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Decomposition study of in vivo phytoplankton absorption spectra aimed at identifying the pigments and the phytoplankton group in complex case 2 coastal waters of the Arabian Sea

by

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Abstract

Phytoplankton modify the optical properties of the seawater by altering the subsurface light field. Information on the accessory pigments present in the phytoplankton helps to differentiate major phytoplankton classes or taxonomic groups. The variability in the absorption spectra of phytoplankton and particulate matter of case 2 coastal waters of the Southeastern Arabian Sea were studied from June 2010 to November 2011. The phytoplankton specific absorption coefficient, at 440 nm and 675 nm, a^*_{ph} (440) and a^*_{ph} (675) varied from 0.018 to 0.32 m^2 mg^{-1} and from 0.0005 to 0.16 m^2 mg^{-1}, respectively. The 4th derivative spectra computed for each *in vivo* absorption spectrum showed that the amplitude of maxima obtained is proportional to the concentration of the chromoprotein which absorbed that wavelength. Regression of pigment concentration against the 4th derivative spectral coefficient showed that the measurements of particulate absorption could provide quantitative information on chlorophyll *a* and other accessory pigment concentrations. Fucoxanthin and diadinoxanthin, the carotenoid pigments found in the diatoms were identified from the derivatives peaks. The study demonstrates the utility of using the 4th derivative analysis as a tool to identify the dominating phytoplankton group and its pigment composition.

Key words: Total particulate absorption, phytoplankton specific absorption coefficient, packaging effect, derivative analysis

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Introduction

Phytoplankton modify the optical properties of the seawater by altering the subsurface light field (Babin et al. 2003, Tzortziou et al. 2007, Bricaud 2012). This property of phytoplankton plays an important role in the remote estimation of aquatic biomass (Morel 1988). Knowledge of phytoplankton light absorption coefficients are used to predict the light energy absorbed by the phytoplankton and its use in photosynthesis (Bricaud et al. 2004). The variations in the optical properties of coastal waters are attributed mainly to the absorption by chlorophyll *a* (Chl-*a*) and other accessory pigments present in the phytoplankton. Coastal waters also receive dissolved and particulate materials from several sources that make them optically complex (IOCCG 2000). The process of light absorption by phytoplankton is a complex process and is a function of shape, size and optical properties of the cell (Ferreira 2013, Fujiki and Taguchi 2002). The important sources of variability in the absorption come from the contributions of cellular pigment composition, accessory pigments, detritus absorption and the pigment packaging effect (Bricaud et al. 2004, Baird 2013).

The accessory pigments play an important role in the optical characteristics of most natural waters, especially in the 460 – 640 nm regions (Bidigare et al. 1989a). Different phytoplankton species have characteristic absorption peaks, which are related to the pigment composition (Mao et al. 2010). In addition, certain accessory pigments are found exclusively in certain phytoplankton groups and could be used as markers (Jeffry, Vesk 1997) to study the seasonal variation of phytoplankton in natural waters. Studying the phytoplankton biomass variability is essential for understanding the food web and its influence on the optical properties of the aquatic ecosystem. Particulate detritus matter also makes large contributions to absorption especially in the blue and ultraviolet region of the spectrum (Stramski 2007; Kishino et al. 1986; Maske, Haardt 1987; Mitchell, Kiefer 1988).

The absorption spectra of phytoplankton are complex due to the mixtures of pigments and associated proteins; it is difficult to identify these components by optical analysis. The phytoplankton specific absorption coefficient (a^*_{ph} (λ)) spectra from the coastal environment often lack sharply defined peaks or shoulders due to the overlapping of pigment absorption bands. The derivative analysis was used as a method to obtain estimates of photosynthetic pigment concentrations in natural samples where absorption can be strongly influenced by detrital matter (Bidigare et al. 1989b). Rosado-Torres (2008) has recently used derivative analysis to study the phytoplankton dynamics and to quantify the chlorophyll *a* in Mayagüez Bay. The utility of derivative analysis for highlighting the accessory pigment absorption during bloom conditions in a coastal bay was demonstrated by Vijayan and Somayajula, (2014). The 4^{th} derivative spectra computed for each *in vivo* absorption spectrum and amplitude of maxima obtained are often proportional to the concentration of the chromoprotein, which provide quantitative information on chlorophyll *a* and other accessory pigments.

The bio-optical data from the region are meager and this study is the first to report on the coastal waters of the Southeastern Arabian Sea coast to characterize the absorption nature of the phytoplankton. Since the information on the accessory pigments can help to distinguish major phytoplankton classes or taxonomic groups (Hoepffner, Sathyendranath 1993; Coupel et al. 2014), it would be a great improvement of the ocean-color remote sensing if the information regarding the accessory pigments can also be retrieved from the water color. For this purpose, one of our approaches in this paper is to retrieve dominant phytoplankton species and its characteristic accessory pigments from the 4^{th} derivative hyperspectral analysis of absorption by phytoplankton (a_{ph}) and thereby to study the variability in phytoplankton absorption in the Arabian Sea.

Materials and methods

Study Area

The study area is located in the coastal waters of the Arabian Sea. The study area covers 8 stations from S1 to S8 (Fig. 1) and monthly samples were collected from June 2010 to November 2011. The sampling was carried out using a Commercial Purse seiner 'MV Bharat Darshan'. The surface water samples were collected using a 2.5 l Hydro-Bios

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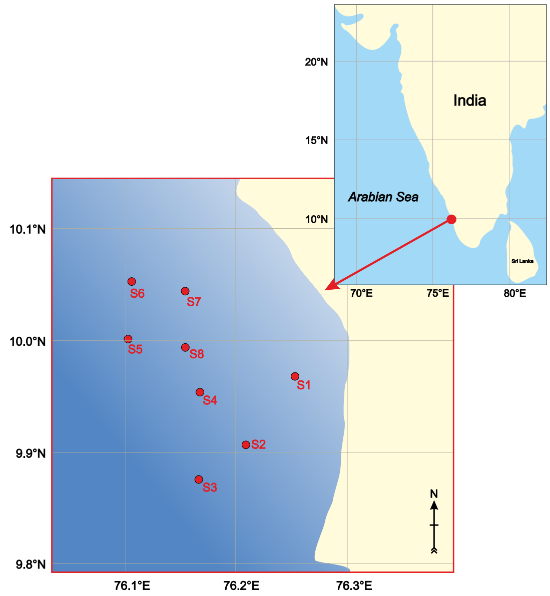

Figure 1

Plot showing the study area with eight different stations of coastal waters of the Southeastern Arabian Sea

Niskin water sampler, stored in dark polyethylene bottles and kept in -20°C till the analysis.

Measurements of chlorophyll *a* concentration

Water samples were filtered through a 47 mm Whatman GF/F filter under low vacuum and extracted with 90% acetone at 0°C for 24 hours in dark. Chl-*a* pigment concentration was measured using a Turner Design 10AU Fluorometer.

Spectral absorption measurements

Particulate absorption coefficients were measured using a quantitative glass fibre filter technique (QFT). Absorption of the sample was measured against a blank filter using a Shimadzu UV-VIS 2450 spectrophotometer attached to an integrating sphere following the procedure of Mitchell (1990). The wavelength scan was done from 300 nm – 750 nm with the resolution of 1 nm before and after rinsing the filter paper with warm methanol for one hour to determine the detrital absorption (Kishino et al. 1985). For each of the measured spectra, the optical density obtained at 750 nm was subtracted from that of all other wavelengths. Optical density of the total suspended matter was corrected for the path length amplification (β effect) and converted into light absorption coefficients by the total particulate matter and detritus matter, $a_p(\lambda)$ and $a_d(\lambda)$, i.e. before and after extraction with methanol, respectively according to Cleveland and Weidemann (1993) and Kyewalyanga et al. (1998). The plankton absorption component $a_{ph}(\lambda)$ derived as a difference between the particulate and detritus absorptions of the total particulate matter by subtraction $a_d(\lambda)$ from $a_p(\lambda)$ (Kishino et al. 1985, Bricaud et al. 2004).

$$a_{ph}(\lambda)= a_p(\lambda)-a_d(\lambda)$$

The Chl-*a* specific light absorption coefficients of phytoplankton $a^*_{ph}(\lambda)$ were then obtained by normalizing $a_{ph}(\lambda)$ with Chl-*a* concentration.

Phytoplankton numerical density

Water samples were collected to identify the phytoplankton taxonomy, stored in high-density polyethylene bottles and preserved using buffered formalin. Leica Generic DMIL inverted microscope with phase contrast was used to estimate the phytoplankton diversity and abundance using standard identification keys (Tomas 1997).

Derivative analysis

The fourth derivative of $a_p(\lambda)$ and $a_{ph}(\lambda)$ was calculated by applying the 41 point fourth degree polynomial smoothing and then by differentiation using the Savitzky-Golay method (Savitzky, Golay 1964). The polynomial smoothing was applied because differentiation tends to amplify the effects of high frequency noise in the spectra (Aguirre-Gomes et al. 2001). The procedure was carried out using the Microcal Origin 8.0 scientific analysis software. Peaks in the fourth derivative curves were selected using the peak finder tool in the software.

Results

Variation in the phytoplankton specific absorption coefficient

Figure 2 shows the variation of the phytoplankton specific absorption coefficient of different months

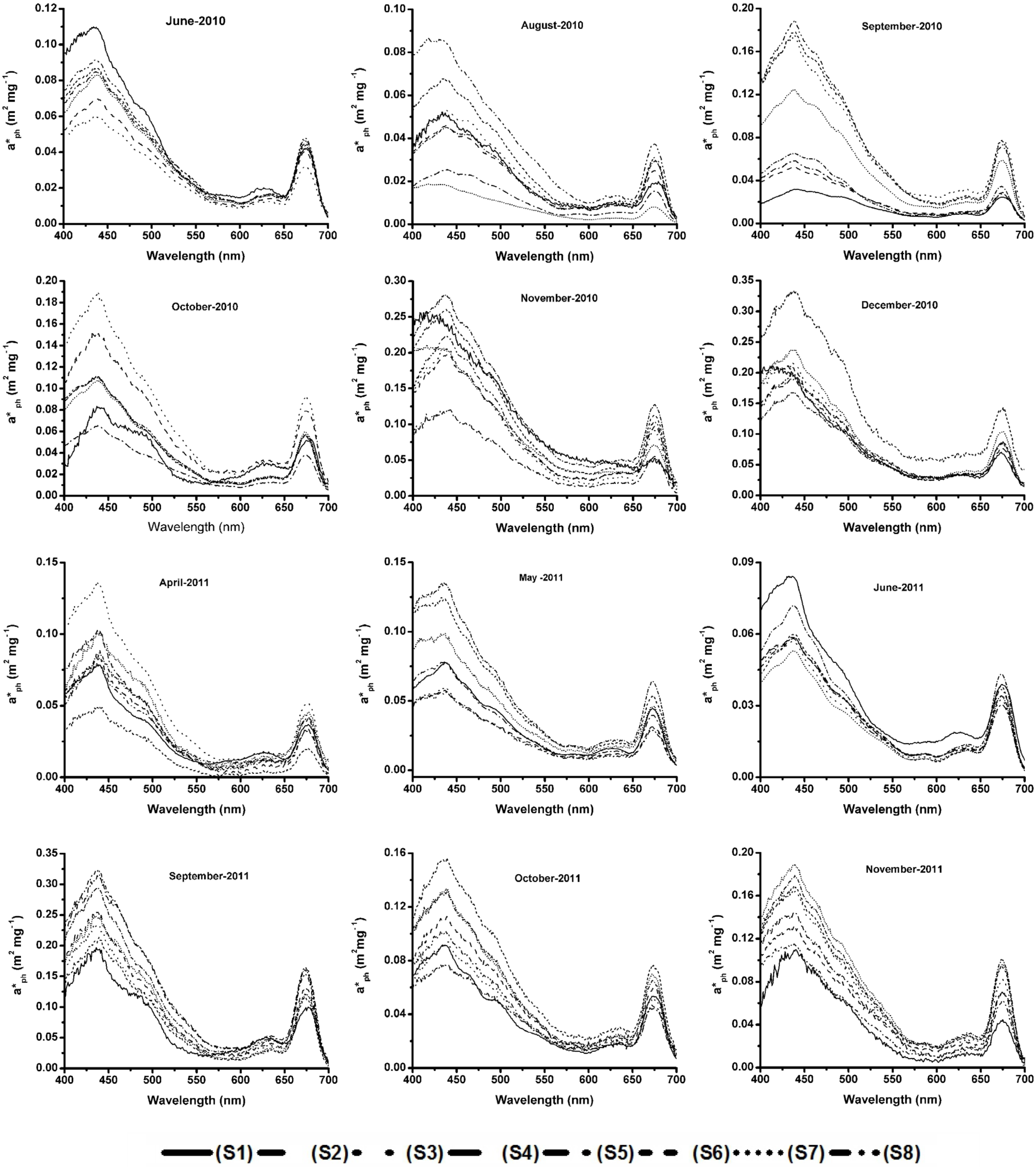

Figure 2

Plots showing the spectra of chlorophyll specific absorption by phytoplankton (a^*_{ph} (λ)) against wavelength (nm) for the study area covering Station 01 to Station 08 in 2010-2011

during the study period and illustrates the spatial and temporal variation in the spectral shape and magnitude. The phytoplankton specific absorption coefficient at 440 nm ($a^*_{ph}(440)$) varied from 0.018 to 0.32 m^2 mg^{-1} with an average value of 0.12 during the study period. In the red region, a^*_{ph} (675) varied from 0.0005 to 0.16 m^2 mg^{-1} with an average value of 0.054 m^2 mg^{-1} and Chl-*a* concentration from 0.05 to 12.4 mg m^{-3} with an average value of 3.73 mg m^{-3}. In June, at the onset of the south west monsoon for both 2010 and 2011, a^*_{ph} (440) exhibited a low value with an average of 0.0.015 m^2 mg^{-1} and 0.062 m^2 mg^{-1} with high Chl-*a* concentration 7.133±1.017 mg m^{-3} and 7.710±3.107 mg m^{-3}, respectively. In November and December, high a^*_{ph} (440) was measured. The relation between a^*_{ph} at 440nm and 675 nm with Chl-*a* are shown in Figure 3.

Spatial and temporal variation of dominant phytoplankton species

The phytoplankton species, which dominated in the southeastern coastal Arabian Sea during the study period, was shown in Table 1. During monsoon 2010, centric diatoms like *Chaetoceros* spp., dinoflagellates like *Dinophysis* spp. and pennate diatoms like *Rhizosolenia* spp. dominated. In October both the pennate and centric diatoms dominated in the study area. In November 2010, centric diatoms dominated while in December pennate diatoms and dinoflagellates dominated. In April, blue green algae, e.g. *Trichodesmium* spp., were abundant while in May it was centric and pennate diatoms. In June, September and November 2011, pennate and centric diatoms dominated in the study area. The detailed species diversity was described elsewhere (Minu et al. 2014).

Table 1

List of dominant species present in the study area during June 2010 to November 2011

Station	2010						2011					
	Jun.	Aug.	Sep.	Oct.	Nov.	Dec.	Apr.	May	Jun.	Sep.	Oct.	Nov.
S1	C	Q	T	O	F	P	E	R	I	K	L	G
S2	E	C	K	C	M	S	U	B	C	B	I	S
S3	K	D	C	C	K	O	U	R	C	T	E	Q
S4	C	G	Q	S	T	S	A	B	C	K	I	K
S5	C	G	Q	S	K	L	C	C	C	S	L	K
S6	C	C	M	C	K	N	U	R	C	K	U	K
S7	C	G	Q	C	K	M	U	R	T	I	H	K
S8	C	G	K	C	K	B	U	R	R	K	I	L

A-*Anabaena* spp., B-*Asterionella* spp., C-*Chaetoceros* spp., D-*Corethron* spp., E-*Coscinodiscus* spp., F-*Cyclotella* spp., G-*Dinophysis* spp., H-*Eucampia* spp., I-*Gyrodinium* spp., J-*Gyrosigma* spp., K-*Leptocylindrus* spp., L-*Navicula* spp., M-*Nitzschia* spp., N-*Peridinium* spp., O-*Prorocentrum* spp., P-*Pseudonitzschia* spp, Q-*Rhizosolenia* spp., R-*Skeletonema* spp., S-*Thalasionema* spp., T-*Thalassiosira* spp., U-*Trichodesmium* spp.

Derivative analysis of phytoplankton absorption

Derivative analysis on the absorption data of a_p and a_{ph} (λ) of 1 nm resolution for the study area are shown in Figure 4 and 5, respectively. The maxima of the 4^{th} derivative occur close to or at wavelengths where there are absorption peaks attributable to photosynthetic pigments. Since the magnitude of the 2^{nd} derivative does not provide a reliable measure of the concentration of photosynthetic pigments, due to the absorption contributed by the effect of overlapping pigments (Aguirre-Gomez et al. 2001), we have performed and considered only the 4^{th} derivative analysis for the identification of absorption peaks. In order to identify the major contributing pigments of the *in vivo* absorption

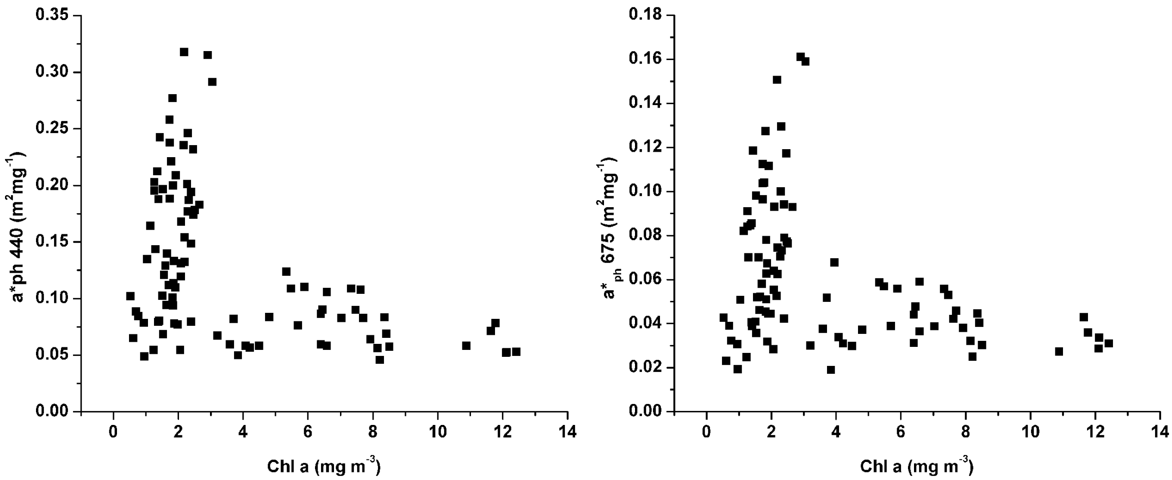

Figure 3

Variation in chlorophyll specific absorption by phytoplankton (a^*_{ph}) at 440 nm and 675 nm (Y axis) as a function of chlorophyll *a* concentration (X axis) in 2010-2011 in the study area

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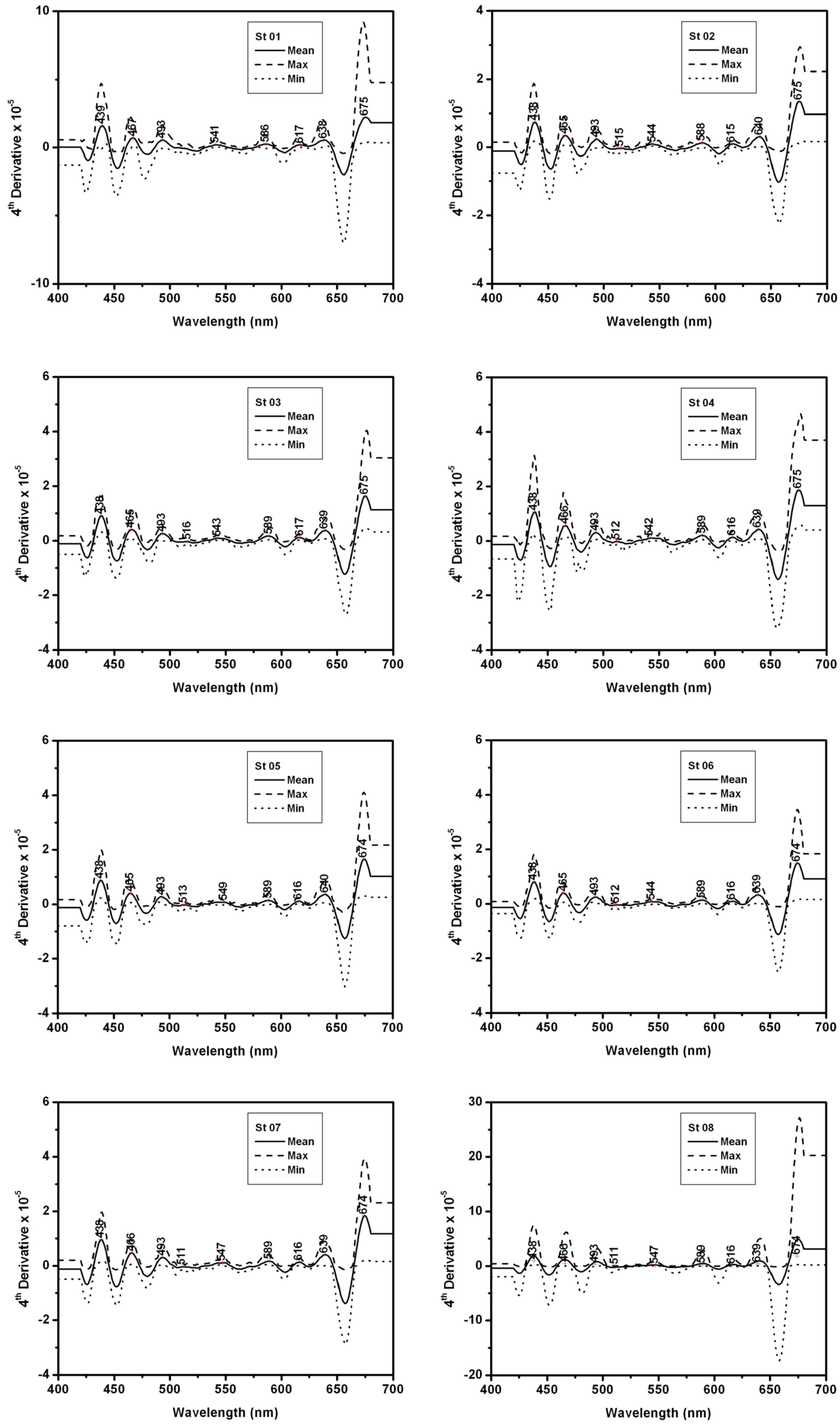

Figure 4

Spectra showing the 4th derivative analysis of the total particulate absorption (a_p (λ)) against wavelength (nm) in the study area

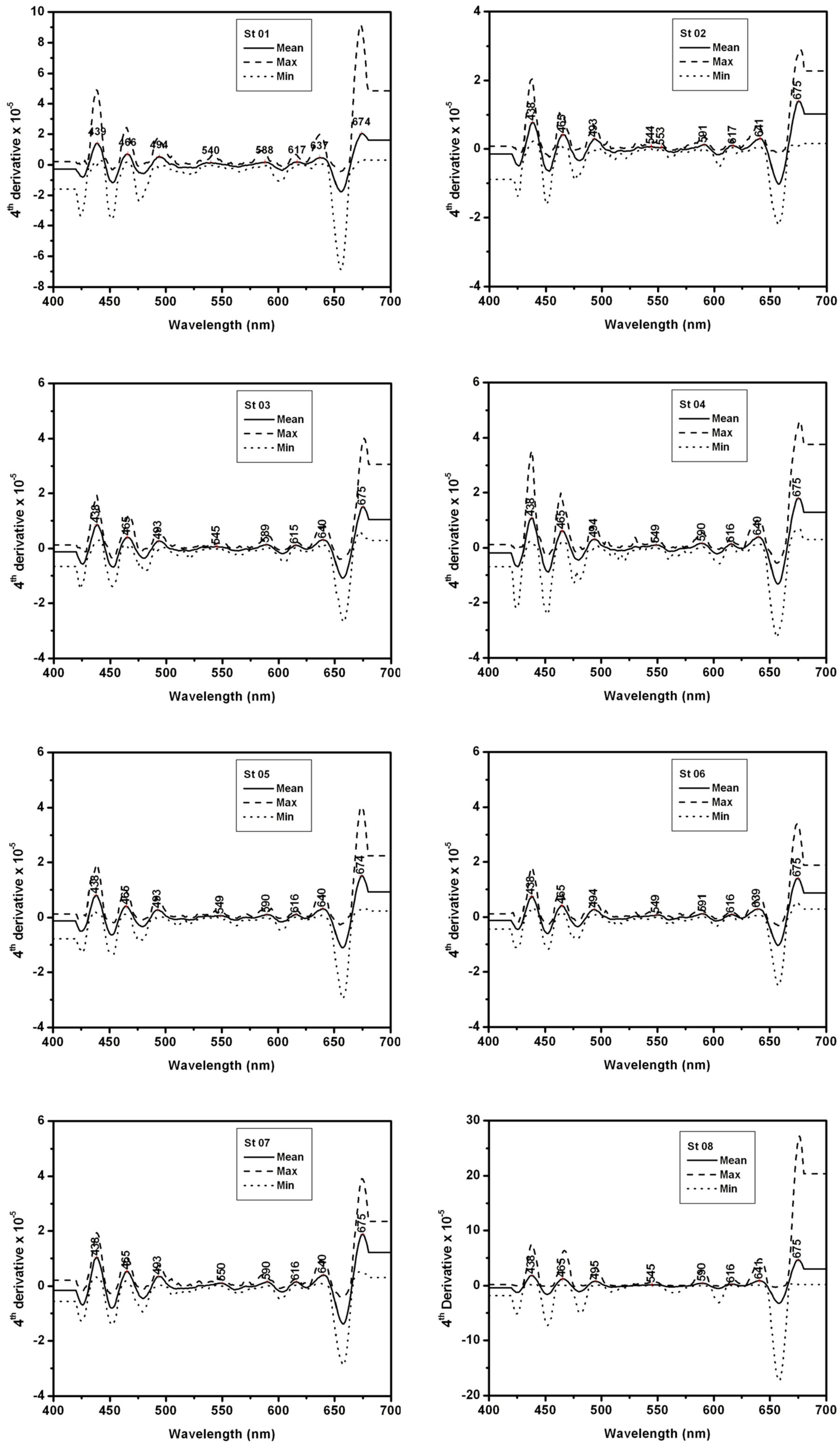

Figure 5

Spectra showing the 4th derivative analysis of absorption by phytoplankton (a_{ph} (λ)) against wavelength (nm) in the study area spectra of phytoplankton in coastal waters, we have compared the wavelength positions of the 4th derivative spectra of the total absorption and phytoplankton absorption with the published values and presented them in Table 2. The identified positions and the corresponding pigments were also presented in Table 2. Phytoplankton with pigments Chl-*a*, Chl-*b*, Chl-*c*, Fucoxanthin, Diadinoxanthin, and Phycoerythrobilin dominated in these waters. The comparison analysis of the peak position of the 4th derivative of a_p and a_{ph} (λ) absorption indicated that the derivative analysis effectively filtered out the contribution of detritus absorption.

The value of the 4th derivative for Chl-*a* was maximum at 675 nm band. The phycoerythrin pigment was present and it was reflected at 495-499 nm peaks. Chl-*a* specific phytoplankton absorption $a^*_{ph}(\lambda)$ and their 4th derivatives for the whole study in the Arabian Sea showed the contribution by major phytoplankton groups from different peaks identified.

Table 2

Summary of photosynthetic pigment absorption maxima determined by derivative analysis

Pigment group	λ_{max} [nm] (Present study)	λ_{max} [nm] (Published)	Reference
Chlorophylls			
Chlorophyll *a*	438-440, 675	438, 675	Prezelin and Alberte (1978) Aguirre- Gomez et al (2001)
Chlorophyll *b*	465-467	470, 652	Kan and Thornber (1976)
Chlorophyll *c*	465, 586, 639	460, 640	Mann and Myers (1968) Millie et al. (1997)
Carotenoids			
Fucoxanthin	495, 512, 541	460 – 530	Mann and Myers (1968)
Diadinoxanthin (+carotene)	495, 512	425 – 500	Mann and Myers (1968)
Phycoerythrin			
Phycoerythrobilin	543, 544	543	Ong et al. (1984)
Phycourobilin	491 – 495	492	Ong et al. (1984) Louchard et al. (2002)

Discussion

Derivative analysis is a powerful tool to study the phytoplankton dynamics, in the absence of microscopy data, especially in the coastal waters. Spectral derivative analysis has been applied successfully to optical data as a method to produce wave crests at wavelengths where shoulders and peaks occur in spectroscopy curves (Millie et al. 1995). These wave crests correspond to the absorption peaks of accessory pigments when derivative analysis is applied to phytoplankton absorption curves. The advantages of this method over solvent extraction and HPLC consist in the fact that natural samples are processed rapidly with a high degree of precision, no extraction of pigments is needed, several pigments are determined at the same time, and errors due to pigment loss in extraction methods are minimized (Faust and Norris 1985).

The spectral absorption coefficients obtained during different seasons and geographical regions lack the sharply defined peaks and shoulders that are apparent in spectra obtained from phytoplankton cultures. The scatter plot between a^*_{ph} at 440 nm and 675 nm with Chl-*a* are presented in Figure 6. It shows that the absorption of Chl-*a* within algal cells is not a linear function of the concentration, and the specific absorption coefficient is not constant. This might be due to either a combined effect of packaging, where the Chl-*a* concentration per phytoplankton cell increases and Chl-*a* absorption efficiency decreases (Bricaud et al. 2012) or internal pigment variation due to a change in the dominant phytoplankton species.

The spectral shape and magnitude of the phytoplankton absorption curve reflects the pigment composition, dynamic variation of the species composition and the packaging effect. Each species has its unique pigment signature and therefore contributes to the shape of the absorption spectrum. The diatoms, i.e. the dominant phytoplankton group during the study period, have marker pigments – fucoxanthin and diadinoxanthin (Hsiu-Ping et al. 2002) with the absorption peak at 495, 512 and 541 nm. Phycobiliproteins, which are the light harvesting pigments found in cyanobacteria, rhodophytes and cryptophytes are evinced by peaks at 491-495 nm and at 544 nm, especially at station S6, S7 and S8, where numerical abundance of *Trichodesmium* was higher. Sometimes the overlapping of photosynthetic pigments with bands of absorption causes masking of spectral absorption features of accessory pigments. This could also occur due to large contributions from detritus matter, which is generally in an oxidized state and lacks well-defined resonances (Babin et al. 2003), and is a characteristic feature of coastal waters.

The accessory chlorophylls and carotenoid pigments have their absorption peaks in the 490-550 nm regions. Since Chl-*a* is the most abundant

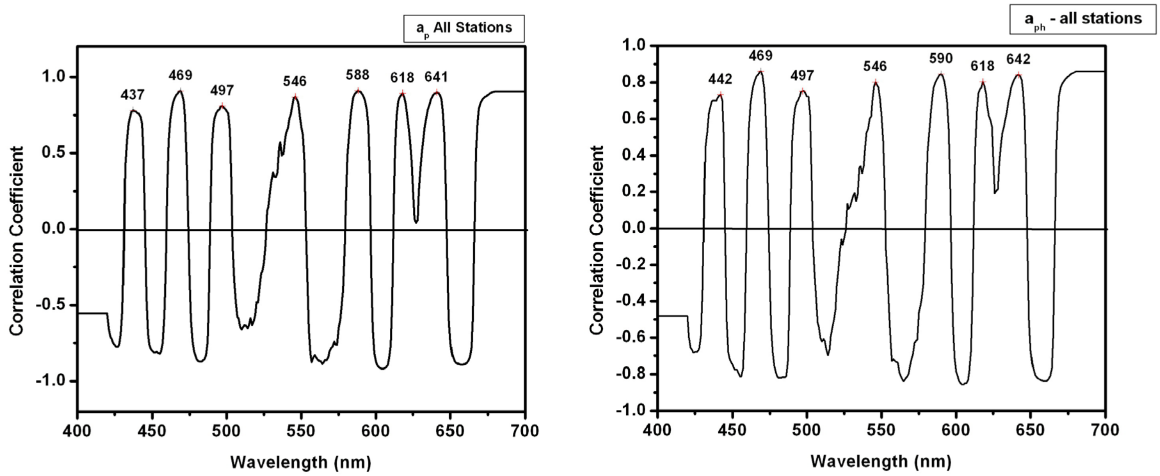

Figure 6

Plots showing the correlation coefficient analysis between chlorophyll *a* concentration and coefficients of the 4th derivative spectra of the total particulate absorption, a_p (λ) and the 4th derivative of absorption by phytoplankton, a_{ph} for the study area in 2010-2011

pigment found in phytoplankton whose absorption maxima are around 438-440 nm and 675-677 nm wavelengths, the accessory pigment's absorption peaks are relatively identified in the 450-550 nm spectral region applying the 4th derivative analysis. Other peaks found in the region from 560-618 nm may be accounted for degradation products and Chl-*c*.

Several works have identified the position of the absorption peaks of phytoplankton pigments with HPLC analysis. Bidigare et al. (1989b) and Coupel et al. (2014) reported absorption peaks for Chl-*a* at 675 nm, Chl-*b* at 650 nm and Chl-*c* at 467 nm. Absorption peaks at 438 and 677 nm were reported for Chl-*a* and at 466, 589 and 639 nm for Chl-*c* by Millie et al. (1995) and Chase et al. (2013). Carotenoids, especially fucoxanthin, diadinoxanthin and β-carotene, have peaks reported at 495 nm and in the case of fucoxanthin – at 550-555 nm (Chase et al. 2013, Louchard et al. 2002, Gómez et al. 2001). Based on the literature, the peaks found with the 4th derivative analysis in the study area are consist of Chl- *a* (440 and 675 nm), Chl-*b* (465 nm), Chl-*c* (586 nm, 639 nm) and carotenoid pigments-fucoxanthin (495 and 547 nm) (Table 2). Chl-*a* is the principal photosynthetic pigment of phytoplankton and is common to all species. Chl-*b* and Chl-*c* are very common photosynthetic pigments, found in diatoms, dinoflagellates and other groups. The absorption peaks identified in the coastal Arabian Sea are consistent with the dominance of these groups in the phytoplankton community (Table 1). Thus the 4th derivative analysis is found as a simple and efficient tool for the monitoring of chemotaxonomic markers in phytoplankton assemblages. Some minor variations in the magnitude of the 4th derivative peaks and the appearance of new peaks in the study area suggest that they might be due to the spatial and temporal difference and the influence of rivers as all the stations in the study area are under riverine input, pigment packaging and photoadaption in the trophic conditions (Shaw, Purdie 2001; Cleveland 1995; Jyothibabu et al. 2006; Roesler, Barnard 2014). The diatom abundance is high in the proximity of river mouths (Suzuki et al. 2014). Bigidare et al. (1989b) reported a good correlation (R^2 = 0.89) between the 4th derivative of $a_{ph}(\lambda)$ at 467,650 and 675 nm vs. Chl-*a* concentration in the Sargasso Sea. In general, the 4th derivative analysis suggests that even though the relative composition of the phytoplankton community was stable throughout the year, the dominant taxa varied. Peaks identified in the 4th derivative of a_{ph} are by the contribution of all the taxa.

The spectral correlation analysis between the 4th derivative of $a_{ph}(\lambda)$ and Chl-*a* in the present study confirm such results. High correlation coefficients were also found for the wavelengths associated with carotenoid accessory pigments. In summary, the 4th derivative of $a_{ph}(\lambda)$ appears to be a valuable

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alternative method for the quantification of Chl-*a* in the Arabian Sea. The result of this study would help in choosing spectral regions for Chl-*a* estimation where interference by accessory pigments is minimal.

Conclusions and remarks

From the optical point of view, the phytoplankton absorption in the coastal Arabian Sea is highly scattered and absorbed, also the phytoplankton absorption in the water varies spatially and temporally with cell size, and taxonomic affiliations. Derivative analysis of the particulate absorption coefficients offers an alternative and efficient method to separate the absorption by specific photosynthetic pigments from that of the detritus matter. This study suggests that the 4^{th} derivative analysis appears to be an efficient and simple tool for monitoring the phytoplankton pigment.

As evidenced by the obtained results, diatoms are the dominant phytoplankton group in the Southeastern Arabian Sea. Phytoplankton communities are highly varied during the study period. Using the fourth derivative plots of *in vivo* phytoplankton absorption spectra, ($d^4a_{ph}(I)$), it is possible to identify several maxima absorption values attributed to different pigments which can be further used for the study of factors affecting the variation in the phytoplankton absorption spectrum to develop the regional algorithm suitable in this region for various remote sensing applications like prediction of harmful algal blooms.

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