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Method Article

# Validating chlorophyll-a concentrations in the Lagos Lagoon using remote sensing extraction and laboratory fluorometric methods



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# A B S T R A C T

Remote sensing data is a viable alternative for mapping pigment concentrations in water body, and consequently, the trophic. Chlorophyll-a (Chl-a) is present in all phytoplankton species. This study therefore uses laboratory fluorometric and remote sensing extraction methods for assessing chlorophyll-a concentration in the Lagos Lagoon. The fluorometer was calibrated with a commercially available chlorophyll-a standard before used in the laboratory to estimates chlorophyll-a concentration. Landsat 7 (ETM+) and Landsat 8 (OLI) were acquired for the remote sensing method. The Landsat data were first geometrically rectified. Then brightness values were converted to reflectance through the radiometric correction process. For the regression models, logarithmically transformed chlorophyll-a was used as the dependent variable. Single bands, band ratios and logarithmically transformed band ratios were the independent variables. R2 values were computed and evaluated.

- Chlorophyll-a contributes to productive water bodies
- laboratory fluorometric and remote sensing extraction methods
- Landsat data acquired for the remote sensing method

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#### A R T I C I E I N E O

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<span id="page-1-0"></span>

### Method Details

Optical satellite datasets have been used to detect freshwater systems for decades however traditionally, satellite remote sensing of freshwater systems has been limited by sensor technology as well as its current and past missions have not provided the measurement resolutions needed to fully resolve freshwater ecosystem properties and processes [[1\]](#page-7-0). Nevertheless, integration of earth observation products derived from satellite imageries that may improve water quality monitoring is one of the feasible methods  $[2-4]$  $[2-4]$ . Several studies methods have demonstrated the relationship between optical properties (reflectance) of water to other water parameters' properties vis-a-vis suspended sediments, chlorophyll concentrations, dissolved organic matter, pigment load, temperature, Secchi disc depth and other laboratory based water quality [5[–](#page-7-0)8]. Satellites sensors can measure the amount of solar radiation at various wavelengths reflected by surface water, which can be compared to water quality parameters for instance, total suspended solids which constitutes an alternative means of estimating water quality [\[9,10](#page-7-0)]. Remote sensing therefore, offers a credible means of estimating water quality measurement. In a comparative study to assess the ability of satellite based sensors to monitor suspended sediment concentration, Secchi disc depth, and turbidity, it was discovered that predictions based on optical measures of water quality are slightly better when using earth observation data [[11\]](#page-7-0). Apart from extremely demanding time and capital investments of traditional methods, its monitoring also requires sequential laboratory and unreliable in situ measurements and analysis [[12](#page-7-0)].

It is on the aforementioned basis that this study established that both laboratory and satellite extraction methods have their merit and demerit.



Table 1 Landsat data and Laboratory estimation of Chl-a.

<span id="page-2-0"></span>

Fig. 1. Estimated chlorophyll-a distribution in the Lagos Lagoon, 2010.





#### Methods

#### Laboratory method

#### Sample collection and storage

The samples points comprise of 7 locations points across Lagos lagoon. The locations include Ibeshe, Egbin, Oworoshoki, University of Lagos-UNILAG front, Ijora, Five Cowries Creek, and Commodore Channel ([Table](#page-1-0) 1,[Figs.1](#page-2-0) and 2). The water samples were collected into clean polyethylene bottles. Water sample measured 200 ml was filtered through a  $0.45 \mu m$  fibre membrane filter, after which the residue on the filter was transferred to a tissue blender, covered with 3 ml of 90% aqueous acetone and macerated for 1 min. the sample was then transferred to a centrifuge tube, capped and allowed to stand for 2 h in the dark at  $4^{\circ}$ C (in a refrigerator). Samples were filtered through 47 mm GF/ F filters using polycarbonate in-line filters (Gelman) and a vacuum of less than 100 mm Hg. Filters are folded in half twice and wrapped in aluminum foil, labeled, and stored in refrigerator until ready for analysis. For fluorometric analysis, we used 25 mm GF/F filters.

After removing the samples from refrigerator, the pigments are extracted by placing the filters in 5.0 ml 100% acetone. For 47 mm GF/F filters, 0.8 ml of water is retained adjusting the final extraction solution to 86% acetone and the final extraction volume to 5.8 ml. The samples are covered with Parafilm to reduce evaporation, sonicated ( $0^{\circ}$ C, subdued light) and allowed to extract for 4 h in the dark at  $-20$  °C. Following extraction, samples are vortexed, filters are pressed to the bottom of the tube with a stainless-steel spatula and spun down in a centrifuge for 5 min to remove cellular debris. For fluorometric analysis (not HPLC), decantation can replace centrifuging.

Chlorophyll-a, fluoresce in the red wavelengths after extraction in acetone are excited by blue wavelengths of light. The fluorometer stimulates the extracted sample with a broadband blue light and the resulting fluorescence in the red is detected by a photomultiplier. The significant fluorescence by phaeopigments is corrected for by acidifying the sample which converts all of the chlorophyll a to phaeopigments. By applying a measured conversion for the relative strength of chlorophyll and phaeopigment fluorescence, the values were therefore used to calculate the chlorophyll-a concentrations.

Apparatus: Filtration system and Whatman GF/F filters Liquid nitrogen and freezer for storage and extraction Glass centrifuge tubes for extraction, 15 ml Turner fluorometer, fitted with a red sensitive photomultiplier, a blue lamp, 5–60 blue filter and 2–

64 red filter. Reagents 100% acetone 90% acetone M HCl (100 ml HCl in 900 ml de-ionized water)

# Laboratory estimation of chlorophyll-a

For laboratory assessment, the fluorometer was calibrated with a commercially available chlorophyll-a standard before used in the laboratory [\[13](#page-7-0)–16[,5](#page-7-0)]. The standard is dissolved in 90% acetone for at least 2 h and its concentration  $(mg l^{-1})$  is calculated spectrophotometrically as follows:

Chl-a =  $[(\text{Amax} - \text{A}_{750 \text{ nm}})/E^*]$  \*(1000 mg/1 g) (1)

where:

 $A_{\text{max}}$  is absorption maximum (664 nm)  $A_{750 \text{ nm}}$  is absorbance at 750 nm to correct for light scattering E is extinction coefficient for chl-a in 90% acetone at 664 nm (87.67 L  $\rm{g^{-1} cm^{-1}}$ )  $l$  is cuvette path length (cm).

From the standard, a minimum of five dilutions are prepared for each door. Fluorometer readings are taken before and after acidification with 2 drops 1.2 M HCl. Thereafter, linear calibration factor  $(K_v)$ are calculated for each door  $(x)$  as the slope of the unacidified fluorometric reading vs. chlorophyll- $a$ concentration calculated spectrophotometrically. The acidification coefficient  $(F_m)$  was calculated by averaging the ratio of the unacidified and acidified readings  $(F_0/F_2)$  of pure chlorophyll-a. Samples are read using a door setting that produces a dial reading between 30 and 100. The fluorometer is zeroed with 90% acetone each time the door setting is changed.

Chlorophyll-a was determined using a Fluorometer equipped with filters for light emission and excitation [[5](#page-7-0),[15](#page-7-0),[17,18\]](#page-7-0). Thereafter, it was centrifuged at 5000 rpm for 20 min. and the supernatant was decanted. Volume left after decanting was noted. Different readings were taken from the Fluorometer (which had been pre-calibrated with 2, 5, 10 and 20  $\mu$ g standard chlorophyll solutions) at  $\times$ 1,  $\times$ 3,  $\times$ 10, and  $\times$ 30 sensitivity settings and noted. The concentrations of chlorophyll-a for the samples were calculated using Eqs. (1) and (3):

Chl 
$$
(\mu g/l) = (F_m/F_m - 1)^* [(F_o - F_a)^* K_x^* (vol_{ex}/vol_{filt})
$$
 (2)

Phaeo (chl equiv.weights) =  $(F_m/F_m - 1)^*[(F_m * F_a) - F_0]K_x - \text{vol}_{\text{ex}}$  (3)

where:

 $F_m$  is acidification coefficient (Fo/Fa) for pure Chl a (usually 2.2).  $F<sub>o</sub>$  is reading before acidification  $F_a$  is reading after acidification  $K_x$  is the door factor from calibration calculations  $vol_{ex}$  is extraction volume vol $_{\text{filt}}$  is sample volume.

#### Remote sensing extraction method

#### Image data processing

Landsat-7 ETM+ image is superior to its predecessors (e.g. Landsat -5), with significant improvement of on-flight geometric and 5% absolute radiometric calibration, and consist of improved panchromatic band with 15 m spatial resolution (band 8), Visible (reflected light) bands in the spectrum of blue, green, red, near-infrared (NIR), and mid-infrared (MIR) with 30 m spatial resolution (bands 1–5, 7), and a 60 m thermal infrared (band 6) spatial resolution (USGS, 2018).

Landsat 8 Operational Land Imager (OLI) and Thermal Infrared Sensor (TIRS) images consist of nine spectral bands with a spatial resolution of 30 m for Bands 1–7 and 9. The resolution for Band 8 (panchromatic) is 15 m. In addition, it also has two Thermal IR bands with a spatial resolution of 100 m (later resampled into 30 m). Since the spectral bands of Landsat ETM are very similar, this study used similar methods for 2007 and 2010 imageries. Using the image metadata, the radiometric calibration was conducted to convert digital numbers into top-of-atmosphere radiance Watanabe et al. [[19](#page-7-0)[,38\]](#page-8-0). The retrieval of the at-surface reflectance was accomplished using the Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes (FLAASH), an atmospheric correction module, implemented in the ENVI software. This tool adopted the MODerate resolution atmospheric TRANsmission (MODTRAN4), an atmospheric radioactive transfer code [\[20,19](#page-7-0),21–[23](#page-7-0)].

#### Image preprocessing and subset

The Landsat 7 and 8 images were imported into the ArcGIS environment and a shape file covering the Lagos lagoon was superimposed on the images and used to extractthe Region of interest (ROI). The extracted images were then stretched using the histogram equalization technique and filtered to remove haze, cloud cover and noise using the Quick atmospheric correction tool in Envi 5.0 software [\[20](#page-7-0)[,24\]](#page-8-0).

Landsat ETM+ data pre-processing followed standard specification including radiometric and geometric calibration and terrain correction [[25](#page-8-0),[26](#page-8-0)]; conversion from digital number to satellite reflectance (for six reflectance bands) or at satellite radiance temperature (the thermal band), and referencing to the National Albers equal-area map projection and resampling using cubic convolution to 30 m resolution. After initial pre-processing, tasselled-cap brightness, greenness, and wetness were derived using satellite reflectance-based coefficients [\[27,26\]](#page-8-0).

#### Estimation of chlorophyll-a using Landsat satellite imageries

Landsat 7 and Landsat 8 images with acquisition dates of November 06, 2010 and November 11, 2015 acquired from USGS Earth Explorer were used for this study. The data were in GeoTiff format with 16bit radiometric resolution (ranges from 0 to 65535).

#### Landsat 7

The band ratios among the first four ETM+ bands as proposed and tested in the literature were computed [28–[34\]](#page-8-0). In the regression models established, the logarithmically transformed chlorophylla concentration was used as a dependent variable [[35](#page-8-0)]. The three types of independent variables were tested: reflectance of a single band, logarithmically transformed band ratios, and ratios of logarithmically transformed single band. R2 values were computed. From the best results, a map was generated showing the chlorophyll-a distribution and concentration in Lagos Lagoon.

# Conversion of Landsat 8 DN values to top of atmosphere (TOA) reflectance

The Landsat 8 DN was then converted to TOA reflectance using the Landsat 8 processing toolbox of ArcGIS 10.3.

Radiometric calibration and atmospheric correction for Landsat 8 required to achieve the purpose of chlorophyll a concentration retrieval [\[36](#page-8-0)] were conducted using the ENVI software in this study. After radiometric calibration, the un-calibrated digital numbers (DN) were converted to radiance values through the formula:

$$
L_{\lambda} = M_L Q_{cal} + A_i \tag{4}
$$

where

 $L_{\lambda}$  is the top-of-atmosphere (TOA) spectral radiance,

 $M_L$  is band specific multiplicative rescaling factor from the metadata,

 $A_i$  is the band specific additive rescaling factor from the metadata, then the dimensionless top-ofatmosphere reflectance  $\rho$ TOA can be calculated as:

$$
\rho T O A = \pi L_{\lambda} d^2 / ES UN_{\lambda} \cos \theta_s \tag{5}
$$

**Where** 

 $L_{\lambda}$  is the spectral radiance at the sensor,  $d^2$  is the Earth-sun distance in astronomical units. ESUN is the mean solar exoatmospheric irradiance for each band and  $\theta$ cos is the solar zenith angle in degrees

#### Band ratio using band 4 and band 5 reflectance

The reflectance band 4 (NIR) and band 5 (MIR) were divided to correct atmospheric distortions in the images and to obtain a band ratio of the both images.

#### Estimation of chl-a content

The band ratio (3\_4.tif) was then divided by  $\pi$  to obtain the chlorophyll-a content using the raster calculator in ArcGIS and the regression method. Finally, the FLAASH module outputs a bottom-ofatmosphere reflectance value for each pixel and an average scene visibility and water amount estimate [[37](#page-8-0)]. It is worth mentioning that the image data used in this work are all processed by FLAASH

<span id="page-7-0"></span>atmospheric correction. This process produced a Landsat image of all individual bands with reflectance values.

#### Conclusion

Chlorophy-a is an indicator of phytoplankton abundance and contributes signicantly to the overall primary productivity of coastal water bodies. Chlorophyll-a are useful in providing information for detail assessment of algal biomass and its spatial and temporal variability. This study estimates Chl-a concentration using laboratory and remote sensing (using Landsat ETM and OLI images) methods. The fluorometric method is extensively used for the quantitative analysis of chlorophyll  $a$  and phaeopigments while remote sensing extraction method is extensively used for the quantitative and qualitative mapping of chlorophyll-a. The procedures in this study are appropriate for all levels of chlorophyll-a concentration in any aquatic environment. These two methods based on their details take into consideration the scientific requirements for assessing historical and current issues about water body.

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