Aquatic particulate absorption coefficient combining extraction and bleaching optimized for inland waters

Bruno Cremella $^{1,2,3}$, Simon Bélanger, $^4$ Yannick Huot$^{1,2,3}$

$^1$Département de Géomatique Appliquée, Université de Sherbrooke, 2500, boul. de l’Université, Sherbrooke, Quebec, J1K 2R1, Canada
$^2$CARTEL—Centre d’Applications et de Recherche en Télédétection, Département de Géomatique Appliquée, Université de Sherbrooke, 2500, boul. de l’Université, Sherbrooke, Quebec, J1K 2R1, Canada
$^3$Département de Sciences Biologiques, GRIL—Groupement de Recherche Interuniversitaire en Limnologie, Université de Montréal, Campus MIL, Montréal, Quebec, H3C 3J7, Canada
$^4$Département de Biologie, Chimie et Géographie, Groupes BORÉAS et Québec-Océan, Université du Québec à Rimouski, 300, allée des Ursulines, Rimouski, Quebec, G5L 3A1, Canada

Abstract

The particulate absorption coefficient is one of the fundamental inherent optical properties describing interactions of light with material in water. Its spectral properties contain important information about chemical and biological constituents. It is often partitioned into algal and non-algal fractions which provide useful information describing phytoplankton. Particulate absorption coefficient has been routinely measured in the ocean particularly to calibrate remote sensing algorithms. However, the methods to measure marine algal and non-algal absorbing fractions might fail in freshwaters due to difficulties extracting green-algae pigments and cyanobacterial phycocyanin and the high organic content of the non-algal particles, making direct bleaching biased. In this work, we describe a method with sequential extraction, bleaching, and post-processing to obtain unbiased pigments and non-algal absorption fractions in freshwater environments, and we compare it against the resulting fractions obtained by only extraction or bleaching, using samples collected from 649 lakes across Canada. The resulting non-algal particles spectra from our method appear free of interfering pigments while maintaining spectral shapes, as verified by the higher correlation coefficient between the 400 and 700 nm exponential coefficient ($S$, often referred to as slope) of the non-algal particles spectra and the organic fraction of total suspended solids, and by having a better correlation between the ratio of absorption coefficient of phytoplankton at 620 and 676 nm and cyanobacterial biomass percentage. Overall, this method solves the two problems in freshwater particulate absorption partitioning associated with (1) unextracted pigments with methanol extraction methods and (2) bias introduced to non-algal absorption spectra from NaOH bleaching.

Although atmospheric correction remains difficult in small lakes (Pahlevan et al. 2021; Pan et al. unpubl.), new approaches make the remote sensing of lakes increasingly viable for monitoring (Pahlevan et al. 2020). This capacity will be further augmented due to improvement in sensors and with recent and upcoming hyperspectral platforms like PRISMA, HyspIRI, and EnMAP (Giardino et al. 2014; Hestir et al. 2015; Lopinto and Ananasso 2020), which will provide data with enough spatial and spectral resolution to account for the different colored constituents of inland water allowing monitoring of lakes on large scale. Arising from increasing nutrient inputs, eutrophication of inland waters is a key process that needs to be monitored (Schindler 2006). Its consequences on phytoplankton biomass and composition are particularly important as they lead to increased dominance of potentially toxic cyanobacteria (Vasconcelos 2006; O’Neil et al. 2012; Rigosi et al. 2014), which have direct consequence to water quality and drinkability (Chorus and Bartram 1999).

Central to the development of the remote sensing approaches is the determination of accurate in situ inherent optical properties (IOP), namely the absorption and backscatter coefficients (see Table 1 for abbreviation and symbols), which underlie the remote sensing reflectance estimated from satellite sensors (Odermatt et al. 2012). As one of the key IOPs, the absorption coefficient of suspended particles ($a_p$, m$^{-1}$) provides one of the richest sources of information retrievable...
Table 1. Symbols and abbreviations.

| Symbol (abbreviation) | Units | Definition | Source |
|-----------------------|-------|------------|--------|
| \( a \) (ac) \( \equiv \) | m\(^{-1}\) | Absorption coefficient | Symbol, abbreviation |
| \( a_p \) \( \equiv \) | m\(^{-1}\) | Total particulate absorption coefficient | Measured |
| \( a_{\text{NAP}} \) \( \equiv \) | m\(^{-1}\) | Non-algal fraction of \( a_p \) | True/unknown fraction of \( a_p \) |
| \( a_{\phi} \) \( \equiv \) | m\(^{-1}\) | Phytoplankton absorption coefficient | True/unknown fraction of \( a_p \) |
| \( a_{\phi}^{\text{NAP}} \) \( \equiv \) | m\(^{-1}\) | Absorption coefficient after methanol extraction \( (V1) \) | Measured |
| \( a_{\phi}^{\text{NAP}} \) \( \equiv \) | m\(^{-1}\) | Absorption coefficient after bleaching \( (V2) \) | Measured |
| \( a_{\phi}^{\text{corr}} \) \( \equiv \) | m\(^{-1}\) | Absorption coefficient after methanol extraction corrected for the unextracted Chl \( a \) and phycobiliproteins \( (V3) \) | \( a_{\phi}^{\text{corr}} = a_{\phi}^E - a_{\phi}^B \) |
| \( a_{\phi}^E \) \( \equiv \) | m\(^{-1}\) | Phytoplankton abs from subtracting extracted NAP \( (V1) \) | \( a_{\phi}^E = a_p - a_{\phi}^{\text{NAP}} \) |
| \( a_{\phi}^B \) \( \equiv \) | m\(^{-1}\) | Phytoplankton abs from bleached NAP spectra \( (V2) \) | \( a_{\phi}^B = a_p - a_{\phi}^{\text{NAP}} \) |
| \( a_{\phi}^{\text{corr}} \) \( \equiv \) | m\(^{-1}\) | Phytoplankton spectra corrected for the unextracted Chl \( a \) and phycobiliprotein \( (V3) \) | \( a_{\phi}^{\text{corr}} = a_p - a_{\phi}^{\text{NAP}} \) |
| \( a_I \) \( \equiv \) | m\(^{-1}\) | Remaining bleachable material spectra | \( a_I = a_{\phi}^{\text{NAP}} - a_{\phi}^{\text{NAP}} \) |
| \( a_{\phi}^{\text{corr}} \) \( \equiv \) | m\(^{-1}\) | Unextracted Chl \( a \) absorption coefficient | Fitted and shifted a pure Chl \( a \) in methanol spectra to match the red peak \( (676 \text{ nm}) \) and the \( 650–700 \text{ nm} \) peak of \( a_p \) (estimated through baseline removal) | |
| \( a_{\phi}^{\text{corr}} \) \( \equiv \) | m\(^{-1}\) | Unextracted non-algal particles absorption coefficient | Estimated through baseline removal between \( 450 \) \( \text{nm} \) and \( 700 \text{ nm} \) of the resulting spectra of \( a_I \) |
| \( a_{\phi}^{\text{corr}} \) \( \equiv \) | m\(^{-1}\) | Unextracted phycobiliproteins absorption coefficient | Estimated through baseline removal between \( 450 \) \( \text{nm} \) and \( 700 \text{ nm} \) of the resulting spectra of \( a_I \) |
| \( a_{\text{Chla}} \) \( \equiv \) | m\(^{-1}\) | Absorption coefficient of Chl \( a \) in MeOH | \( a_{\text{Chla}} \) |
| \( a_{\text{Chla}} \) \( \equiv \) | m\(^{-1}\) | Absorption coefficient of Chl \( a \) in MeOH | \( a_{\text{Chla}} \) |
| \( \log (a_{\text{NAP}}^{400–700}) \) \( \equiv \) | m\(^{-1}\) | Slope of log \( a_{\phi}^{\text{NAP}} \) as a function of wavelength for the \( 400–700 \text{ nm} \) range | \( \log (a_{\text{NAP}}) = \log (a_{\text{NAP}}) = \log \left( a_{\phi}^{\text{NAP}} \right) \) \( + \text{const} \) |
| NAP | Non-algal particles | Abbreviation |
| CDOM | Colored dissolved organic matter | Abbreviation |
| TSS | Total suspended solids | Abbreviation |
| OSPM | Organic suspended particulate matter | Abbreviation |
| \( \lambda \) | nm | Wavelength | Symbol |

†The \( V1, V2, V3 \) notation refers to the three methods used to retrieve \( a_{\phi}^{\text{NAP}} \) and \( a_p \) herein.

by remote sensing, and its optical properties provide information about phytoplankton composition (Hoepffner and Sathyendranath 1993), particulate matter transport in coastal waters (D’Sa et al. 2007; Bélanger et al. 2013) and is a fundamental cause of underwater light extinction affecting major biogeochemical processes (Kirk 1980; Babin et al. 2003). Its separation into its components of algal particles \( (a_p, \text{m}^{-1}) \) and non-algal particles \( (a_{\phi}^{\text{NAP}}, \text{m}^{-1}) \) is a key starting point to understand the bio-optical characteristics of lakes and the links to phytoplankton composition. However, while this separation is easily achieved in most seawater samples (Kishino et al. 1985; Briceaud et al. 1995), freshwater phytoplankton assemblages and pigmentation make this separation difficult (Tassan and Ferrari 1995).

The main basis for fractioning particulate absorption in marine and coastal water has been methanol extraction (Kishino et al. 1985): following soaking in methanol of the particulate matter collected on filters, the algal pigments are solubilized in the methanol solution while the non-algal particulate (NAP) remains on the filter \( (a_{\phi}^{\text{NAP}}, \text{m}^{-1}) \), where the superscript “E” refers to the methanol extraction). The difference between the absorbance measured of the filter before extraction, which represent \( a_p \), and after extraction provides the absorption coefficient of phytoplankton pigments in vivo \( (a_{\phi}^{\text{corr}}, \text{m}^{-1}) \). However, phycobilins and pigments of some chlorophytes are poorly extracted with this approach (Bricaud and Stramski 1990; Ferrari and Tassan 1999). For example, up to 13% of \( a_p \) \( (680 \text{ nm}) \) remain unextracted in samples from reservoirs...
of the Neuse River basin in North Carolina (Vaehaetalo et al. 2005). Since phycobilins-containing phytoplankton—mostly cyanobacteria and cryptomonads (Becker et al. 2002; Seppälä et al. 2005)—are often an important fraction of the total biomass in lakes (Chow-Fraser et al. 1994; Reynolds 1997), methanol extraction has limited applicability in inland waters (Binding et al. 2008). An alternative to methanol extraction was developed to address these unextractable pigments, by treating the filters with sodium hypochlorite (NaOCl) (Tassan and Ferrari 1995; Ferrari and Tassan 1999). This procedure achieves complete depigmentation of the algal fraction by chemical oxidation of the light absorbing structures of organic molecules; however, some or all of the absorption of the NAP (particularly the organic fraction) will be lost or modified too (see Fig. 1A, top panel, yellow line; Binding et al. 2008). Other oxidations techniques also produce similar changes in $a_{NAP}$ (Estapa et al. 2012). A selective filter pad depigmentation using $\beta$-cyclocitral that bleaches chlorophylls and carotenoids but not the phycobiliproteins has been successful (Simis and Kauko 2012) but the effects on natural samples and $a_{NAP}$ has not been studied yet. Another method described by Simis et al. (2005) used a hot ethanol double extraction that left no trace of unextracted phycobilins in the non-algal fraction but did not extract all chlorophylls. In those cases, $a_{NAP}$ was substituted for an exponential fitted to the post-extraction filter $a_p$ spectra, which provides $a_{NAP}$ values with little bias, but some of the $a_{NAP}$ spectral shape can be lost if $a_{NAP}$ does not follow an exponential shape. This can happen when samples contain high concentrations of iron oxides terrigenous dust (Stramski et al. 2007), which are abundant in some inland waters, and in other coastal waters with high iron sediment loads (Estapa et al. 2012). Roesler and Perry (1995) removed the phycobilins with hot water after a hot methanol extraction in marine samples. The most recent International Ocean Colour Coordinating Group (IOCCG) reviews these and other methods for fractioning but they acknowledge that methanol extraction remains the most widely used in ocean science because it effectively removes most algal pigments in most environments (IOCCG Protocol Series 2018).

For upcoming hyperspectral remote sensing platforms, maintaining all the spectral signatures as unbiased as possible is key for developing algorithms that can exploit the small nm-level variations in the spectra (Giardino et al. 2014), potentially allowing even the determination of phytoplankton community composition (Xi et al. 2015; Zheng and DiGiacomo 2018; Reynolds and Stramski 2019). In this work, we describe a new approach of sequential methanol extraction and NaOCl bleaching that retrieves the NAP and algal fractions which, after appropriate fitting procedures, avoids most of the biases introduced by these methods separately.

![Fig. 1. Schematics of the steps used in the method proposed with synthetic spectra magnifying the spectral shape changes.](image-url)
Materials and procedures

Chlorophyll a concentration

Chlorophyll a (Chl a) samples were obtained by filtration on GF/F from the same water sample as the absorption and measured both by fluorometry (two duplicates per lake; McIntyre and Cullen 2005) and high-performance liquid chromatography (HPLC, one measurement per lake; Vinebrooke and Leavitt 1999). The chlorophyll measurements were averaged to obtain a three-replicate average. This was justifiable due to HPLC and fluorometry high collinearity (log-log type II major axis regression slope: $1.044 \pm 0.036$, intercept $-0.369 \pm 0.046$, Pearson’s $R = 0.918$) and close magnitude: fluorometry duplicates A and B root mean square percent error was 33.4%, while between HPLC and the mean of the fluorometry duplicates was 56.7%. The potential effect of chlorophyll b (Chl b) affecting the fluorometry measured was confirmed to not be present (as expected from the fluorescence protocol used, Welschmeyer 1994) after comparing the correlation of the mean percent error between the fluorometry and HPLC Chl a, with the logarithm of the ratio of Chl b: Chl a (Pearson’s $R$: $-0.051$, $p$-value = 0.2173, df = 579), and with the percent Chl b per Chls $a + b$ (Pearson’s $R$: 0.054, $p$-value = 0.1893, df = 579).

Total suspended solids mass and partitioning

Total suspended solids (TSS) measured gravimetrically on pre-weighed 47 mm Whatman GF/F filters after rinsing with distilled water. The organic fraction of TSS (OSPM) was determined as the weight lost by heating the filters for 3 h at 500°C (loss-on-ignition method), corrected by blank filters equally treated 47 samples had a OSPM slightly larger than TSS (a difference on the order of 0.1 mg in usually light weight samples with less than 1 mg L$^{-1}$) and in these cases OSPM values were decreased to match TSS.

Phytoplankton taxonomy

Phytoplankton taxonomy was quantified following Utermöhl’s sedimentation method (Lund et al. 1958) with a Zeiss Axiovert 40 CFL inverted microscope at $\times250$ and $\times500$ magnifications. A minimum of 400 units were counted; these being single-celled individuals, filaments, or colonies depending on the organization of the algae. Weight biomass was calculated from recorded abundance and specific volume estimates, based on geometric solids (Rott 1981).

Laboratory measurement methodology and absorption coefficient calculation

Surface water was collected in dark containers at the deepest station of the lakes (664 lakes visited, depth ranging from $1 \times 10^{2}$ to more than $1 \times 10^{3}$ m and areas from $0.1 \times 10^{2}$ to $266 \times 10^{3}$ km$^{2}$) as part of the NSERC Canadian Lake Pulse Network field campaign (see Huot et al. 2019 for details on lake selection and NSERC Canadian Lake Pulse Network 2021 for the field sampling protocols) which occurred over three summers (2017, 2018, and 2019).

Collected water was filtered (two filter duplicates per water sample were analyzed for 101 lakes, one for all other lakes) in dim light in temporary (tents) laboratories on the lake’s shore using 25 mm GF/F filters (Whatman). The filters were immediately stored at $-80°C$ in individual plastic petri dishes. Volume to be filtered was determined from the Secchi depth (see Table 2; see Supporting Information Appendix S1 for effectiveness of the method) to obtain absorbance values that are within appropriate range ($0.1–0.5$) for the integrating sphere method (Rotting and Gehnke 2012). Blank filters were also collected by filtering deionized water occasionally during the sampling campaign. Frozen samples were shipped on dry ice to the laboratory for analysis.

In the laboratory, filters were thawed under dim light at room temperature for 5 min and remoistened by placing filters on top of a drop of deionized water (MilliQ® system) on aluminum covered petri dishes to minimize pigment degradation (Stramski 1990). The diameter of the filtration area was measured with a vernier caliper on select filters to ensure consistency (identical filtration kits were used for all filtration). Absorbance ($A$, unitless) scans from 800 to 300 nm (only the 800–400 nm range was kept for this study) were performed in a PerkinElmer’s LAMBDA™ 650 UV/Vis spectrophotometer equipped with a 150-mm integrating sphere with a center-mounted sample holder to obtain the total particle absorption spectra ($a_{p}$). New filters moistened with deionized water were used for the baseline and reference. After the scan, samples were transferred to filtration racks where they were washed by adding 5 mL of absolute methanol which was immediately pumped under low vacuum to remove moisture. Another 5 mL of methanol were added, and the filters were left to soak for 15 min for extraction (Kishino et al. 1985). After the extraction, the methanol was removed by pumping under low vacuum and rinsed with 15 mL of deionized water. Post-extraction filters were scanned in the spectrophotometer. This measurement, after computation of the absorption coefficient and correction for pathlength amplification (see below), will provide the measurement of $a_{\text{SAM}}$. The filters were then bleached by putting a drop ($\sim 200 \mu L$) of sodium hypochlorite (NaOCl, 1% active Cl) in a Petri dish and putting the filter on

Table 2. Filtered volume used for particulate absorption according to the Secchi depth measured at the sampling location.

| Secchi depth (m) | Volume filtered (mL) |
|-----------------|----------------------|
| $< 1$           | 100                  |
| 1–2.4           | 150                  |
| 2.5–3           | 200                  |
| 3–6             | 350                  |
| $> 6$           | 500                  |

Source: NSERC Canadian Lake Pulse Network (2021).
top (Tassan and Ferrari 1995) for 5 min. The filters were rinsed by setting them on top of a drop of deionized water. The filters were scanned again, which will provide $d^b_{\text{NAP}}$.

At least 1 filter was measured from 649 lakes (out of the 664 sampled lakes), which, after including the 78 blanks and 106 duplicate samples, leads to a total of 835 filters analyzed (2505 spectra). The average absorbance of the field blanks of each treatment (untreated, post-extraction, and post-bleaching scans) was computed for each year (6 for 2017, 37 for 2018, and 37 for 2019). The mean blanks were subtracted from each filter scan of the corresponding treatment and year. Two corrections were then applied. First, if the average total particulate absorbance between 750 and 800 nm was negative, that average was subtracted at all wavelengths (10 total particulate filters, 17 post-extracted filters, and 47 post-bleaching filters). Methodologically, this can occur, for example, if the blank filters have higher absorbance compared to the particulate filters: for the filters with mean negative near-infrared absorption, the median value were $-0.026$, $-0.057$, and $-0.016$ for untreated, post-extracted, and post-bleached filters, respectively, while the blanks had a range of $-0.002$ to 0.019, $-0.014$ to 0.020, and $-0.030$ to 0.019, respectively. Drift might be behind the more negative values. Second, a pseudo-null point correction was performed by (1) computing Drift might be behind the more negative values. Second, a pseudo-null point correction was performed by (1) computing $\phi_0$ (Stramski et al. 1999). $\phi_0$ includes the bleachable portion of the NAP.

Three absorption spectra are thus made directly with the spectrophotometer (see Fig. 1B, measurement column): the total particulate ($a_p$); the methanol extraction containing bleachable and non-bleachable non-algal colored material and unextracted pigments ($a^B_{\text{NAP}}$); and the bleached filter which contains the non-bleachable colored material ($a^b_{\text{NAP}}$). From these three basic measurements, three derived primary spectrum were calculated (Fig. 1B, “derived primary” column): (1) the “phytoplankton” absorption obtained from the methanol extraction NAP, $\phi_p = a_p - a^B_{\text{NAP}}$; (2) the “phytoplankton” absorption obtained from the bleached NAP $a_p = a_p - a^b_{\text{NAP}}$; and (3) remaining “bleachable material spectra” $a^U = a^B_{\text{NAP}} - a^b_{\text{NAP}}$, which represents the spectra of the non-extracted pigments and bleachable organic NAP matter. Note that we use quotation mark above when referring to phytoplankton absorption as neither is a good measure of the true phytoplankton in freshwaters as $a^U$ is missing unextracted pigments while $a^b$ includes the bleachable portion of the NAP.

**Differential data processing**

Three different post-processing approaches were used to obtain the best $a_p$ and $a_{\text{NAP}}$. For the 1st (V1), representing the traditional extraction used in oceanography, $a_p$ was assumed equal to $a^B_{\text{NAP}}$ and $a_{\text{NAP}}$ to $a^b_{\text{NAP}}$. For the 2nd (V2), using the bleaching procedure, $a_p$ was assumed equal to $a^b$ and $a_{\text{NAP}}$ to $a^B_{\text{NAP}}$, and the effects of the intermediate extraction were assumed to have a little impact in comparison to a direct bleaching. This assumption is supported by the equality of residual spectra obtained by both methods applied in parallel and sequentially in cultures without unextractable pigments (such as chromophyte algae) shown by Ferrari and Tassan (1999).

A 3rd method (V3), incorporating both extraction and bleaching with a series of numerical processing steps to account for the biases inherent to the treatments, was developed and the steps are described below.

The processing for the V3 method can be described as a series of four steps (numbers refer to the numbers on Fig. 1):

1. Compute $a^U$ by subtracting $a^B_{\text{NAP}}$ from $a^b_{\text{NAP}}$, as described above.
2. Isolation of unextracted Chl $a$ spectra. First, to remove the noise (which might heavily affect the next step), the $a^U$ spectra were passed through a Savitzky–Golay smoothing filter (Savitzky and Golay 1964, sgolayfilt in R library signal) with a bandwidth of 31 nm and using a 3rd-degree polynomial function. Second, a continuum removal function (method of Clark and Roush 1984, as implemented in the continuum Removal function of the R package spectrap) was applied between 650 and 750 nm, and the resulting peak was retrieved. The shorter waveband at 650 nm was chosen to avoid the plateauing/downward part of the phyocyanin absorption peak at 620 nm. A pure Chl $a$
(\(a_{\text{Chla}}\)) absorption spectra in methanol (Taniguchi and Lindsey 2021) was multiplied and shifted so the Chl \(a\) Q-band peak (more precisely the \(Q_p\) band, the red absorption peak of chlorophylls which in phytoplankton is almost entirely attributable to Chl \(a\), Roesler and Barnard 2013) matches the absorption value and maximum wavelength of the retrieved peak, as:

\[
a^U_{\text{Chla}}(\lambda) = a_{\text{Chla}}\left(\lambda + (666 - \lambda^U_{Q \text{-band}})\right) \times a^U_{Q \text{-band}}(\lambda),
\]

where \(a^U_{\text{Chla}}(\lambda)\) is the unextracted Chl \(a\) spectra at wavelength \(\lambda\); \(a_{\text{Chla}}\) is the absorption of Chl \(a\) in methanol at wavelength \(\lambda\) shifted by the difference between the Q-band (longer wavelength) peak wavelength of the pure Chl \(a\) in methanol (\(\lambda_{\text{Chla}} = 666\)) and the unextracted bleached fraction Q-band estimated peak wavelength (\(\lambda^U_{Q \text{-band}}\), \(\lambda^U_{Q \text{-band}}\)) and \(a^U_{Q \text{-band}}\) is the Q-band peak height of the same fraction. The resulting adjusted and wavelength-shifted Chl \(a\) spectrum (\(a^U_{\text{Chla}}\)). If the maximum wavelength of the isolated peak was below 660 nm or above 700 nm, \(a^U_{\text{Chla}}\) was considered 0.

3. Isolation of unextracted phycocyanin spectra. The \(a^U_{\text{Chla}}\) were subtracted from \(a^U\). On the resulting spectra, a Savitzky–Golay smoothing filter (similar as in step 2) was applied, and a continuum removal function was applied between 750 and 450 nm, to isolate the unextracted phycobilins absorption (\(a^U_{\text{PB}}\)).

Correction of \(a^E_{\text{NAP}}\) for unextracted pigments. The \(a^U_{\text{Chla}}\) and \(a^U_{\text{PB}}\) were subtracted from \(a^E_{\text{NAP}}\) to obtain the corrected \(a_{\text{NAP}}\), referred to as \(a_{\text{NAP}}^{\text{corr}}\). This \(a_{\text{NAP}}^{\text{corr}}\) was then subtracted from \(a_{\phi}\) spectra to obtain the \(a_{\phi}\) for the V3 method: \(a_{\phi}^{\text{corr}}\).

The three resulting \(a_{\phi}\) spectra were null-point corrected between 750 and 800 nm (near-infrared [NIR]), and this null-point absorption was added to the respective \(a_{\phi}\), since \(a_{\phi}\) does not have significant absorption in this range, and natural suspended particles have a NIR absorption of yet unknown source (Röttgers et al. 2014; Utschig and Röttgers 2020). For the 758 non-blank samples, the 750–800 nm mean values after blank removal were negative for only 8, 12, and 12 spectra for \(a_{\phi}\), \(a^E_{\text{NAP}}\), and \(a^E_{\phi}\), respectively. For around 80 \(a_{\phi}\) measurements, NIR absorption was lower than the corresponding \(a^E_{\text{NAP}}\). The choice of baseline addition instead of scaling the spectra is mentioned in the Supporting Information Appendix S1.

Quality control and outlier removal

A set of quality control tests were applied to flag bad spectra based on spectral shape properties and outlier tests based on other variables measured in LakePulse tied to equivalent IOPs. Two specific absorptions were 1st calculated. First, the Chl \(a\)-specific absorption coefficient (\(a^E_{\phi}\) in \(m^2 \text{mg}^{-1}\)) was calculated as the \(a^E_{\phi}\) (mean when there were duplicates) divided by the Chl \(a\) concentration.

Second, the mean TSS (two duplicates) were used to calculate TSS-specific absorption coefficient of NAP (\(a^E_{\text{NAP}}\) (443) \(m^2 \text{g}^{-1}\)), calculated as the mean lake \(a_{\text{NAP}}\) divided by the TSS weight. Outlier tests were performed on \(a^E_{\text{NAP}}\) (443).

Individual spectra were assigned with one or more of these six flags. Three flags relating to the shape of \(a_{\phi}\) specifically: (flag #1) \(a_{\phi}\) (676) being higher than \(a^E_{\text{NAP}}\) (443); (flag #2) \(a_{\phi}\) with substantial negative absorption (defined as > 10% of the maximum in the 400–700 nm range); and (flag #3) high NIR absorption in \(a_{\phi}\) (defined as \(a_{\phi}\) (790) > 1/3 \(a_{\phi}\) (676)). One flag (flag #4) duplicates with coefficient of variation > 50%. One flag (flag #5) testing for outliers using multiple criteria: 1.5 inter-quartile range, Rosner’s test of multiple outliers (Rosner 1975), and absolute Z-score > 2 were performed on \(a^E_{\phi}\) (443) and \(a^E_{\phi}\) (676), if any of these outlier tests was positive, flag #6 was added (3 for each \(a^E_{\phi}\) wavelength) and the specific positive outlier tests were attached as comments for further consideration. The same three outlier tests of flag #5 were performed on \(a^E_{\text{NAP}}\) (443) as flag #7, and comments were added to the spectra. If a filter had two or more flags the data was discarded. If the filter had flag #4, and one of the duplicates had no other flags while the other had one or more, the bad duplicate was discarded. If the filters were flagged as outliers, but the duplicates were coincident (no flag 4) and no shape anomaly was flagged, the filters were kept. Only seven filters were discarded from further analysis, two of them had a duplicate that was kept. Four additional tests were applied directly to the post-processed spectra with direct elimination: (1) to flag bad extractions, if the minimum value of \(a^E_{\phi}\) in the range 650–400 nm was more negative than 0.1 * \(a^E_{\phi}\) (676), the filter was eliminated (6 filters, 2 with a duplicate that was kept); (2) to eliminate bad extractions (loss of NAP material with the extraction), if the minimum value of \(a^E_{\phi}\) in the range 650–400 nm was larger than 0.75 * \(a^E_{\phi}\) (676), the filter was eliminated (4 filters); (3) to exclude filters where the filtered volume in the field was too high for pathlength correction, if the \(a_{\phi}\) (620) multiplied by the filtered volume (in mL) was higher than 250, the filter was eliminated (1 filter). After eliminating the flagged spectra, we estimated the averages, standard deviations (SD), and the coefficient of variation (100*SD/mean) of the \(a_{\text{NAP}}\) and \(a_{\phi}\) fractions for each lake when sample duplicates were measured, giving a total of 636 lake spectra.

The three methods for partitioning the absorption coefficient were evaluated by examining the relationship between the retrieved absorption coefficients by each method and variables such as Chl \(a\) concentration and TSS. To evaluate the changes in shape between treatments, two analyses were performed: First, the correlation between the organic fraction of
suspended material (OSPM%) and $S_{NAP}^{400-700}$ given that is has been shown that the slope increases with the proportion of organic particulate matter across coastal (Babin et al. 2003) and freshwaters (Riddick et al. 2015; Rodrigues et al. 2020; Shang et al. 2021), although in boreal lakes the relationship is either negative (Binding et al. 2008) or non-significant (Yacobi et al. 2015); while Babin et al. (2003) omitted the 400–480 and 620–710 nm ranges to diminish the effect of remaining pigments, we include those regions precisely to visualize said effects. Second, we analyzed the correlation between $a_p(620)/a_p(676)$ ratio (indicator of phycocyanin per Chl $a$, Yacobi et al. 2015) and the cyanobacterial fraction of biovolume (CBV%), given that phycocyanin is well correlated with cyanobacterial biovolume (Randolph et al. 2008).

Assessment and results

The lakes studied had a mean Chl $a$ concentration of 10.3 $\mu$g L$^{-1}$ (range: 0–441.8 $\mu$g L$^{-1}$), TSS of 12.3 mg L$^{-1}$ (range: 0–3241.6 mg L$^{-1}$), and a mean OPSM% of 66.4%. The mean $a_p(443)$ was 0.749 m$^{-1}$ (range: 0.023–45.0 m$^{-1}$; Fig. 2). The mean coefficient of variation for the 101 measured filter duplicates was 6.0% for $a_p$, 7.7% for $a_{E NAP}$, and 13.9% $a_{B NAP}$, showing the fidelity of the sampling, filtration and measuring process. Biases in the extraction method and the bleaching method

![Fig. 2. Measured $a_p$ spectra from 636 lakes across Canada (A) in logarithmic scale and (B) normalized at 443 nm.](image)

![Fig. 3. The $a_{NAP}$ and $a_p$ computed with the three methods. Top row: estimates of $a_{NAP}$ normalized at 443 nm from (A) the extraction method ($a_{E NAP}$, V1); (B) the bleaching method ($a_{B NAP}$, V2); and (C) the new combined method ($a_{Corr NAP}$, V3). Bottom row: estimates of $a_p$ normalized at 676 nm from (D) the extraction ($a_p$, V1); (E) bleaching ($a_p$, V2); and (F) the new combined method ($a_{Corr p}$, V3).](image)
appear as differences in the absorption spectral shapes and amplitude of the different methods. The presence of unextracted pigments is evident in many of the normalized $a_{E\text{NAP}}$ where the phycocyanin and unextracted Chl a absorption signatures superpose onto the near exponential nature of $a_{E\text{NAP}}$ (Fig. 3A). This is supported by $a_{E\phi}^b$ (676) being on average 12.1% lower than $a_{E\phi}^r$ (676) (Fig. 4B). However, part or all of the higher 676 nm absorption obtained by the bleaching procedure comes from the bleaching of non-algal organic material. The bleaching of organic non-algal material is evidenced by an 11.5% average decrease of $a_{B\text{NAP}}$ (443) relative to $a_{E\text{NAP}}$ (443) (Fig. 4A), or for a less phytoplankton impacted wavelength (560 nm) by an average reduction of $a_{B\text{NAP}}$ (560) of 22.1%, and by a 13.5% mean reduction of $\text{S}_{\text{NAP}}^{400-700}$ (Fig. 4C) by bleaching relative to extraction. By analyzing a set of 3 filters with very low Chl a (< 1.0 μg L⁻¹), where more than 90% of the absorption at 443 nm is attributable to NAP, we found that the reduction in absorption at 443 nm in comparison with $a_{p}$ was 6.2% for $a_{E\text{NAP}}^r$, 19.3% for $a_{B\text{NAP}}^r$, and 6.5% for $a_{\text{Corr}\text{NAP}}^r$; while the change in the 400–700 nm slope (in absolute value) in comparison to $a_{p}$ was 5.6% for $a_{E\text{NAP}}^r$, 16.6% for $a_{B\text{NAP}}^r$, and 7.9% for $a_{\text{Corr}\text{NAP}}^r$. This demonstrates that the overcorrection of the bleaching step is recovered in the secondary processing steps (see Fig. 1).

Regarding the primary spectral differences between $a_{E\text{NAP}}^r$ and $a_{B\text{NAP}}^r$, the $a_U$ spectra show a decreasing exponential with wavelength reflecting the oxidation of at least a fraction of the organic matter on which phycobilins and unextracted chlorophylls are superimposed (Fig. 5B). The ratio of the Q-band absorption between the unextracted and the extracted Chl a, $a_{U\text{Chl a}}(669) : a_{\text{Corr} \phi}(676)$, ranged from 0 to 0.79 (mean ± SD = 0.044 ± 0.07, Fig. 5C). After the isolated unextracted Chl

![Fig. 4](https://via.placeholder.com/150)

**Fig. 4.** Relative differences in absorption coefficient across treatments; (A) relative difference between the non-algal particles by extraction ($a_{E\text{NAP}}$) and bleaching ($a_{E\text{NAP}}^b$) and (D) between extraction ($a_{E\text{NAP}}$) and the combined method ($a_{\text{Corr}\text{NAP}}$) at 443 nm. (B) Relative difference between the phytoplankton fraction by bleaching ($a_{\phi}^b$) and extraction ($a_{\phi}^r$) and (E) between bleaching ($a_{\phi}^b$) and the combined method ($a_{\text{Corr} \phi}$) at 676 nm. (C) Relative difference between the log of the non-algal particles absorption slope by extraction ($\text{S}_{\text{NAP}}^{400-700}$) and bleaching ($\text{S}_{\text{B\text{NAP}}}^{400-700}$), and (F) between extraction ($\text{S}_{\text{E\text{NAP}}}^{400-700}$) and the combined method ($\text{S}_{\text{Corr\text{NAP}}}^{400-700}$).
a was fitted, the remaining unextracted pigments (Fig. 5D), isolated by continuum removal, shows the shape and expected peaks of phycocyanins, with some rare cases where phycoerythrins might be the dominant phycobiliprotein (Ficek et al. 2004).

The new, combined method produces $a_{NAP}$ estimates that do not show the spectral signature of unextracted pigments (Fig. 5C), while keeping an almost unchanged amplitude with respect to $a_{NAP}^E$ (443) by having a mean reduction of only 1.7% (7.4% for $a_{NAP}^E$ [560]) in comparison to the extraction method (Fig. 4D). This reduction, although overall small, is higher than 0 due to the removal of unextracted Chl $a$, which for some filters reached more than 20% of the total unextracted 443 nm absorption (Fig. 4D). On the other hand, the distribution of the differences between $a_{φ}^B$ (676) and $a_{φ}^{Corr}$ (676) show a very high frequency of no difference (0%) but on average $a_{φ}^{Corr}$ (676) was 5.9% smaller (Fig. 4E), showing the actual bleached organic non-algal material present in $a_{φ}^B$ (676) since the continuum removal used to isolate the phycobilins isolates the pigment peaks found in that range and excludes the NAP background.

The differences in values among the three methods for $a_{φ}^*(676)$ and $a_{NAP}^*(443)$ were small in comparison with the variation between lakes and compared well with other studies, showing similar values to eutrophic lakes (Sun et al. 2010; Xue et al. 2017). The values were significantly larger than their marine (Bowers et al. 1996; Babin et al. 2003) counterparts or cultures of freshwater phytoplankton (Table 3).

Linear regressions between (1) the organic percentage of suspended solids (OSPM, %, Fig. 6, top row) and $S_{NAP}^{400—700}$; and (2) between $a_{φ}^B(620)/a_{φ}^*(676)$ and the cyanobacterial fraction of biovolume (CBV%, Fig. 6, bottom row) were fitted to check which method better reflects the lakes' physiochemistry and biology. For $S_{NAP}^{400—700}$ and OSPM% it was found that, although overall they are poorly correlated, bleaching ($R^2 = 0.111$) and the new combined method ($R^2 = 0.109$) have a higher coefficient of determination than extraction ($R^2 = 0.053$), while for $a_{φ}^B(620)/a_{φ}^*(676)$ and CBV%, extraction ($R^2 = 0.333$) and the new combined method ($R^2 = 0.346$) were better than bleaching ($R^2 = 0.096$). The root mean square error (RMSE) for fitted $S_{NAP}^{400—700}$ vs. OSPM% and $a_{φ}^B(620)/a_{φ}^*(676)$ vs. CBV% linear regressions, also supports this, with the new combined

![Fig. 5. Intermediate processing absorption coefficient spectra products for the proposed method (V3). (A) Total unextracted, bleached fraction ($a^U$) as a percentage of $a_{NAP}^E$ at the same wavelength, (B) $a^U$ normalized by $a_{NAP}^E$ (443), (C) unextracted Chl $a$ ($a^U_{Chl}$), and (D) unextracted phycobilins ($a^U_{PB}$) normalized by the extracted total pigment absorption ($a_{φ}^E$) at 676 nm.](image-url)
method having a lower RMSE than the other methods for OSM% (RMSE = 0.00139), and slightly higher than extraction for CBV% (RMSE = 0.105 in comparison with extraction RSME, 0.093). These improvements, although mild (considering most spectra will not have significant differences across the three methods), support the idea that the combined methods have a strong specificity and produce spectral fractions that represent better the underlying water constituents especially when chlorophytes or cyanobacteria are dominant.

**Discussion, comments, and recommendations**

The issues of correct assignment of unextracted pigments to \( a_\phi \) and correct assignment of bleachable NAP to \( a_{NAP} \) have been recognized in the literature (IOCCG Protocol Series 2018), but to our knowledge no practical methods were available. One solution for these issues was to fit an exponential function to \( a_{NAP} \) by excluding the spectral range where pigments are absorbing (i.e., 400–480 and 620–710 nm ranges; Babin et al. 2003). However, applying this method to our freshwater data produced 15 spectra with negative \( a_\phi \) (676) and 120 spectra with negative \( a_\phi \) (620) and therefore was not explored further. This is likely due to a higher complexity of \( a_{NAP} \) spectral shape in freshwaters (due to previously mentioned iron oxides and unextracted pigments) in comparison to marine equivalents.

The cut-offs used to estimate the filtered volume to obtain absorbances in the optimal range of the pathlength amplification correction (0.1–0.5; Rötting and Gehnke 2012), were on average successful, with a new suggested category for Secchi depths <1 m where the volume filtered should be 50 mL rather than 100 mL as adopted during LakePulse (see Supporting Information Appendix S1).

The presence of a chlorophyll Q-band is evident in the \( a_{NAP} \) spectra (443) (mean ± SD)

\[
\begin{array}{ccc}
\text{Dataset} & a_{NAP} (443) (\text{m}^2 \text{g}^{-1}) & a_{\phi} (676) (\text{m}^2 \text{mg}^{-1}) & \text{Source} \\
\hline
\text{V1 (E, extraction)} & 0.113 ± 0.084 & 0.026 ± 0.010 & \text{Lakepulse V1} \\
\text{V2 (B, bleaching)} & 0.101 ± 0.082 & 0.031 ± 0.014 & \text{Lakepulse V2} \\
\text{V3 (Corr, corrected extraction)} & 0.111 ± 0.084 & 0.028 ± 0.011 & \text{Lakepulse V3} \\
\text{Open ocean} & 0.009–0.035 & 0.0288 & \text{Bricaud et al. (1995)} \\
\text{Shallow eutrophic lake} & 0.095±,† & 0.147 & \text{Sun et al. (2010)} \\
\text{Shallow eutrophic lake (spring)} & 0.149 ± 0.147 & 0.021 ± 0.008 & \text{Xue et al. (2017)} \\
\text{Shallow eutrophic lake (summer)} & 0.091 ± 0.030 & 0.018 ± 0.007 & \text{Xue et al. (2017)} \\
\text{Freshwater cultures} & 0.016 ± 0.004 & 0.010 ± 0.002 & \text{Cremella et al. (unpubl.)} \\
\text{Marine cultures} & 0.0265* & \text{Giardino et al. (2017)} \\
\text{Three lakes (oligotrophic to eutrophic)} & 0.031 & \text{Babin et al. (2003)} \\
\text{Coastal ocean} & 0.0235 & \text{Bowers et al. (1996)} \\
\end{array}
\]

*440 nm.
†Assuming no Chl \( a \).

Table 3. Comparison of total suspended solids specific absorption coefficient \( a_{NAP} \) (443) (m^2 g^-1) and the Chl \( a \) specific absorption coefficient \( a_{\phi} \) (676) (m^2 mg^-1) from this study and the literature. It includes the three methods of this work: non-algal particles absorption fraction by extraction (\( a_{NAP}^E \), V1), bleaching (\( a_{NAP}^B \), V2) and the new combined method (\( a_{NAP}^{Corr} \), V3). Phytoplankton absorption fraction by extraction (\( a_{\phi}^E \), V1), bleaching (\( a_{\phi}^B \), V2), and the new combined method (\( a_{\phi}^{Corr} \), V3). For \( a_{NAP} \) and TSS, values provided are from Giardino et al. (2017) and Mishra et al. (2017) using log \( a_{NAP} \) (440) = 0.0265 log TSS (\( R^2 = 0.65 \)); Sun et al. (2010) using \( a_{NAP} \) (443) = 0.095 (TSS = 0.07 Chl\( \alpha \)); Xue et al. (2017) using the \( a_{NAP} \) (443) values from the summer and spring; Babin et al. (2003) using \( a_{NAP} \) (443) = 0.031 TSS (\( R^2 = 0.80 \)); and Bowers et al. (1996) using \( a_{NAP} \) (443) = 0.0235 TSS. For \( a_{\phi} \) and Chl \( a \), we compared it against Sun et al. (2010) using \( a_{\phi} \) (676) = 0.0288 Chl\( \alpha \); Xue et al. (2017) using the \( a_{\phi} \) (675) values from the summer and spring; Bricaud et al. (1995) using \( a_{\phi} \) (676) = 0.009 Chl\( \alpha \) as the lower bound and \( a_{\phi} \) (676) = 0.035 Chl\( \alpha \) as the upper bound; Marie-Rose Vandenhecke et al. (2015) from cultures of marine phytoplankton species and Cremella et al. (unpubl.) from cultures of freshwater phytoplankton species.
Taniguchi and Lindsey (2021); (2) the 2nd-derivative spectra showing low to no spectral features of other pigments (Fig. 7); and (3) the absorption of NaOCl and bleached biomass in the 400–450 nm range (Ferrari and Tassan 1999) impedes the application of continuum removal functions to \( a_U \) in that range. The application of such corrections on \( a_U \) also avoids the interference of the continuum removal function on the \( \sim 500 \text{ nm} \) shoulder observed commonly on mineral NAP (Bowers and Binding 2006). The increase in \( S_{\text{NAP}}^{400-700} \) by using the bleaching process suggests that complex organic material, usually of higher absorption in the visible range and lower exponential slope (Uyguner and Bekbolet 2005; Binding et al. 2008; Helms et al. 2008), is oxidized by the bleaching process. The combination of extraction and bleaching could, in theory, be used to distinguish between organic and inorganic fractions of \( a_4 \), however, in addition to the mentioned NaOCl treatment, the 400–450 nm absorption increase, the level of oxidation of non-algal matter attained by NaOCl is unknown, and therefore should remain as a supporting addition of the extraction method since the oxidation of pigments is known and certain. The retention of the shifted Q-band chlorophylls might be due to extraction resistance on some green algae (Shoaf and Lium 1976; Wood 1985; Ferrari and Tassan 1999; Wiltshire et al. 2000), likely by their cellulose-algaenan cell walls (Baudelet et al. 2017).

The magnitude of the differences between spectra produced by the different methods justifies the application of the new corrections, as the cross-method error is larger than the duplicate error, and, for some samples, the difference between extraction and bleaching calculated fractions can be up to 100% of the smaller estimator. Since these differences are associated with high phycobilins and/or high bleachable non-algal material,
which might indicate underlying human-induced impacts, obtaining the best possible separation will prevent misdiagnosis of lakes based on remote sensing reflectance. The proposed new method was shown to provide a more realistic fractioning both in shape and in magnitude, as supported by the higher correlation coefficient between the 400 and 700 nm log slope (S) of the non-algal particles’ spectra and the organic fraction of the TSS, and by having a better correlation between the ratio between the absorption coefficient of phytoplankton at 620 and 676 nm and cyanobacterial biomass percentage (Fig. 6).

In terms of the logistical and laboratory complexity, this method requires one more filter measurement and 5 min of NaOCl bleaching over the methanol extraction method. This approach is faster than soaking the filters in methanol for a longer period of time to remove more pigments (Bélanger et al. 2013). Although we tested it on an integrating sphere configuration, other filter-pad measurement configurations (transmittance, transmittance-reflectance) will most likely produce similar results.

Overall, this method successfully solves two methodological problems: (1) unextracted pigments with methanol extraction methods and (2) the bleached fraction of the organic non-algal fraction, without introducing biases. Once this improved fractioning method is applied, $a_p$ and $a_{\text{NAP}}$ exhibit more realistic relationships with other underlying measured biogeochemical properties.

Fig. 7. Comparison of the 2nd derivative of chlorophyll containing spectra normalized by Chl a concentration: (A) six cultures collection $a_p$ (five lights × three replicates each, Cremella et al. unpubl.), (B) $a_p$ (this study), (C) $a_U$ (this study) and (D) $a_{\text{Chla}}$ in MeOH (Taniguchi and Lindsey 2021). Dotted lines indicate the Soret band and Q-band mean maxima.

References

Babin, M., D. Stramski, G. M. Ferrari, H. Claustre, A. Bricaud, G. Obolensky, and N. Hoepffner. 2003. Variations in the light absorption coefficients of phytoplankton, non-algal particles, and dissolved organic matter in coastal waters around Europe. J. Geophys. Res. 108: 3211. doi:10.1029/2001JC000882

Baudelet, P. H., G. Ricochon, M. Linder, and L. Muniglia. 2017. A new insight into cell walls of Chlorophyta. Algal Res. 25: 333–371. doi:10.1016/j.algal.2017.04.008

Becker, A., A. Meister, and C. Wilhelm. 2002. Flow cytometric discrimination of various phycobilin-containing phytoplankton groups in a hypertrophic reservoir. Cytom. J. Int. Soc. Anal. Cytol. 48:45–57. doi:10.1002/cyto.10104

Bélanger, S., S. A. Cizmeli, J. Ehn, A. Matsuoka, D. Doxaran, S. Hooker, and M. Babin. 2013. Light absorption and partitioning in Arctic Ocean surface waters: Impact of multiyear ice melting. Biogeosciences 10: 6433–6452. doi:10.5194/bg-10-6433-2013

Bending, C. E., J. H. Jerome, R. P. Bukata, and W. G. Booty. 2008. Spectral absorption properties of dissolved and particulate matter in Lake Erie. Remote Sens. 112: 1702–1711. doi:10.1016/j.rse.2007.08.017

Bowers, D. G., G. E. L. Harker, and B. Stephan. 1996. Absorption spectra of inorganic particles in the Irish Sea and their relevance to remote sensing of chlorophyll. Int. J. Remote Sens. 17: 2449–2460. doi:10.1080/01431169608948782

Bowers, D. G., and C. E. Binding. 2006. The optical properties of mineral suspended particles: A review and synthesis. Estuar. Coast. Shelf Sci. 67: 219–230. doi:10.1016/j.ecss.2005.11.010

Bricaud, A., and D. Stramski. 1990. Spectral absorption coefficients of living phytoplankton and nonalgal biogenic matter: A comparison between the Peruvian upwelling area and
O’Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14: 313–334. doi:10.1016/j.hal.2011.10.027

Pahlevan, N., and others. 2020. Seamless retrievals of chlorophyll-a from Sentinel-2 (MSI) and Sentinel-3 (OLCI) in inland and coastal waters: A machine-learning approach. Remote Sens. Environ. 240: 111604. doi:10.1016/j.rse.2019.111604

Pahlevan, N., and others. 2021. ACIX-Aqua: A global assessment of atmospheric correction methods for Landsat-8 and Sentinel-2 over lakes, rivers, and coastal waters. Remote Sens. Environ. 258: 112366. doi:10.1016/j.rse.2021.112366

Randolph, K., J. Wilson, L. Tedesco, L. Li, D. L. Pascual, and E. Soyeux. 2008. Hyperspectral remote sensing of cyanobacteria in turbid productive water using optically active pigments, chlorophyll a and phycocyanin. Remote Sens. Environ. 112: 4009–4019. doi:10.1016/j.rse.2008.06.002

Reynolds, C. S. 1997. Vegetation processes in the pelagic: A model for ecosystem theory. Ecology Institute Oldendorf.

Reynolds, R. A., and D. Stramski. 2019. Optical characterization of marine phytoplankton assemblages within surface waters of the western Arctic Ocean. Limnol. Oceanogr. 64: 2478–2496.

Riddick, C. A. L., P. D. Hunter, A. N. Tyler, and others. 2015. Spatial variability of absorption coefficients over a biogeochemical gradient in a large and optically complex shallow lake. J. Geophys. Res. Ocean. 120: 7040–7066. doi:10.1002/2015JC011202

Rigosi, A., C. C. Carey, B. W. Ibelings, and J. D. Brookes. 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnol. Oceanogr. 59: 99–114. doi:10.4319/lo.2014.59.1.0099

Rodrigues, T., E. Alcantara, L. Rotta, N. Bernardo, and F. Watanabe. 2020. An investigation into the relationship between light absorption budget and trophic status in inland waters. Ecol. Indic. 115: 106410. doi:10.1016/j.ecolind.2020.1064107066.

Roessler, C. S., and M. J. Perry. 1995. In situ phytoplankton absorption, fluorescence emission, and particulate backscattering spectra determined from reflectance. J. Geophys. Res. Ocean. 100: 13279–13294. doi:10.1029/95JC00455

Roessler, C. S., and A. H. Barnard. 2013. Optical proxy for phytoplankton biomass in the absence of photophysiology: Rethinking the absorption line height. Methods Oceanogr. 7: 79–94. doi:10.1016/j.mio.2013.12.003

Rosner, B. 1975. On the detection of many outliers. Dent. Tech. 17: 221–227. doi:10.1080/00401706.1975.10489305

Rott, E. 1981. Some results from phytoplankton counting intercalibrations. Schweiz. Z. Hydrol. 43: 34–62. doi:10.1007/BF02502471

Röttgers, R., and S. Gehnke. 2012. Measurement of light absorption by aquatic particles: Improvement of the quantitative filter technique by use of an integrating sphere approach. Appl. Optics 51: 1336–1351. doi:10.1364/AO.51.001336

Röttgers, R., C. Dupouy, B. B. Taylor, A. Bracher, and S. B. Woźniak. 2014. Mass-specific light absorption coefficients of natural aquatic particles in the near-infrared spectral region. Limnol. Oceanogr. 59: 1449–1460. doi:10.4319/lo.2014.59.5.1449

Savitzky, A., and M. J. E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. Anal. Chem. 36: 1627–1639. doi:10.1021/ac60214a047

Schindler, D. W. 2006. Recent advances in the understanding and management of eutrophication. Limnol. Oceanogr. 51: 356–363. doi:10.4319/lo.2006.51.1_part_2.0356

Seppälä, J., P. Ylöstalo, and H. Kuosa. 2005. Spectral absorption and fluorescence characteristics of phytoplankton in different size fractions across a salinity gradient in the Baltic Sea. Int. J. Remote Sens. 26: 387–414. doi:10.1080/01431160410001723682

Shang, Y., P.-A. Jacinthe, L. Li, and others. 2021. Variations in the light absorption coefficients of phytoplankton, non-algal particles and dissolved organic matter in reservoirs across China. Environ. Res. 201: 111579. doi:10.1016/j.envres.2021.111579

Shoa, W. T., and B. W. Lium. 1976. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. Limnol. Oceanogr. 21: 926–928. doi:10.4319/lo.1976.21.6.0926

Simis, S. G. H., S. W. M. Peters, and H. J. Gons. 2005. Remote sensing of the cyanobacterial pigment phycocyanin in turbid inland water. Limnol. Oceanogr. 50: 237–245. doi:10.4319/lo.2005.50.1.0237

Simis, S. G. H., and H. M. Kauko. 2012. In vivo mass-specific absorption spectra of phycobilin pigments through selective bleaching. Limnol. Oceanogr. Methods 10: 214–226. doi:10.4319/mom.2012.10.214

Stramski, D., M. Babin, and S. B. Woźniak. 2007. Variations in the optical properties of terrigenous mineral-rich particulate matter suspended in seawater. Limnol. Oceanogr. 52: 2418–2433. doi:10.4319/lo.2007.52.6.2418

Stramski, D., S. Kaczmarek, R. A. Reynolds, J. Uitz, and G. Zheng. 2015. Correction of pathlength amplification in the filter-pad technique for measurements of particulate absorption coefficient in the visible spectral region. Appl. Optics 54: 6763–6782. doi:10.1364/ao.54.006763

Stramski, D. 1990. Artifacts in measuring absorption spectra of phytoplankton collected on a filter. Limnol. Oceanogr. 35: 1804–1809. doi:10.4319/lo.1990.35.8.1804

Sun, D., Y. Li, Q. Wang, H. Lv, C. Le, C. Huang, and S. Gong. 2010. Partitioning particulate scattering and absorption into contributions of phytoplankton and non-algal particles in winter in Lake Taihu (China). Hydrobiologia 644: 337–349. doi:10.1007/s10750-010-0198-7

464
Taniguchi, M., and J. S. Lindsey. 2021. Absorption and fluorescence spectral database of chlorophylls and analogues. Photochem. Photobiol. 97: 136–165. doi:10.1111/php.13319

Tassan, S., and G. M. Ferrari. 1995. An alternative approach to absorption measurements of aquatic particles retained on filters. Limnol. Oceanogr. 40: 1358–1368. doi:10.4319/lo.1995.40.8.1358

Utschig, C., and R. Röttgers. 2020. Mass-specific light absorption coefficients of mineral particles in aqueous suspension for the ultraviolet to near-infrared radiation spectral region (200–2500 nm). Appl. Optics 59: 10554–10564. doi:10.1364/AO.393289

Uyguner, C. S., and M. Bekbolet. 2005. Evaluation of humic acid photocatalytic degradation by UV–vis and fluorescence spectroscopy. Catal. Today 101: 267–274. doi:10.1016/j.cattod.2005.03.011

Vaehaetalo, A. V., R. G. Wetzel, and H. W. Paerl. 2005. Light absorption by phytoplankton and chromophoric dissolved organic matter in the drainage basin and estuary of the Neuse River, North Carolina (USA). Freshw. Biol. 50: 477–493. doi:10.1111/j.1365-2427.2004.01335.x

Vasconcelos, V. 2006. Eutrophication, toxic cyanobacteria and cyanotoxins: When ecosystems cry for help. Limnetica 25: 425–432. doi:10.23818/limn.25.30

Vinebrooke, R. D., and P. R. Leavitt. 1999. Phytobenthos and phytoplankton as potential indicators of climate change in mountain lakes and ponds: A HPLC-based pigment approach. J. North Am. Benthol. Soc. 18: 15–33. doi:10.2307/1468006

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnol. Oceanogr. 39: 1985–1992. doi:10.4319/lo.1994.39.8.1985

Wiltshire, K. H., M. Boersma, A. Möller, and H. Buhtz. 2000. Extraction of pigments and fatty acids from the green alga Scenedesmus obliquus (Chlorophyceae). Aquat. Ecol. 34: 119–126. doi:10.1023/A:1009911418606

Wood, L. W. 1985. Chloroform–methanol extraction of chlorophyll a. Can. J. Fish. Aquat. Sci. 42: 38–43. doi:10.1139/f85-005

Xi, H., M. Hieronymi, R. Röttgers, H. Krasmann, and Z. Qiu. 2015. Hyperspectral differentiation of phytoplankton taxonomic groups: A comparison between using remote sensing reflectance and absorption spectra. Remote Sens. (Basel) 7: 14781–14805. doi:10.3390/rs7114781

Xue, K., Y. Zhang, H. Duan, and R. Ma. 2017. Variability of light absorption properties in optically complex inland waters of Lake Chaohu. China. J. Great Lakes Res. 43: 17–31. doi:10.1016/j.jglr.2016.10.006

Yacobi, Y. Z., J. Köhler, F. Leunert, and A. Gitelson. 2015. Phytocyanin-specific absorption coefficient: Eliminating the effect of chlorophylls absorption. Limnol. Oceanogr. Methods 13: 157–168.

Zheng, G., and P. M. DiGiacomo. 2018. Detecting phytoplankton diatom fraction based on the spectral shape of satellite-derived algal light absorption coefficient. Limnol. Oceanogr. 63: S85–S98.

Acknowledgments

The authors are grateful to the NSERC Canadian Lake Pulse Network and its contributors at the coordination, sampling, and analysis levels. We also thank Geneviève Potvin for numerous exchanges regarding particulate absorption and CDOM and Anaïs Oliva for measuring the TSS. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Canadian LakePulse Network, BC was partially funded by the Fonds de recherche du Québec—Nature et technologies (FRQNT) GRIL Program for Regular Collaborative Projects: GRIL-PCR-18E03.

Submitted 28 December 2021
Revised 08 April 2022
Accepted 13 May 2022

Associate editor: Ivona Cetinic