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Spectrofluorophotometer, UV-Vis Spectrophotometer

Application News

Measuring Chlorophyll *a* with a Spectrofluorophotometer

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User Benefits

- The seawater concentration of chlorophyll *a* or total chlorophyll *a* can be measured by fluorometry with sample pretreatment.[1)]
- When measuring chlorophyll *a*, fluorescence measurements with a spectrofluorophotometer give higher signal intensities, better sensitivity, and can detect lower concentrations compared with absorbance measurements using a spectrophotometer.

■ Introduction

The oceans are inhabited by a variety of organisms, each of which is located on an ecological pyramid. Positioned at the very bottom of this ecological pyramid is a class of organisms called phytoplankton. Phytoplankton are mainly found close to the surface in coastal zones fed by rivers, and are less common in non-coastal tropical or subtropical zones.[2)] Phytoplankton are an important indicator organism as they provide us with information about the lowest strata of the marine ecosystem. The amount of phytoplankton in seawater can be determined by measuring the concentration of chlorophyll.[1)]

The Guideline of Ocean Observations (The Oceanographic Society of Japan)[1)] describes several methods for measuring chlorophyll, which it classifies as an algal pigment. This article describes measuring the amount of chlorophyll in various water samples using the fluorometric method presented in these guidelines. This fluorometric method is also compared with an absorption spectrophotometry method for measuring chlorophyll that is also presented in the guidelines.

Fig. 1 RF-6000 Spectrofluorophotometer

■ Sample Collection and Pretreatment

Samples were collected from two locations: close to the mouth of the Tama River (Tokyo), and next to Daishi Bridge, which is a bridge on the Tama River 2.4 km upstream from the first sample collection point (Fig. 2). Samples were also collected under two different climatic conditions: on a day of good weather, and after a typhoon. The samples were analyzed at the Shimadzu Tokyo Innovation Plaza (STIP), which is located near the first collection point.

Samples were prepared for analysis by filtering under suction through a glass fiber filter (diameter: 47 mm, pore size: 1.0 µm).*1 After filtration, 10 mL of an organic solvent (DMF, methanol, or 90 % acetone) was used to transfer the filter residue to a container, which was then left in a cold dark place (-20 °C) for a period of time (typically 24 hours) to allow extraction to occur.

*1 Guidelines require filtration of around 50 to 200 mL for coastal waters, and around 200 to 300 mL for oceanic waters. 200 mL was filtered in this study.

Fig. 2 Sample Collection Points

■ Absorbance Measurements with a Spectrophotometer

After extraction, the resulting supernatant samples were analyzed using a UV-2600i Plus spectrophotometer under the conditions shown in Table 1. The resulting absorbance spectra are shown in Fig. 3. Each solvent was also used as a control (blank).

Table 1 Measurement Conditions

Instrument:	UV-2600i Plus
Photometric Data:	Absorbance
Measured Wavelength Range:	350 to 850 nm
Sampling Interval:	1 nm
Scanning Speed:	Medium
Slit Width:	1 nm

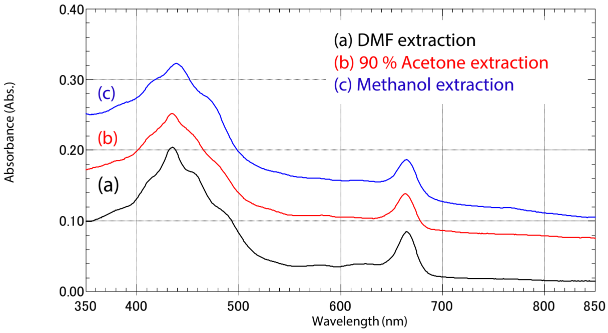

Fig. 3 Absorbance Spectra of Extracted Samples (good weather, river mouth)
(A) DMF extraction, (b) 90 % acetone extraction, (c) methanol extraction

A chlorophyll *a* signal (absorbance) was recorded at 663 to 665 nm. Chlorophyll *a* concentration was calculated by subtracting the absorbance at 750 nm from the absorbance at 663 to 665 nm (difference).[1] This method was used to calculate the concentration of chlorophyll *a* in each of the samples. The results of these measurements are shown in Table 2. Note that chlorophyll *a* does not produce an absorbance signal at 750 nm, and the absorbance signal at 750 nm is assumed to be created by light scatter from impurities in the extracted sample.

Although DMF is considered the most effective of the three solvents at chlorophyll *a* extraction, the results obtained in this study (Table 2) did not always match this assumption. The chlorophyll *a* levels calculated in each solvent extract were not proportional across the samples, but exhibited a degree of variation. This suggests a high absorbance signal at 750 nm and that light scatter is having a major impact on the readings. In these circumstances, low absorbance signals for chlorophyll could result in large errors. This indicates that appropriate sample standing times and centrifugation conditions are needed to ensure useful absorbance data.[3] Small shifts in absorbance peaks are also apparent between the extraction solvents.

Table 2 Chlorophyll *a* Absorbance Measurements (663–665 nm - 750 nm)

	River Mouth		Upstream Bridge	
	Good weather	After typhoon	Good weather	After typhoon
DMF Extraction	0.066	0.028	0.025	0.063
90 % Acetone Extraction	0.057	0.025	0.022	0.052
Methanol Extraction	0.068	0.039	0.048	0.058

■ Fluorescence Measurements with a Spectrofluorophotometer

After extraction, the resulting supernatant samples were analyzed using an RF-6000 spectrofluorophotometer under the conditions shown in Table 3.[1] The results are shown in Fig. 4. The fluorescence peak wavelength and intensity are shown in Table 4.

Table 3 Measurement Conditions

Instrument:	RF-6000
Excitation Wavelength:	436 nm
Fluorescence Wavelength Range:	600 to 800 nm
Sampling Interval:	1.0 nm
Scanning Speed:	200 nm/min
Slit Width:	Excitation 5 nm/Fluorescence 5 nm
Sensitivity:	Low

Chlorophyll fluorescence was recorded at 670 nm. The fluorescence measurements show that DMF extracted the most chlorophyll *a* from three out of four samples, followed by acetone, while ethanol extracted the least chlorophyll *a* from every sample.

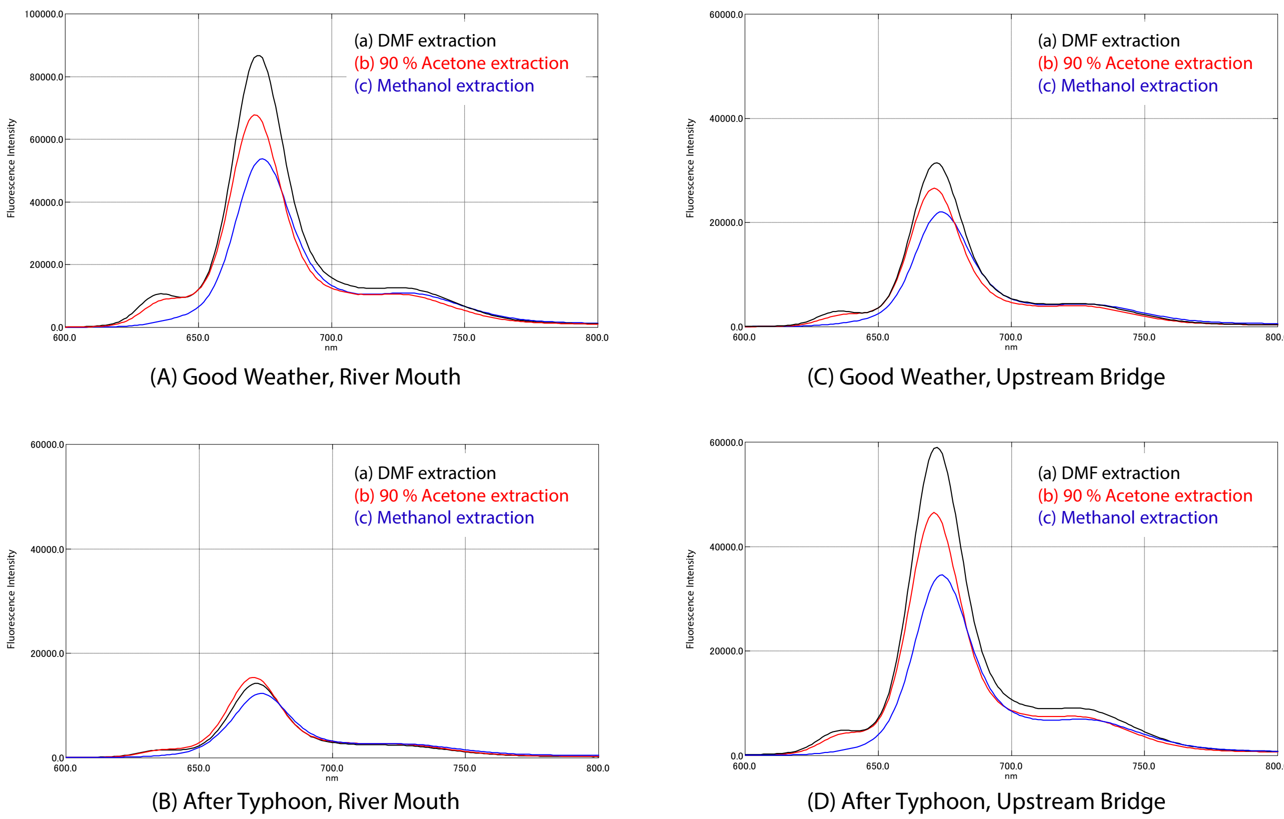

Fig. 4 Fluorescence Spectra of Extracted Samples

High intensity fluorescence signals were recorded even from samples that gave an absorbance signal of 0.05 Abs (approx. 89 % transmittance). Obtaining accurate absorbance readings from this same sample after 10-fold dilution (to 0.005 Abs. or 98.9 % T) would be very difficult due to high relative sample noise and poor reproducibility. Conversely, even after 10-fold dilution, highly accurate fluorescence readings could still be obtained from this sample with a spectrofluorophotometer due to the higher intensity of fluorescence signals relative to noise. The findings also show that the effect of light scatter due to impurities is smaller when measuring fluorescence with a spectrofluorophotometer compared with absorbance using a spectrophotometer.

Although samples were only collected on two days, less chlorophyll was detected in the river mouth sample after the typhoon than during good weather. This is believed to be due to factors such as the water being more turbid after the typhoon due to upstream sediment runoff. Although sediment runoff is also present further upstream, the same decrease in chlorophyll levels after the typhoon was not observed in the upstream bridge sample. This could be due to the impact of wood and other drifting debris on the sample after the typhoon.

Compared with absorbance signals, fluorescence signals were higher in sensitivity, and the effect of light scatter due to impurities was also smaller, showing that fluorescence analysis can offer more stable measurements at lower sample concentrations.

Table 4 Chlorophyll *a* Fluorescence Peak Wavelength and Intensity

	River Mouth		Upstream Bridge	
	Good weather	After typhoon	Good weather	After typhoon
DMF Extraction (672 nm peak)	86747	14246	31430	58947
90 % Acetone Extraction (671 nm peak)	67805	15424	26568	46497
Methanol Extraction (674 nm peak)	53813	12311	22080	34585

■ Conclusion

Water samples were prepared and chlorophyll *a* levels were measured by fluorometry according to the methods presented in Guideline of Ocean Observations.[1)]

Chlorophyll *a* can be measured at lower sample concentrations by fluorescence with a spectrofluorophotometer than by absorbance with a spectrophotometer.

Of the three organic solvents used for extraction (DMF, 90 % acetone, and methanol), the fluorometric method results showed that DMF was capable of extracting the most chlorophyll.

Various methods are available for measuring chlorophyll, and Shimadzu analytical instruments allow users to select the optimal approach from these methods for their experimental needs.

References

1) The Oceanographic Society of Japan, "Guideline of Ocean Observations 5th Edition," Vol. 4, Chap. 4, 2023
2) Nitrogen compounds in PM2.5 atmospheric pollution are growing ocean phytoplankton, Ocean and Earth Information Site JAMTEC BASE (accessed on June 1, 2026)
3) Water Standard Test Methods 2020 Edition III Organic Materials, Japan Water Works Association

Related Applications

1. Chlorophyll Quantitative Measurement: Using Equations from Japanese Water Standard Test Methods, Application News No. 01-01101

01-01245-EN First Edition: Aug. 2026

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