The Effects of Pheophytin a on Absorption Properties of Phytoplankton in Dalian Bay, China

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Abstract. We examined the effects of pheophytin a (Phe-a) on absorption properties of phytoplankton obtained from the red tide prone area of Dalian Bay in the North Yellow Sea, China, in June 2007 and July 2011. When the proportion of Phe-a relative to chlorophyll-a (Chl-a) increased, the absorption peak in the blue-violet region shifted from 438nm to 409nm. The relationship between blue-violet absorption maximum and Phe-a concentration is more tightly correlated than the relationship between blue-violet absorption maximum and Chl-a concentration. However, we found the opposite results for the red absorption maximum. These findings suggest that Phe-a mainly effects the blue-violet absorption maximum, while Chl-a primarily controls the red absorption maximum. Our results are determined by specific absorption coefficients of Phe-a and Chl-a in living algae cells. A multivariate linear regression analysis revealed that the specific absorption coefficients of Phe-a accounts for the absorption peaks at 407 nm and 673 nm, and specific absorption coefficient of Chl-a accounts for the absorption peaks at 440 and 673 nm, furthermore, the specific absorption coefficients of Phe-a are much larger than those of Chl-a in the blue-violet region, but they are lower in the red region.

1. Introduction

Phytoplankton is ubiquitous free-floating organisms in the upper layers of the ocean. Along with nonalgal particles, phytoplankton determines the optical properties in oceanic and coastal waters and is referred to as colored dissolved organic matter (CDOM) or ocean color constituents. An inherent optical property (IOP), the absorption coefficient of phytoplankton, is the foundation for developing semi-analytical ocean color algorithms [1-5] and influenced by the package effect [6] and pigment composition of phytoplankton cells [7].

The package effect accounts for the way that because pigments are packaged in phytoplankton cells, the absorption spectrum is flattened compared to the absorption spectrum of the same pigments in solution [8]. The package effect varies nonlinearly with cell size and intracellular pigment concentration [9-11] The pigment composition is known to differ among phytoplankton species, but phytoplankton cells are rich in Chl-a. Therefore, most phytoplankton absorption spectra display the absorption characteristics of Chl-a, with obvious absorption peaks at 440 nm and 675 nm.

In some coastal areas water eutrophication induces rapid propagation of phytoplankton or even red

tide outbreaks. The combination of water pollution and phytoplankton population booms can lead to large-scale phytoplankton declines. After the death of phytoplankton cells under acidic, heat or lighting conditions, the magnesium ion ((Mg2+)) at the center of the porphyrin ring in the Chl-a molecule is generally replaced by two hydrogen ions (2H+), resulting in the conversion of Chl-a to Phe-a. The conversion to Phe-a can be considered an indication of the death of the phytoplankton cells. If water contains a large amount of Phe-a then the water quality is generally poor. The high concentration of Phe-a changes the absorption properties of phytoplankton and influences the bio-optical information inversion.

Many studies have focused on the absorption properties of Phe-a in solution [12-14], while the absorption properties in living algae cells have only been mentioned in a few papers [15-17]. As the ratio of Phe-a to Chl-a gradually increases, the absorption peak in the blue band shifts toward a shorter band [1, 18] at 410 nm or 420 nm [19, 20]. Additionally, the specific absorption coefficients of Phe-a and Chl-a are different, and varying ratios of Phe-a to Chl-a leads to variations in the absorption peak height. Previous studies have shown that the specific absorption coefficient of Phe-a is greater than Chl-a in the visible domain [15, 16] or only in the blue band [21, 22]. Some studies have pointed out that the specific absorption coefficient of Phe-a is less than Chl-a in the red band [17, 23, 24]. These conclusions were briefly mentioned in previous research, not discussed in depth and more focused on mormal water, but rarely related to the red tide water with high chlorophyll concentration. The objective of this study was to examine effects of Phe-a on the absorption properties of phytoplankton in the red tide prone area of Dalian Bay in the North Yellow Sea, China. We first discuss the effect of Phe-a on absorption peak wavelengths and absorption maximums, as well as variation of the pigment concentration as a function of the absorption maximum. Then, we determine the specific absorption coefficients of Phe-a and Chl-a in living algae cells to potentially explain changes in the absorption spectra of phytoplankton.

2. Method

2.1. Study Area and Field Work

Dalian Bay is on the southeast side of the Liaodong Peninsula of northeast China and is exposed to the North Yellow Sea to the east. The coast is an industrial area that includes shipbuilding, petroleum processing, chemical manufacturing and other industries. The industrial and domestic wastewater of Dalian City discharges into the bay and the main pollutants are inorganic nitrogen, active phosphate, petroleum and heavy metals. As coastal development increases, the environmental quality of Dalian Bay is gradually getting worse, resulting in serious eutrophication and frequent red tides. We collected samples from Dalian Bay seven times between June, 2007 and July, 2011. There were six expeditions that obtained 24 samples in 2007, and another cruise in 2011 that obtained 11 samples (**Figure 1**).

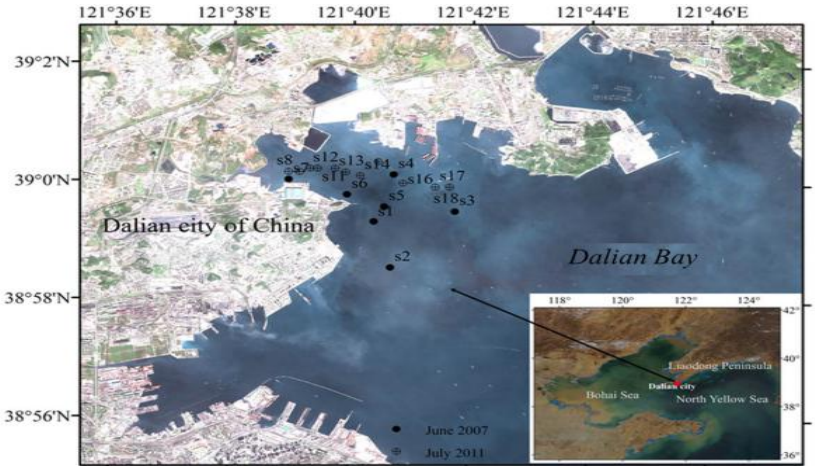

Figure 1. Location of the Dalian Bay Field Stations in the North Yellow Sea, China. ● is Dalian Bay, ● marks the location of water samples collected in June, 2007, ⊕ marks the location of water samples collected in July, 2011.

2.2. Particulate Absorption Measurements

Particle absorption was measured by filtering a known volume of sea water, through Whatman GF/F filters and then measuring the absorption coefficient of the particles on the filter in a Hitachi U-3010 dual beam spectrophotometer, using a clean filter as a reference blank. Methods employed for particulate absorption measurements are described in detail in the work of Bricaud et al. [7; 20; 25]. Total particulate absorption coefficients $a_p(\lambda)$ were determined first, then nonalgal particle absorption coefficients $a_{nap}(\lambda)$ were determined after pigment bleaching with MeOH. The phytoplankton absorption coefficient $a_{ph}(\lambda)$ was obtained from the equation ($a_{ph}(\lambda) = a_p(\lambda) - a_{nap}(\lambda)$).

2.3. Pigment Analyses

For pigment analyses, seawater samples were filtered through 25 mm Whatman GF/F glassfiber filters. Chl-a and Phe-a concentrations (hereafter denoted as [Chl a] and [Pheo a]) were determined by fluorescence [26] using a TD-700 fluorometer (Turner Designs). The filters were flooded with 90% acetone for the extraction and kept for 24 h in a dark fridge. The fluorescence values were measured before and after acidification, then [Chl a] and [Pheo a] were obtained according to data processing. In what follows, [Pheo a] is added to [Chl a] and the sum is referred to as "Total chlorophyll concentration" and noted as [TChl].

3. Results

3.1. Statistical Description of Pigment Concentrations

Both normal and red tide waters were encountered during the fieldwork. The pigment concentrations ([Chl a], [Pheo a], [TChl]) measured in the study areas reveal a range of more than two orders of magnitude (**Table 1**). Almost half of the samples had [TChl] in excess of 20 $mg \cdot m^{-3}$, a threshold value represented red tides.

Table 1. Statistics of Pigment Concentrations

Pigment	Min.	Max.	Average	SD
Chl a/mg·m^{-3}	0.88	156.59	26.04	42.37
Pheo a/mg·m^{-3}	1.19	177.25	28.49	45.96
TChl/mg·m^{-3}	2.11	318.47	54.53	87.67

The [Pheo a]-to-[Chl a] ratios varied between 0.4-2.2 (Figure. 2 most of the measurements were between 0.6-1.8, and approximately 70% of the measurements were above 1). This suggests that Phe-a can substantially interfere with ocean color remote sensing, especially when measuring Chl-a.

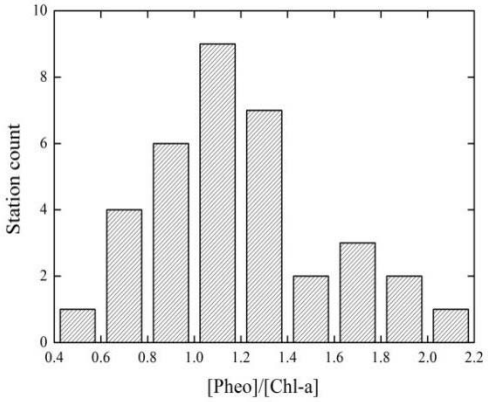

Figure 2. Histogram Plot of [Pheo a]-to-[Chl a] Ratios

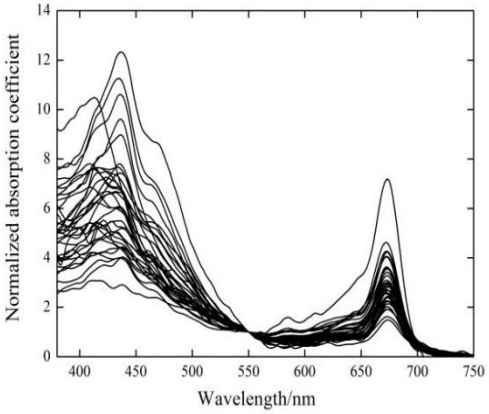

Figure 3. Pytoplankton Normalized Absorption Spectra

3.2. Variation of Phytoplankton Absorption Spectra

The variation of phytoplankton absorption spectra is too large to reveal the features of some low spectra in usual absorption spectrogram. Therefore, the normalized absorption coefficients were calculated as $a_{ph}(\lambda)/a_{ph}(550)$, as shown in **Figure 3** Absorption peaks around 675 nm correspond to the red absorption of Chl-a are present in all curves. It is worth noting that in the blue-violet region, the absorption peaks ranged from 438 to 409 nm. These peaks were either a main peak with less obvious sub-peaks, or a relatively smooth peak. The peak characteristics are related to interference between Chl-a and Phe-a[15, 19, 20].

3.3. Blue-violet Absorption Peak Shift is Affected By Phe-a

We found a negative correlation between blue-violet absorption peak wavelength and the ratio of [Pheo a] to [Chl a], except for several outliers (red dots in **Figure 4**). The linear regression shows that the determination coefficient is 0.58, indicative of a significant covariation. This implies that the presence of Phe-a affects the absorption peak as it shifts to shorter wavelengths (from 438 to 413 nm), as there is a fixed absorption peak around 440 nm for Chl-a. But the outlier samples (at approximately 413 nm) do not follow this trend, and there may be several explanations for this, such as the predominant algae species in the sample and intracellular Phe-a content. The cause of these outlier readings need further study.

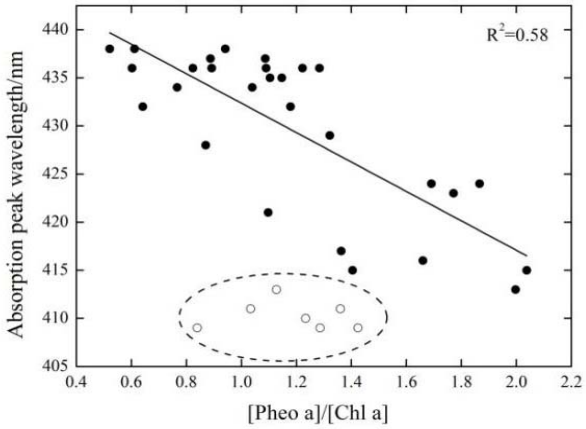

Figure 4. Absorption Peak Wavelength as a Linear Function of the Ratio of [Pheo a] to [Chl a]. The hollow dots are not included the in regression analysis.

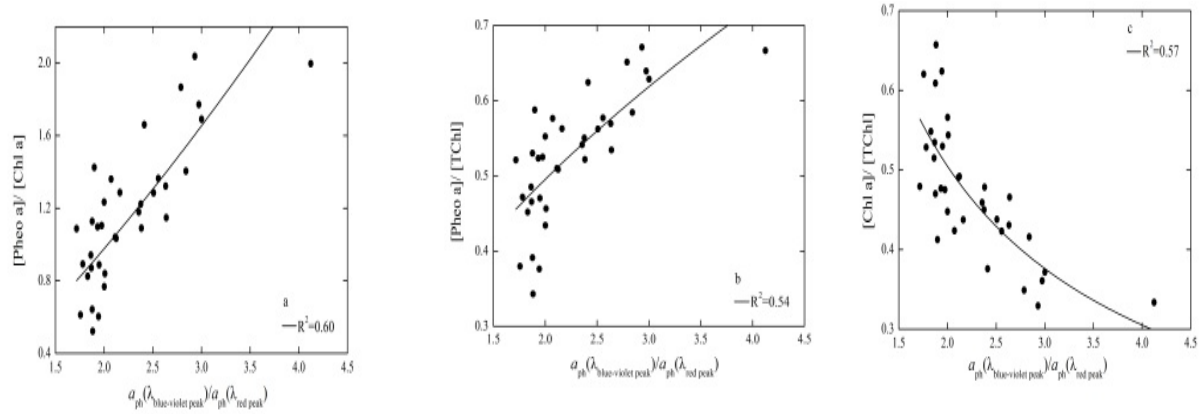

Figure 5. [Pheo a]/[Chl a], [Pheo a]/[TChl] and [Chl a]/[TChl] as a power function of the ratio of blue-violet to red absorption maximums. $\lambda_{\text{blue-violet}}$ peak and λred peak denote the blue-violet absorption peak wavelength and the red absorption peak wavelength.

3.4. Effect of Phe-a on The Ratio of Blue-violet to Red Absorption Maximums

Due to the package effect, the ratio of blue-violet to red absorption maximum generally decreases as chlorophyll concentration increases, but this trend was not observed in our study. Instead, the ratio is related to [Pheo a]/[Chl a], [Pheo a]/[TChl] and [Chl a]/[TChl] (**Figure 5**), and tends to increase nonlinearly with [Pheo a]/[Chl a] or [Pheo a]/[TChl], but decreases nonlinearly with [Chl a]/[TChl]. The regression analyses show that the determination coefficients for these regressions are 0.60, 0.54 and 0.57, respectively. These findings suggest that Phe-a mainly affects the blue-violet absorption maximum, but Chl-a primarily controls the red absorption maximum.

3.5. Relationship between Pigment Concentration and Absorption Maximum

Previous studies have shown that absorption coefficients tend to increase nonlinearly as pigment concentrations increase as a result of the decreasing contribution of nonphotosynthetic pigments and increasing pigment packaging [19, 20, 27]. Therefore, these pigment concentrations can be expressed as power functions of blue-violet absorption maximum or red absorption maximum [28]:

$$[C] = A \times a_{ph}(\lambda)^{B} \quad (1)$$

Where [C] is the pigment concentration of [Pheo a], [Chl a] or [T Chl], $a_{ph}(\lambda)$ is the absorption coefficient of phytoplankton (blue-violet absorption maximum or red absorption maximum), and A and B are regression coefficients.

We found determination coefficients over 0.9 for all regressions, indicative of significant covariations (**Figure 6**). At the blue-violet absorption peak wavelength, the a_{ph} versus the [Pheo a] relationship (**Figure 6(a)**, R^2=0.966) is more highly correlated than the a_{ph} versus the [Chl a] relationship ((**Figure 6(b)**, R^2=0.927); but the opposite occurs at the red absorption peak wavelength ((**Figure 6(d)**, R^2=0.960; (**Figure 6(e)**, R^2=0.976). These results contribute further evidence that Phe-a makes a greater contribution to the blue-violet absorption maximum than Chl-a; and chlorophyll makes a greater contribution to the red absorption maximum. The strongest determination coefficient ((**Figure 6(f)**, R^2=0.983) was between [TChl] and $a_{ph}(\lambda_{red}$ peak) and the strength of this relationship is due to the stable absorption peak wavelength in the red region, the absorption maximum of Phe-a and Chl-a both contribute to absorption at this wavelength, but the absorption is rarely affected by other pigments. In contrast, many pigments affect the absorption in the blue-violet range ((**Figure 6(c)**, R^2=0.962).

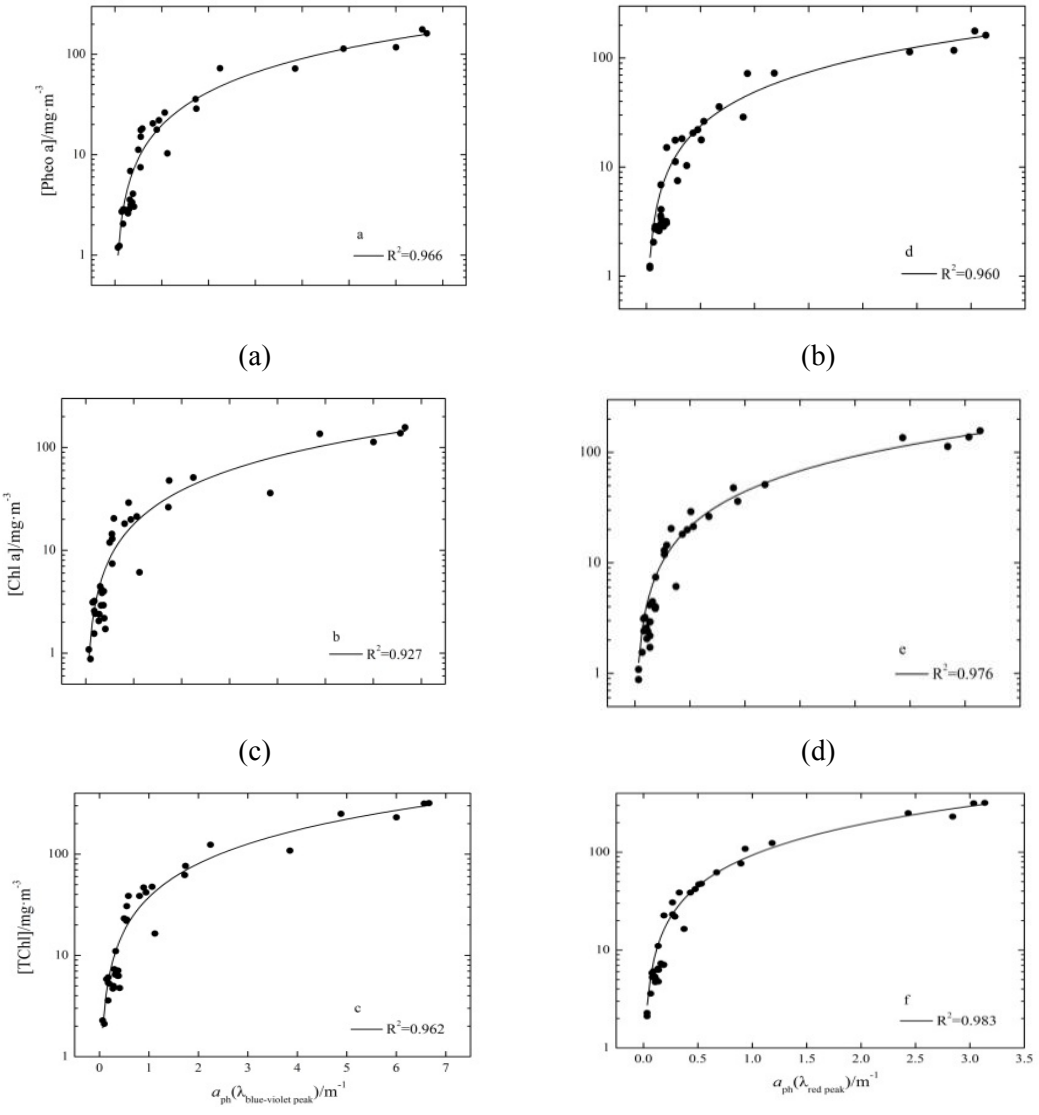

Figure 6. [Pheo a], [Chl a] and [TChl] as a power function of blue-violet absorption maximum (a-c) and red absorption maximum (d-f) respectively.

4. Discussions and Conclusions

The results obtained in our study suggest that the absorption spectral changes of phytoplankton are affected by Phe-a. In fact, these changes are caused by the specific absorption coefficients of Phe-a and Chl-a in living algae cells.

There were two absorption peaks at approximately 440 nm and 675 nm in the absorption spectra of Chl-a in living algae cells, but there is no consistent conclusion about the absorption peak wavelengths of Phe-a in the previous study. Mitchell et al. [29] measured the absorption spectra of senescing diatoms containing large amounts Phe-a. They found that the absorption peak wavelength shifted to 415 nm in the blue-violet region and almost disappeared in the red region. Babin et al. [30] pointed out that there is no evidence that Phe-a contributes to the absorption peak at 415 nm.

For many years scientists have been dedicated to how to quantify the specific absorption coefficients of Phe-a and Chl-a in living algae cellos [15-17]. These absorption components can be expressed as the product of a specific absorption coefficient and the concentration for each pigment [17]. Here we only consider the Phe-a and Chl-a:

$$a_{ph}(\lambda) = a^*_{\text{Pheo a}}(\lambda) \cdot [\text{Pheo a}] + a^*_{\text{Chl a}}(\lambda) \cdot [\text{Chl a}] \quad (2)$$

Where $a_{ph}(\lambda)$ is the absorption coefficients by Phe-a and Chl-a, $a^*_{\text{Pheo a}}(\lambda)$and $a^*_{\text{Chl a}}(\lambda)$are the specific absorption coefficients of Phe-a and Chl-a. To estimate $a^*_{\text{Pheo a}}(\lambda)$ and $a^*_{\text{Chl a}}(\lambda)$, we pooled all of the pigment and $a_{ph}(\lambda)$ determinations and determined the least-squares fit to the above equation with $a^*_{\text{Pheo a}}(\lambda)$ and $a^*_{\text{Chl a}}(\lambda)$ as adjustable parameters.

The $a^*_{\text{Pheo a}}(\lambda)$ and $a^*_{\text{Chl a}}(\lambda)$ each have two absorption peaks in the visible domains, located at 407 and 673 nm for Phe-a and 440 and 673 nm for Chl-a (**Figure 7**). In addition, $a^*_{\text{Pheo a}}(\lambda)$ are found at 0.0366, 0.0186, 0.0073 at 407, 440, 673 nm respectively, and $a^*_{\text{Chl a}}(\lambda)$ are found at 0.0051, 0.0193, 0.0133 at the same wavelengths as above. Overall, $a^*_{\text{Pheo a}}(\lambda)$ is much larger than $a^*_{\text{Chl a}}(\lambda)$ in the blue-violet region, but is less in the red region.

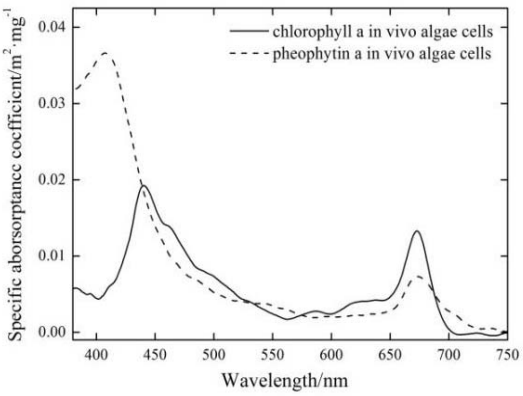

Figure 7. Specific Absorption Spectra of Phe-a and Chl-a in Vivo Algae Cells

Studies have consistently found that specific absorption coefficients of Phe-a are higher than that of Chl-a in the blue- violet region. We obtained similar results in this paper, but the values are far lower than those found in other studies. We hypothesize that this is the case because the samples collected in this study have high pigment concentrations, which leads to lower absorption coefficients compared with similar pigment concentrations for a stronger package effect 31]. Furthermore, we found a different result in the red region where the specific absorption coefficients of Chl-a are higher than that of Phe-a, as have others [17, 23, 24].

The Chl-a and Phe-a concentrations measured in this study indicate that absorption coefficients are mostly influenced by Phe-a in the blue-violet region, whereas they are mostly determined by Chl-a in the red region. Thus, specific absorption coefficients of Chl-a and Phe-a are helpful to master

absorption properties of phytoplankton and optimize the retrieval algorithms of ocean color constituents in coastal waters.

5. Acknowledgement

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