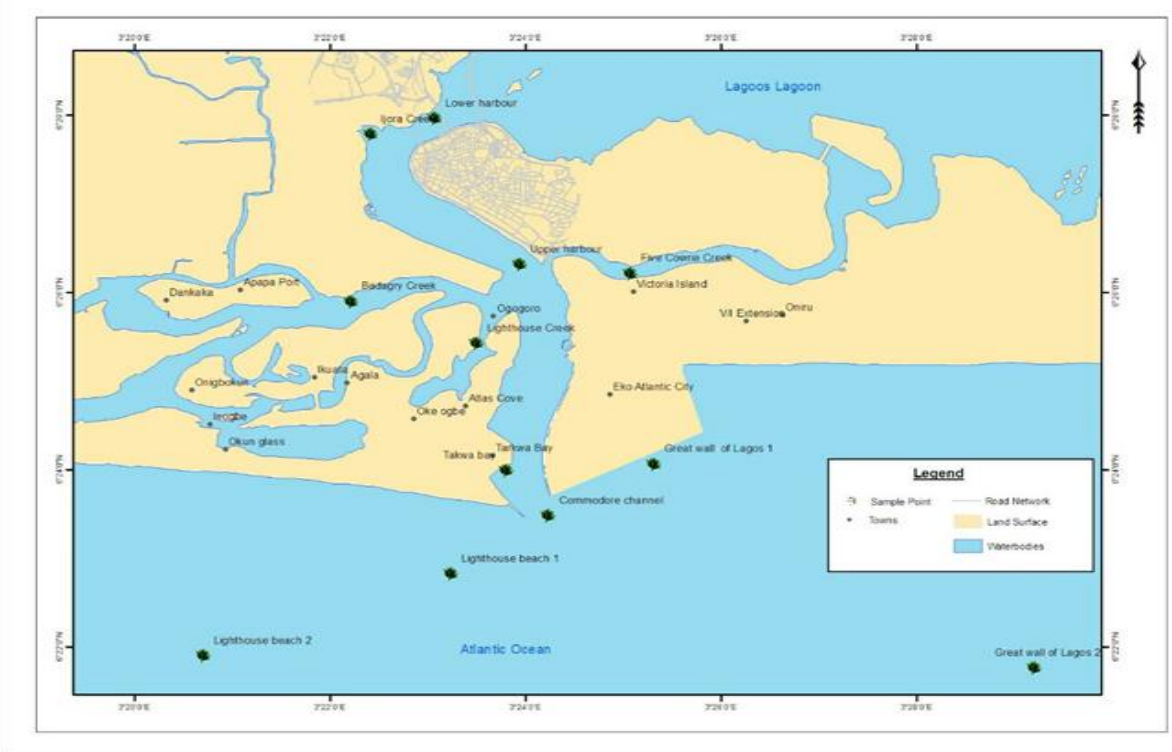


Figure 1: The Lagos Harbour showing the study sites

Where $\tilde{C}$ is the amount of pigment injected (in units of nanograms per injection), $\hat{A}_{pi}$ is the area of the parent peak for pigment and R_{pi} is the purity-corrected response factor. Pigment concentration is thus, expressed as;

$$C_{pi} = \frac{V_x}{V_f}\frac{\tilde{C}_{pi}}{V_c}$$

(2)

Where V_x is the extraction volume (in microliters), V_c is the volume (in microliters) of the sample injected into the HPLC column and V_f is the filtration volume (in milliliters). The internal standard is used to correct V_x for residual water retained on the filter paper (plus any variations in volume caused by evaporation):

$$V_x = \frac{\hat{A}_{c1}}{\hat{A}_{s1}}V_m$$

(3)

Where $\hat{A}_{cl}$ is the peak area of the internal standard when it is injected into the HPLC column before its addition to the sample, $\hat{A}_{sl}$ is the peak area of the internal standard in the sample and V_m is the volume of the internal standard batch mixture added to each filter sample. Percentage composition from pigment concentrations (biomass) was computed using the formula adopted by Vidussi *et al*. (2001) and Artuso *et al*. (2016):

$$(DP / \sum DP) * 100$$

(4)

Where *DP* is the concentration of a diagnostic pigment (pigment marker) and $\sum DP$ is the summation of concentrations of all identified diagnostic pigments (pigment markers).

RESULTS

Microscopic Observations of Phytoplankton

A total of 96 phytoplankton taxa belonging to five classes were recorded for this study (Table 1). Bacillariophyceae (diatoms) comprised seventy-two (72) taxa (78%), Chlorophyceae (green algae) had two (2) taxa (2%), Cyanophyceae (blue-green algae) were represented by 11 taxa (10%), Dictyochophyceae (silicoflagellates) had one (1) taxon (1%) and Dinophyceae (dinoflagellates) had 10 taxa (9%) (Figure 2). Whereas blue-green algae, diatoms, dinoflagellates and silicoflagellates were observed via microscopy for all the stations, the green algae were only reported at Lower Lagos Harbour, Ijora, Badagry, Lighthouse and Five-cowrie creeks. Generally, diatoms were found to be the most abundant group across seasons for all stations. In terms of numerical abundance, Badagry creek recorded the highest

Table 1: Assemblage of phytoplankton in the Lagos harbour (May, 2015 – April, 2016).

	Class 1: Bacillariophyceae				
1	*Achnanthes brevipes* C. Agardh	48	*Mastogloia binotata* Grunow	5	*Nostoc* sp.
2	*Achnanthes eureka* Alvarez -Blanco & Blanco	49	*Mastogloia cuneata* Meister	6	*Oscillatoria curviceps* C. Agardh ex Gomont
3	*Achnanthes longipes* C. Agardh	50	*Mastogloia emarginata* W. Smith	7	*Oscillatoria limosa* C. Agardh
4	*Achnanthidium exiguum* Grunow	51	*Mastogloia* sp.	8	*Oscillatoria magaritifera* Kutzing
5	*Actinocyclus subtilis* W. Gregory	52	*Melosira moniliformes* O. F. Muller	9	*Oscillatoria* sp.
6	*Actinoptychus splendens* Shadbolt	53	*Melosira mummuloides* Ehrenberg	10	*Oscillatoria tenius* C. Agardh ex Gomont
7	*Amphora hyalina* Kutzing	54	*Navicula cryptocephala* Kutzing	11	*Oscillatoria trichodes*
8	*Amphora ovalis*	55	*Navicula expansa* Hagelstein		
9	*Asterionella japonica* Cleve	56	*Navicula formenterae* Cleve		Class 4: Dictyochophyceae
10	*Aulacoseira granulata var angutissima* Ehrenberg	57	*Navicula mutica* Kutzing	1	*Dictyocha fibula* Ehrenberg
11	*Aulacoseira* sp.	58	*Navicula rhyncocephala* Kutzing		
12	*Bacillaria paxillifer* O. F. Muller	59	*Navicula* sp.		Class 5: Dinophyceae
13	*Bacteriastrum delicatulum* Cleve	60	*Nitzschia linearis* W. Smith	1	*Ceratium bicephalum*
14	*Biddulphia aurita* Lyngbye	61	*Nitzschia longissima* Brebisson	2	*Ceratium furca* Ehrenberg
15	*Biddulphia obtusa* Kutzing	62	*Nitzschia palea* Kutzing	3	*Ceratium fusus* Ehrenberg
16	*Biddulphia sinensis* Greville	63	*Nitzschia sigma* Kutzing	4	*Ceratium macroceros* Ehrenberg
17	*Caloneis* sp.	64	*Odontella* sp.	5	*Ceratium* sp.
18	*Chaetoceros atlanticum* Cleve	65	*Palmerina hardmaniana* Greville	6	*Ceratium trichoceros* Ehrenberg
19	*Chaetoceros convolutus* Castracane	66	*Parlibellus delognei* Van Heurck	7	*Ceratium tripos* O. F. Muller
20	*Chaetoceros decipens* Cleve	67	*Pinnularia major* Kutzing	8	*Dinophysix* sp.
21	*Chaetoceros radicans* F. Schutt	68	*Plagiogramma* sp.	9	*Gymnodinium* sp.
22	*Cocconeis diaphana* W. Smith	69	*Pleurosigma angulatum* J. T. Quekett	10	*Prorocentrium* sp.
23	*Cocconeis littoralis* R. Subrahmanyan	70	*Pleurosigma* sp.		
24	*Cocconeis placentula* Ehrenberg	71	*Podosira montagnei* Kutzing		
25	*Coscinodiscus centralis* Ehrenberg	72	*Pseudonitzschia* sp.		
38	*Fragilaria* sp.				
39	*Gomphonema* sp.		Class 2: Chlorophyceae		
40	*Grammatophora marinum* Lyngbye	1	*Gonatozygon monoteanium*		
41	*Guinardia flaccida* Castracane	2	*Spirogyra africanus* F. E. Fritsch		
42	*Gyrosigma* sp.				
43	*Halamphora* sp.		Class 3: Cyanophyceae		
44	*Hemidiscus cuneiforms* Wallich	1	*Anabaena spiroides* Klebhan		
45	*Hyalosynedra laevigata* Grunow	2	*Lyngbya martensiana* Meneghini ex Gomont		
46	*Licmophora lyngbyei* Kutzing	3	*Microcystis aeruginosa* Kutzing		
47	*Licmorpha abbreviata* C. Agardh	4	*Microcystis flos-aque* Wittrock		

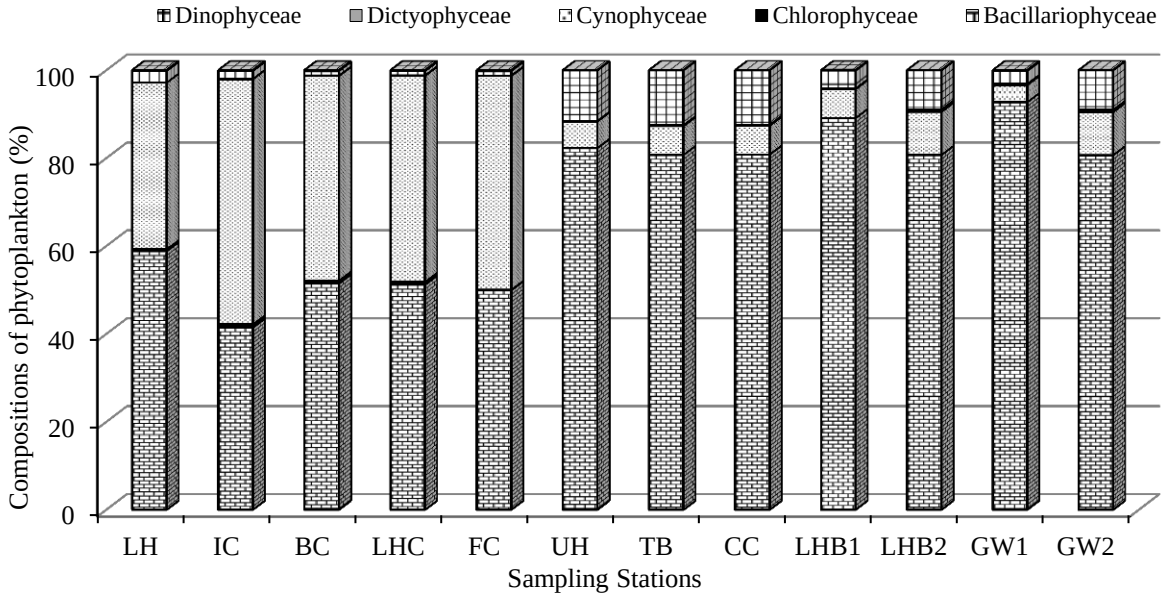


Figure 2: Microscopic Percentage compositions of phytoplankton across sampling stations in the Lagos Harbour and Adjacent Sea, Southwest, Nigeria.

number of species. More importantly, *Achnanthes eureka*, *Hyalosynedra laevigata*, *Mastogloia cuneata*, *M*astogloia *emarginata*, *Navicula formenterae* and *Palmerina hardmaniana* are new records of diatoms in Nigerian waters.

HPLC Pigments Characterization

The results are shown as pigment concentrations in µg/L (Figures 3, 4 and 5). The samples showed clear signs of degradation (relatively increased levels of pheophorbide *a* and pheophytin *a*). Results were stored and registered in DHI laboratory, Denmark. In this study, nineteen pigments were identified, ranging from chlorophylls to carotenoids. Three out of these pigments (chlorophyllide *a*, pheophorbide *a*, and pheophytin *a*) were degradation products of chlorophyll a hence, their values were added to the values of chlorophyll *a* to give total chlorophyll *a* (TChl *a*) values.

Six pigment markers (alloxanthin, chlorophyll *b*, fucoxanthin, peridinin, zeaxanthin and 19-Hexanol-fucoxanthin) were reported for this study; these pigments are diagnostic of Cryptophyceae (cryptophytes), Chlorophyceae (green algae), Bacillariophyceae (diatoms), Dinophyceae (dinoflagellates), Cyanophyceae (blue-green algae) and Prymnesiophyceae (coccolithophores) respectively.

The percentage compositions (in terms of biomass) computed from the concentrations of these diagnostic pigments revealed that fucoxanthin, which is the diagnostic pigment for diatoms, had the highest concentrations across stations except Ijora creek and Lighthouse beach 1 and 2 which recorded highest biomass for zeaxanthin (Cyanophyceae) (Figure 6). In cases where fucoxanthin, which bio-indicated the presence of diatoms (Bacillariophyceae), recorded highest concentrations, zeaxanthin (Cyanophyceae) followed in terms of biomass; but in Great Wall of Lagos 2, peridinin – a marker of dinoflagellates was next to fucoxanthin in concentrations (Figure 6).

Comparison between Chemotaxonomic and Microscopic Assessments of Phytoplankton Assemblage Structures in the Lagos Harbour

The results from HPLC pigment-labeling technique validated microscopic observations for diatoms, dinoflagellates, blue-green and green algae. However, there were pigment-detections of coccolithophores and cryptophytes by HPLC which were not seen under the microscopes (Table 2).

DISCUSSION

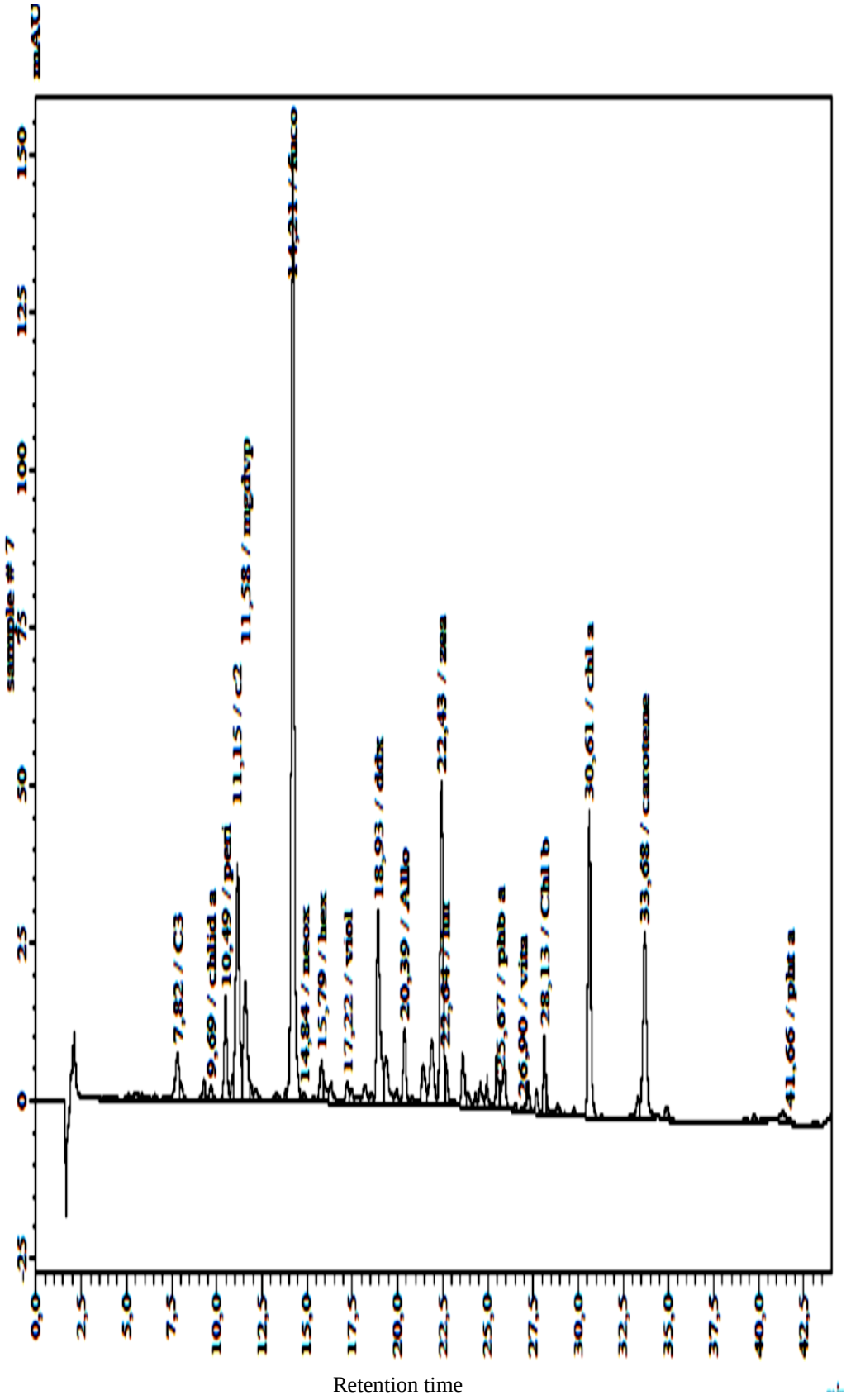
Investigations into the phytoplankton pigment pool by HPLC revealed the relative biomass compositions of various groups while microscopy gave an insight into the relative numerical abundance. In this study, both methods were found to be greatly complementary to each other. Strong agreements between microscopic and chemotaxonomic assessments of phytoplankton were also reported by Andersen *et al*. (1996), Schlüter *et al*. (2000) and See *et al*. (2005).

Whereas microscopy established the dominance of diatoms, cyanobacteria and dinoflagellates in order of magnitude, with occurrences of two and one species of green algae and silicoflagellates respectively, HPLC pigment analysis (chemotaxonomy) ascertained that diatoms were generally most abundant and usually followed by cyanobacteria and then dinoflagellates. However, there were exceptions to these observations. For instance, at the Ijora creek, Lighthouse Beach 1 and 2, cyanobacteria were found to be the most abundant in terms of biomass. This observation could be attributed to the excessive growth rate of the blue-green algae in these phosphate-rich stations (Elegbeleye and Onyema, 2019).

The Ijora creek is known to be polluted and thus, encourages high levels of epipelic algae (Onyema and Nwankwo, 2006). Unlike the microscopic method which revealed two species of chlorophytes in only the creeks and the Lower Lagos harbour, pigment-based chemotaxonomy established the occurrences of this group of phytoplankton in all stations.

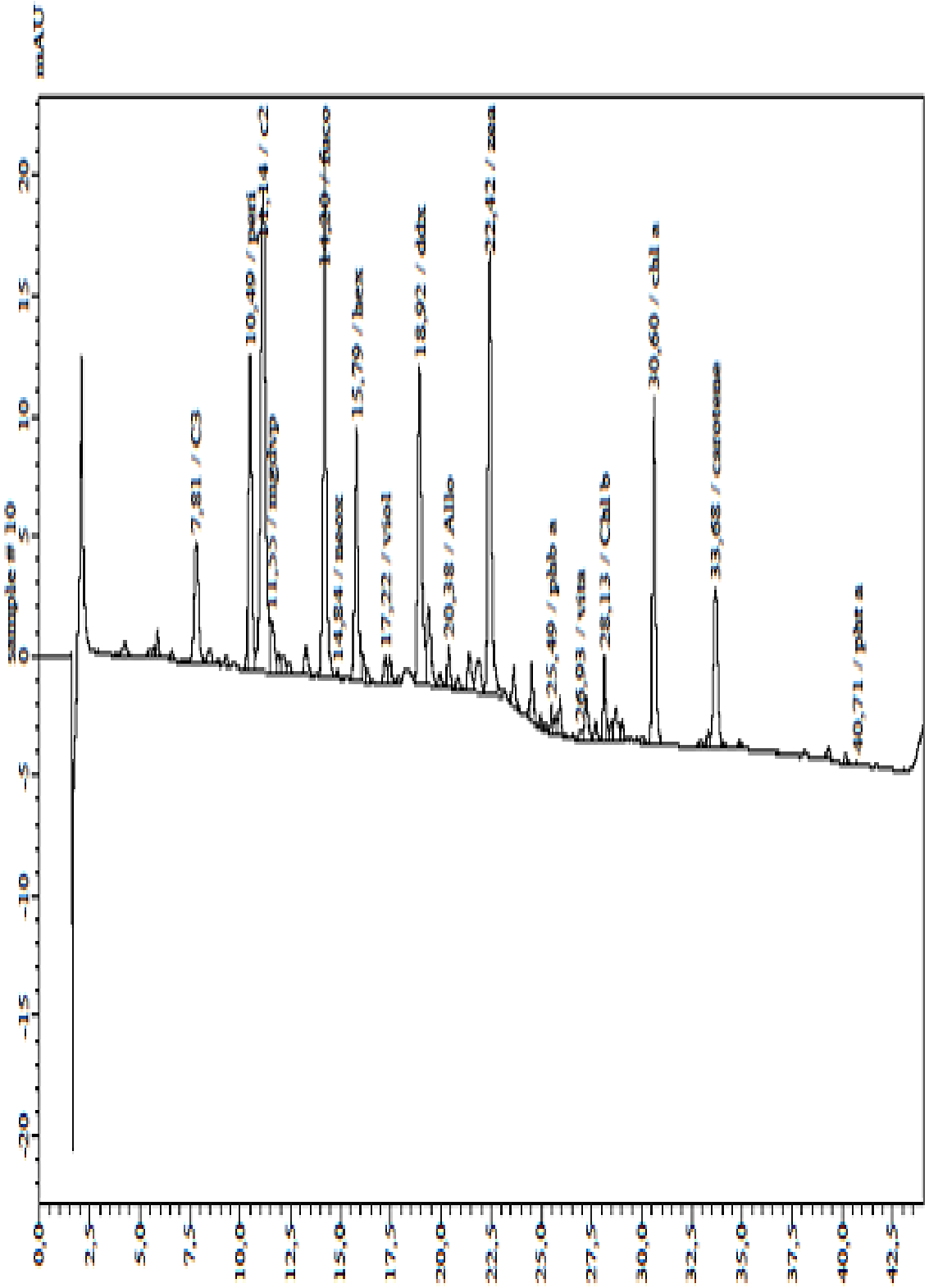
The detection of 19-hexanol-oxy-fucoxanthin by HPLC established the occurrence of living coccolithophores in the Lagos harbour. Coccolithophores which were not detected via microscopy were found to be conspicuously present in stations with very high levels of salinity. These microalgae are typical marine species as rightly revealed by HPLC analysis of phytopigments. Similarly, cryptophytes were recorded for all stations from chemotaxonomy but surprisingly not revealed by microscopic analyses. Wright *et al*. (1996) also reported chemotaxonomic detection of cryptophytes which were missed by microscopy.

The sizes of these two groups (coccolithophores and cryptophytes) could be a hindrance to their detection by microscopic methods. The use of specific biomarker pigments analysed by HPLC method of water samples collected from the Lagos harbour provided considerable insight into the variability of



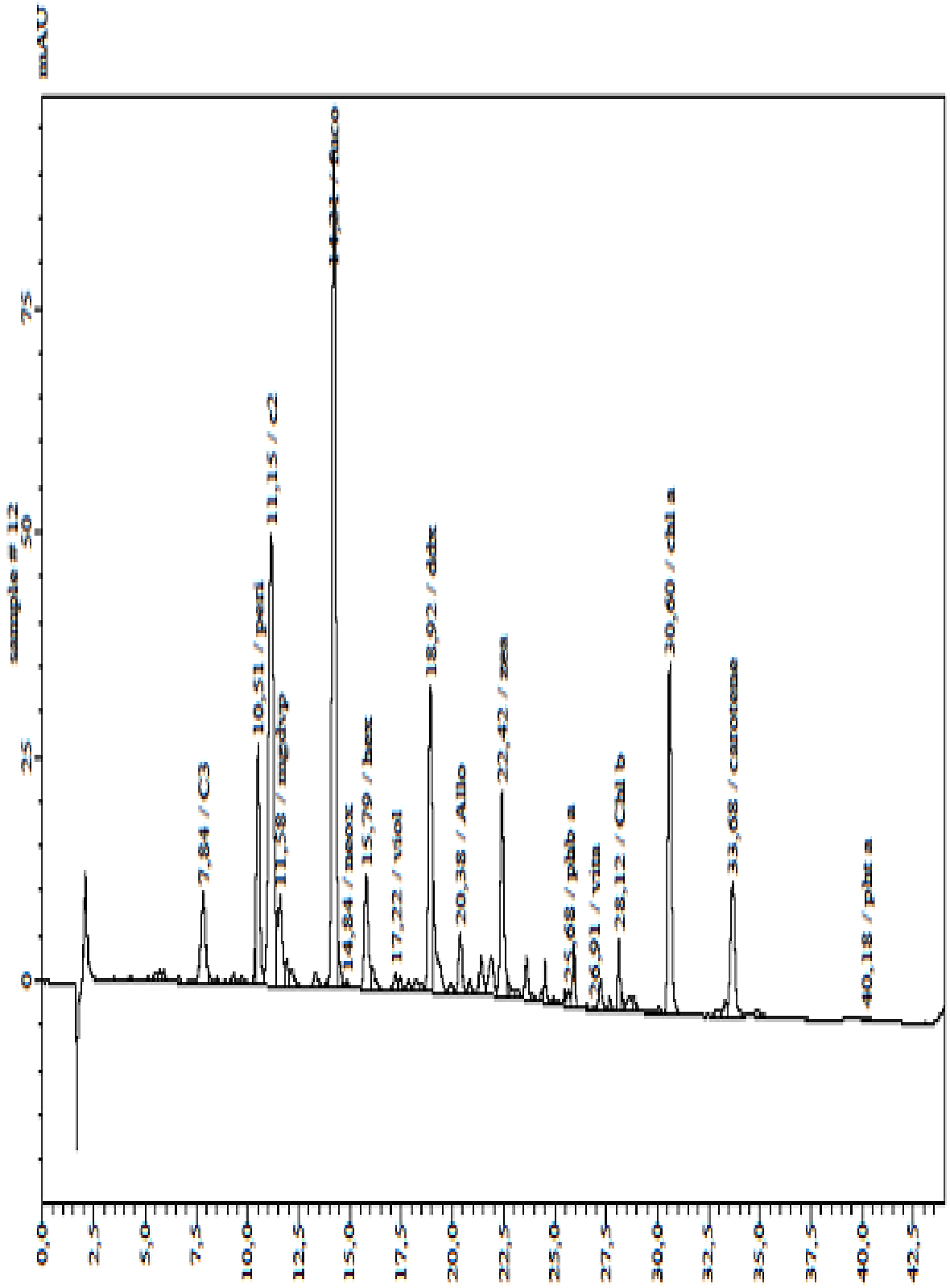
C3=Chlorophyll c3; chld a=Chlorophyllide *a*; peri=Peridinin; c2=Chlorophyll *c*2; mgdvp=Chlorophyll *c*1; fuco=Fucoxanthin; neox=Neoxanthin; hex=19'hexanol-oxy-fucoxanthin; viol=Violaxanthin; ddx=Diadinoxanthin; Allo=Alloxanthin; zea=Zeaxanthin; lut=Lutein; phba=Phaeophorbide *a*; vita=Vitamin E(buffer); Chl b=Chlorophyll *b*; Chl a=Chlorophyll *a*; carotene=α+β Carotene; pht a=Phaeophytin *a*

Figure 3: Chromatogram of HPLC analysis of phytoplankton pigments at Tarkwa bay.



C3=Chlorophyll c3; chld a=Chlorophyllide *a*; peri=Peridinin; c2=Chlorophyll *c*2; mgdvp=Chlorophyll *c*1; fuco=Fucoxanthin; neox=Neoxanthin; hex=19'hexanol-oxy-fucoxanthin; viol=Violaxanthin; ddx=Diadinoxanthin; Allo=Alloxanthin; zea=Zeaxanthin; lut=Lutein; phba=Phaeophorbide *a*; vita=Vitamin E(buffer); Chl b=Chlorophyll *b*; Chl a=Chlorophyll *a*; carotene=α+β Carotene; pht a=Phaeophytin *a*

Figure 4: Chromatogram of HPLC analysis of phytoplankton pigments at Lighthouse Beach 2.



C3=Chlorophyll c3; chld a=Chlorophyllide *a*; peri=Peridinin; c2=Chlorophyll *c*2; mgdvp=Chlorophyll *c*1; fuco=Fucoxanthin; neox=Neoxanthin; hex=19'hexanol-oxy-fucoxanthin; viol=Violaxanthin; ddx=Diadinoxanthin; Allo=Alloxanthin; zea=Zeaxanthin; lut=Lutein; phba=Phaeophorbide *a*; vita=Vitamin E(buffer); Chl b=Chlorophyll *b*; Chl a=Chlorophyll *a*; carotene=α+β Carotene; pht a=Phaeophytin *a*

Figure 5: Chromatogram of HPLC analysis of phytoplankton pigments at Great Wall of Lagos 2.

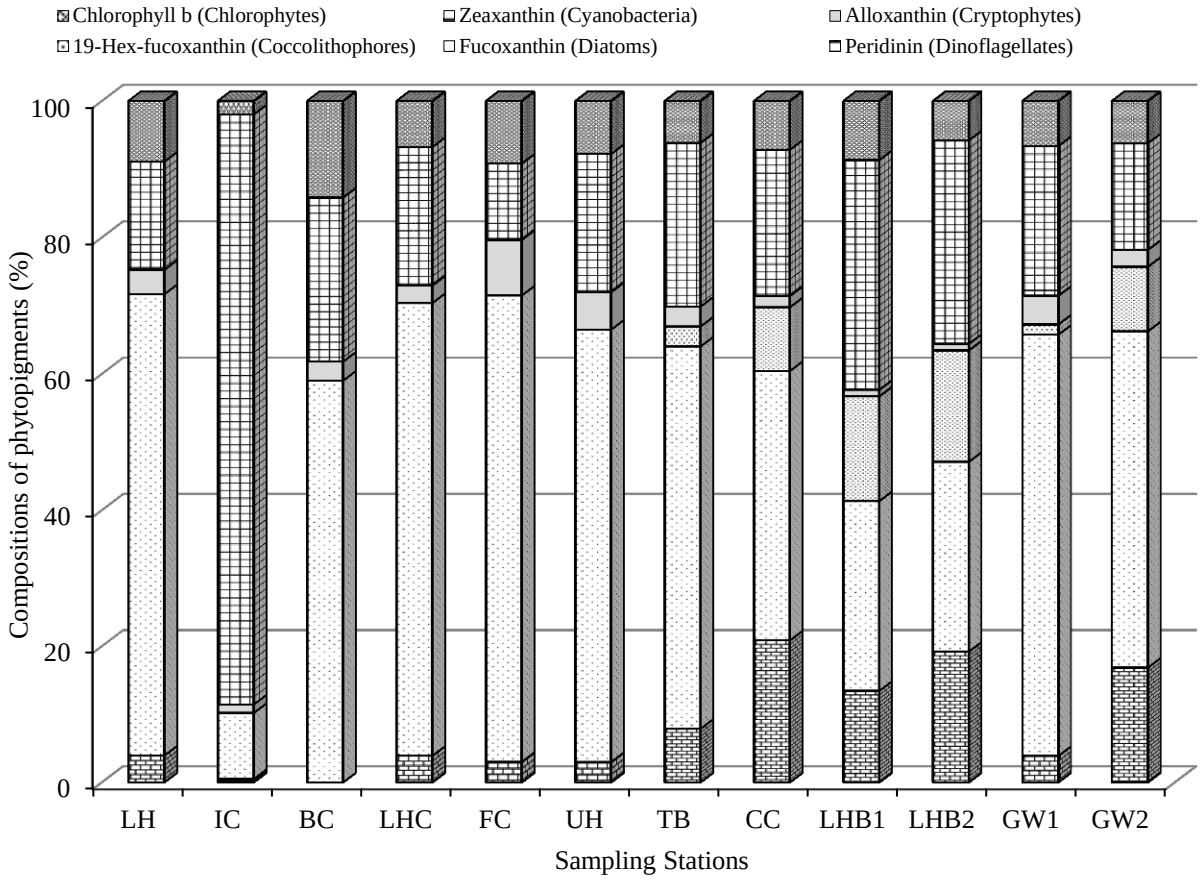


Figure 6: Relative percentage (%) composition of each of the six pigment markers of phytoplankton taxonomic groups observed in the Lagos harbour.

Table 2: Comparison between chemotaxonomic and microscopic assessments of phytoplankton assemblage structures in the Lagos harbor

Phytoplankton Functional Groups	Chemotaxonomy	Microscopy
Chlorophytes	+	+
Coccolithophores	+	-
Cryptophytes	+	-
Cyanobacteria	+	+
Diatoms	+	+
Dinoflagellates	+	+
Silicoflagellates	-	+

Key: + = Detected, - = Not Detected

phytoplankton community composition.

This study documented a pioneering effort at the chemotaxonomic assessment of phtoplankton in Nigeria, leading to the identification and quantification of nineteen phytopigments. Microscopic observations are, however, still needed to identify the taxa contributing to these specific accessory pigments as recommended also by Fujiki *et al*. (2014) and Tamm *et al*. (2015). As such, further research is needed to assess the correct application of chemotaxonomy to ecological studies of natural phytoplankton assemblages in Nigerian waters.

ACKNOWLEDGEMENTS

The author is grateful to Professor Mathew L. Julius of St. Cloud State University (SCSU), Minnesota and Dr. Marina Potapova of the Academy of Natural Science of Drexel University, Philadelphia, USA for their guidance with respect to microscopy. The author is also grateful to Dr. Louise Schlüter of DHI laboratory in Denmark for her supervision of the HPLC analysis of phytopigments. This research was partly funded by the NEEDS ASSESSMENT FUND and the Doctoral Grant of the University of Lagos.

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Conflicts of Interest: The authors declare that no conflicts of interest exist in respect to publishing these research findings.

Citation: Elegbeleye, O. W. (2024) Phytoplankton Assemblage in a Tropical Harbour: Microscopy versus Chemotaxonomy. *West African Journal of Fisheries and Aquatic Sciences*, *4*, 1-11

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