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UV spectrometry for measuring particulate organic matter in natural water

Nozomi Ytow, Motoo Utsumi and Humitake Seki

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305 Japan

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ABSTRACT

Ultraviolet (UV) spectra were measured to characterize the dissolved and particulate organic matter in a bog and to investigate the seasonal fluctuation of this organic matter. The optical density spectra in the UV region of dissolved organic matter (DOM) were proportional to λ^{-4}, where λ is the wavelength of incident light. There was also small absorption on the λ^{-4} spectra. The optical density at a wavelength of 280 nm was proportional to the concentration of dissolved organic carbon (DOC). The spectra and the dependency of optical density on DOC did not vary throughout the investigation period. The UV spectra of acid extractions of particulate organic matter (POM) showed two absorption peaks at the wavelengths of 220 nm and 335 nm. These peaks are well represented by the sum of two Lorentz functions. The ratio of the absorbances, A_{280}/A_{335}, was dependent on the nitrogen/carbon ratio of POM and the chlorophyll fraction in particulate organic carbon (POC), and not dependent on POC itself. The dependency of POC absorption at 280 nm varied with this ratio which fluctuated seasonally.

Introduction

In a natural water body such as an estuary or swampy bog, there are two major sources of organic debris, i.e., phytoplankton and higher plants (Ytow et al., 1994). This debris comprises dissolved and particulate organic matter in the water body. Characterization of the dissolved and particulate organic matter may help to elucidate the transformation pathway of this organic matter in the cycling of biological elements in a natural water body.

Optical characterization of dissolved organic matter has been studied in both freshwater (e.g. Davis-Colley and Vant, 1987) and seawater (e.g. Blough et al., 1993; Bricaud et al., 1981; Prieur and Sathyendranath, 1981) environments, using optical absorption or fluorescence (e.g. Ferrari and Tassan, 1991; Green and Blough, 1994; Hoge et al., 1993) with special relation to yellow substances or humic substances.

The concentration of dissolved organic carbon (DOC) in seawater has been measured optically at wavelengths of 280 nm (e.g. Krom and Sholkovitz, 1977; Naganuma and Seki, 1993) and 250 nm (e.g. Stewart and Wetzel, 1981) to determine DOC. The same method was also applied to the measurement and characterization of DOC in inland water (e.g. Rostan and Cellot, 1995; Zumstein and Buffle, 1989).

Those former studies have been mainly dealing with the fraction of humic substances in DOC which are highly resistant to biodegradation and hence have slow turnover rates (Seki, 1982). In this study, we focused on these particular organic substances which are moderately resistant to biodegradation either with a more rapid turnover rate or rapid turnover rates (Seki, 1982). We used UV absorption spectra to characterize and quantify DOC, particulate organic carbon (POC) and particulate organic nitrogen (PON). The seasonal fluctuation of particulate organic matter is also discussed.

Materials and methods

The field investigation has been performed almost weekly at Station 2 of Matsumiike Bog in the campus of the University of Tsukuba (Naganuma and Seki, 1985; Ytow et al., 1994; Utsumi et al., 1994) from April 1991 to June 1991 (n = 16), September 1991 to November 1991 (n = 16) and from May 1992 to December 1992 (n = 30).

Water samples were collected aseptically at several cm depth from the bog surface with Hyroht sampling bottles. A 350 ml aliquot of each water sample was filtered through Whatman GF/C glass fiber filters (pore size of 1 μm) which were treated at 450 °C for 30 min to burn out organic substances prior to the filtration (Parsons et al., 1984). At least four filtered samples were obtained for each water sample to measure POC, PON and chlorophylls, and to extract particulate organic matter (POM) by an acid treatment. These filtered samples and filtrates were used for chemical analyses of POC, PON and DOC. The POC and PON (only for samples in 1992) were measured by combustion method and gas chromatography with thermal conductivity detection using a CN Analyzer (SUMIGRAPH NC-90A, Sumika Analysis Center Co., Tokyo). Two filters for each water sample were burned directly in the CN Analyzer.

The DOC in filtrates was measured as difference of total carbon and inorganic carbon with a combustion-infrared method using a TOC Analyzer (TOC-5000, Shimadzu, Kyoto). Optical spectra of filtrates were measured with a UV-VIS spectrometer (UV-200, Shimadzu, Kyoto) using cells with 10 cm path length.

The GF/C filters were also used to determine the chlorophyll concentration by the method of Jeffrey and Humphrey (1975). Pigments on each filter were extracted with 15 ml of 90% (v/v) acetone in centrifuge tube. After overnight extraction in a refrigerator, each tube was centrifuged at 2,500 g for 5 min to separate filters. Optical absorbance of the supernatant at 750, 664, 647, 630 nm was measured with the UV-VIS spectrometer. Chlorophyll concentrations were calculated according to Jeffrey and Humphrey (1975).

Acid extraction of POM from the GF/C filters were used for spectrometry. The filters were soaked in 25 ml of 1 N HCl for 3 days. The extracts were centrifuged and decanted to separate the acid soluble fraction from the glass fiber and insoluble fraction. The optical absorption of supernatant was measured with the UV-VIS spectrometer.

Adjusting the pH of these extractions to higher than 3 by adding 1 N NaOH aggregated and precipitated basic components. These aggregates were separated by centrifugation (2,500 g, 10 min). The absorption spectra of the supernatant were also measured with the UV-VIS spectrometer.

Extraction ratio of POM by acid treatment was determined as follows. A water sample was filtered on 20 GF/C filters as described above. Ten of these filters were used to determine POC and PON before acid extraction with the CN analyzer. The other 10 of those filters were treated by acid extraction as described above. After decantation, each extracted filter was re-suspended in 1 N HCl and filtered on GF/C glass fiber filter pre-acidified by 1 N HCl in a filtering apparatus. These filters were washed on the filtering apparatus with 12 ml of 1 N HCl to eliminate residual extracts on filters. Optical absorbance of washing filtrate at 335 nm were monitored with the UV-VIS spectrometer to ensure elimination of extracts. Acid washed filters were washed again with 60 ml of distilled water to eliminate chloride and neutralize the filter. The pH of filtrate was monitored to confirm neutralization ($\mathrm{pH} > 5$). These washed filters were used to determine the unextracted fraction of POC and PON using the CN Analyzer.

We also tried separation of acid extracted POM by HPLC. Due to the aggregation of POM at higher pH, however, the HPCL column became clogged so that the chromatography was impossible.

Results

DOC

An optical density spectrum of the filtrate is shown in Fig. 1. The optical density OD_λ at wavelength λ was proportional to λ^4, expressed by the following regression ($r^2 = 0.963 \pm 0.038$);

$$OD_\lambda = \frac{a}{\lambda^4},$$

where the value of a varied with samples. The residue to the λ^{-4} fitting is also shown in Fig. 1. It has a small peak at the wavelength of 280 nm and a shoulder at 320 nm.

The optical density at $\lambda = 280$ nm was proportional to the DOC concentration throughout the year as shown in Fig. 2. It is expressed by the following regression ($r^2 = 0.898$);

$$OD_{280} = 0.172\ \mathrm{DOC} + 0.131.$$

Acid exctract of POM

The absorption spectrum of an acid extract is shown in Fig. 3. There were two absorbance peaks at 220 nm and 335 nm. The absorption spectrum (A_λ at

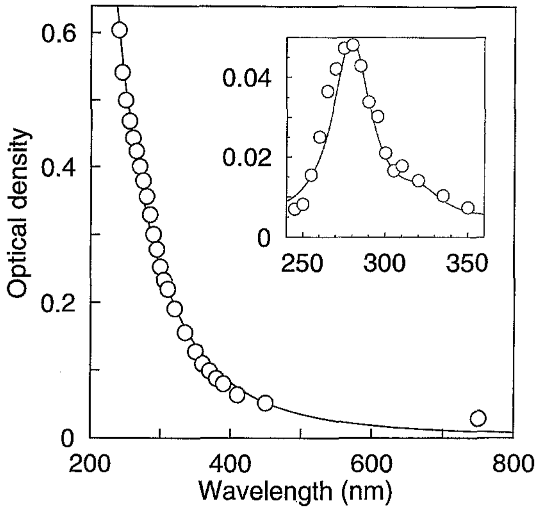

Figure 1. Optical density spectrum of DOM. Symbols indicate measured optical density at the corresponding wavelength. The spectrum shows λ^{-4} dependency (solid line). This dependency indicates that molecular scattering is a major contribution to the optical density. The residue to the regression is shown in the insertion. It shows two absorption maxima at 280 nm and 320 nm

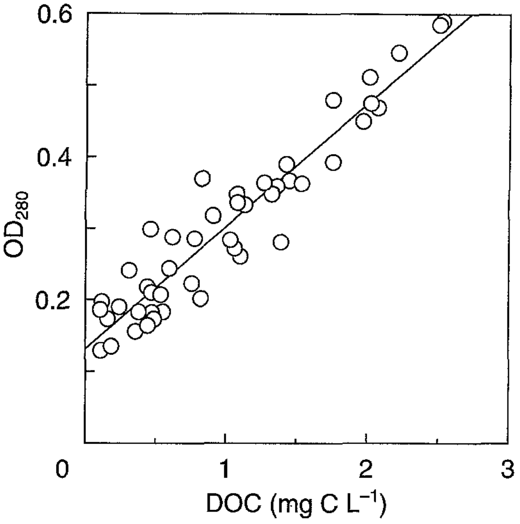

Figure 2. Optical density at the wavelength of 280 nm depends linearly on DOC. The dependency was expressed by a regression of $OD_{280} = 0.172$ DOC $+ 0.131$ ($r^2 = 0.898$), where OD_{280} is optical density at the wavelength of 280 nm and DOC is dissolved organic carbon in mg C L^{-1}, respectively. There was no seasonal variation in the regression

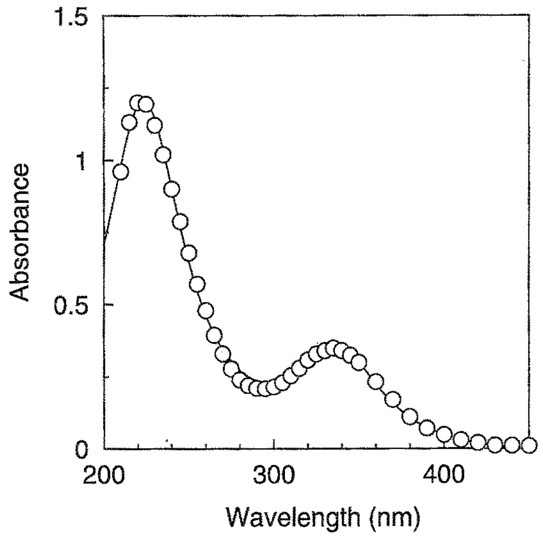

Figure 3. Absorption spectrum of the acid extract from POM. Symbols indicate measured optical density at corresponding wavelength. There were two peaks at 220 nm and 335 nm. The absorption spectrum fits the sum of two Lorentz functions (solid line, see text for details)

wavelength of λ) was expressed by the sum of two Lorentz functions ($r^2 = 0.996 \pm 0.003$);

$$A_\lambda = b + \frac{c_{223}}{1 + \frac{(\lambda - \lambda_{223})^2}{w_{223}^2}} + \frac{c_{340}}{1 + \frac{(\lambda - \lambda_{340})^2}{w_{340}^2}},$$

where each λ is the central wavelength of absorption, w is the half width at half maximum, c is the absorption intensity and b is a constant. Suffixes indicate the central wavelength, $\lambda_{223} = 222.6 \pm 1.0$ nm and $\lambda_{334} = 339.5 \pm 0.5$ nm, respectively. The value of b and c varied according to the sample.

Making the pH of the extracts to be higher than pH 3, resulted in the formation of brown flocculates. The absorption spectra of supernatants had no peaks at wavelengths of 220 nm and 335 nm (data are not shown). These absorption maxima in the acid extract were due to the precipitates. The flocculates were re-solubilized by acidification and the re-solubilized samples formed flocculates again by neutralization. This repetitive flocculation and re-solubilization indicates that the flocculates are basic substances, since the re-solubilized sample had the same absorption as that of the original acid extraction.

The absorption at several wavelengths exponentially increased depending on the duration of acid treatment as shown in Fig. 4. The time constant of extraction was 0.5 days. With 3 days extraction, the absorption reached a plateau. Hence we used 3 days extraction as analytical procedure. During 3 days extraction, POC and PON on the filter decreased 24 ± 8% and 21 ± 7%, respectively.

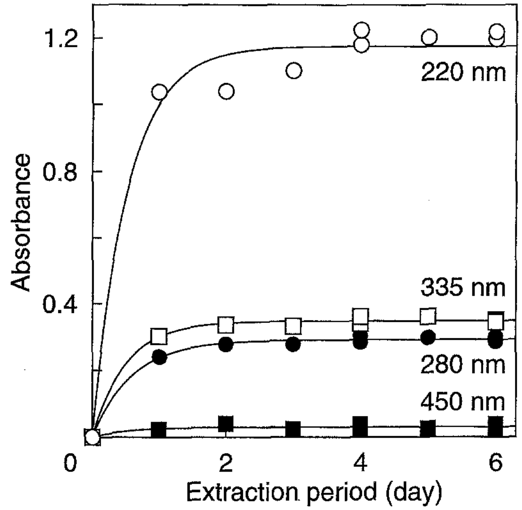

Figure 4. Time course of acid extraction from particulate organic matter on a glass fiber filter monitored by absorption at several wavelengths. The absorption increased exponentially in the initial stage of the extraction. After 3 days extraction, the absorption reached a constant level

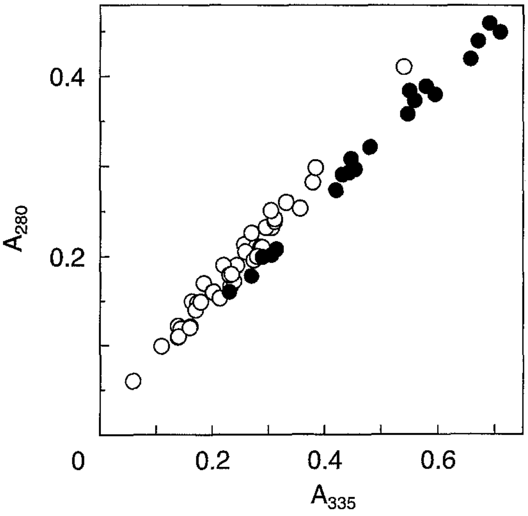

Figure 5. Correlation of absorption maxima of acid extract at wavelength of 280 nm and 335 nm. Open and closed symbols indicate an absorption ratio A_{280}/A_{335} greater or less than 0.7, respectively. There was a tendency that A_{280}/A_{335} were smaller than 0.7 for higher A_{335}

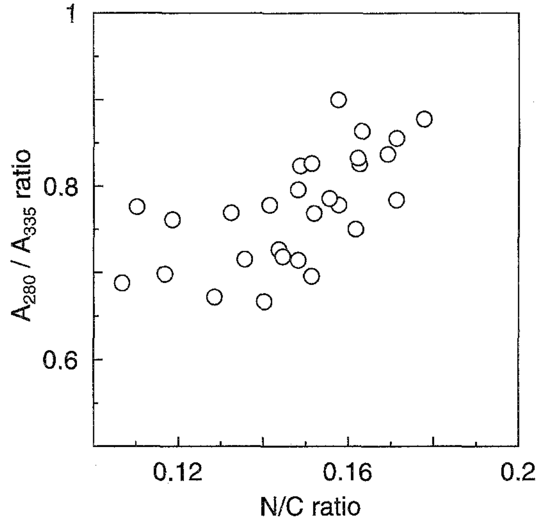

Figure 6. The absorption ratio A_{280}/A_{335} depended on the nitrogen/carbon (N/C) ratio. For N/C ratios less than 0.14 (one nitrogen atom per 7 carbon atoms), the absorption ratio was constant. For N/C ratio greater than 0.14, on the other hand, the ratio increased

In the following comparison of optical absorption of POM acid extraction and PON/POC ratio, we measure absorption at 280 nm and 335 nm since these absorption bands are more stable than that at 220 nm in the time course of extraction. Absorption at 280 nm was selected since it is used in DOC measurement and it is near the bottom of the POM extraction spectrum.

Absorption at the wavelengths of 280 nm (A_{280}) was almost proportional to absorption at 335 nm (A_{335}) (Fig. 5). There was a tendency that the A_{280}/A_{335} ratio were smaller than 0.7 for higher A_{335}. Although the absorptions ratio was independent from the particulate organic carbon concentration ($r^2 = 1.6 \times 10^{-4}$, data are not shown), it was dependent on the nitrogen/carbon (N/C) ratio (Fig. 6). The ratio increased above a value of 0.14 which corresponds to one nitrogen atom to 7 carbon atoms. The increase of the nitrogen fraction in acid extractable/hydrolysable portion of POM was obvious at a A_{280}/A_{335} ratio greater than 0.7. The frequency distribution of the absorbance ratios was divided into two groups around the ratio of 0.7 (Fig. 7). The ratios fluctuated throughout the year, as shown in Fig. 8, with two periods of lower and higher A_{280}/A_{335} ratios. Two pronounced high ratios on April and June corresponded to the phytoplankton bloom (Ytow et al., 1994).

The correlations between POC and chlorophyll concentrations are shown in Fig. 9. The dependency of chlorophyll-*a* and -*b* on POC was different during the seasons, and could be estimated by high and low A_{280}/A_{335} ratios. In the season with the lower ratio, the fraction of chlorophyll-*a* and -*b* in POC was smaller and vice versa. For chlorophyll-*c*, on the other hand, no obvious difference in regression was found.

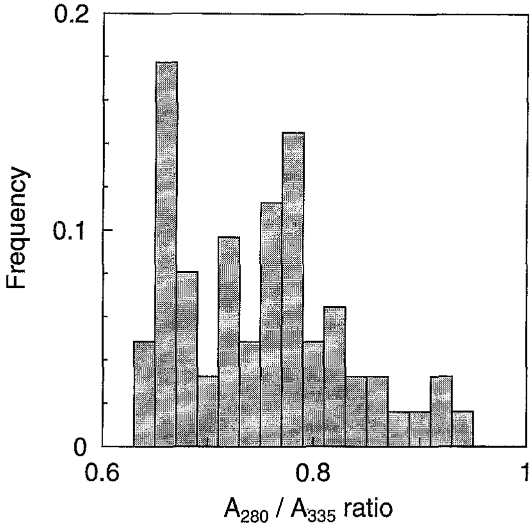

Figure 7. The distribution of the absorption ratio A_{280}/A_{335}. The measurements can be divided into two groups with A_{280}/A_{335} above and below 0.7

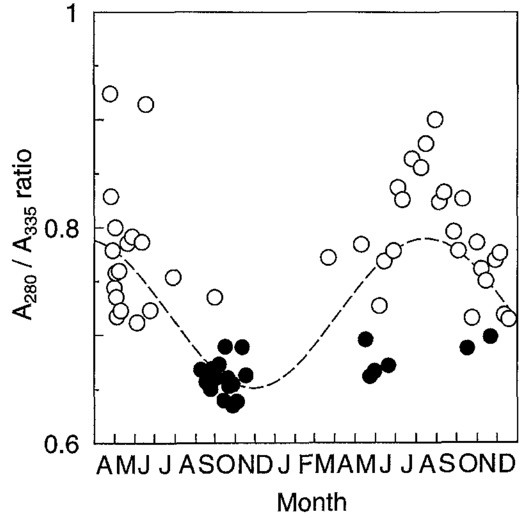

Figure 8. Seasonal fluctuation of the ratio of absorption at wavelengths of 280 nm/335 nm. Open and closed symbols indicate a ratio greater or less than 0.7, respectively. The ratio could be expressed as an annual sinusoidal curve with a minimum during autumn. High ratios in April and June corresponded to phytoplankton blooms

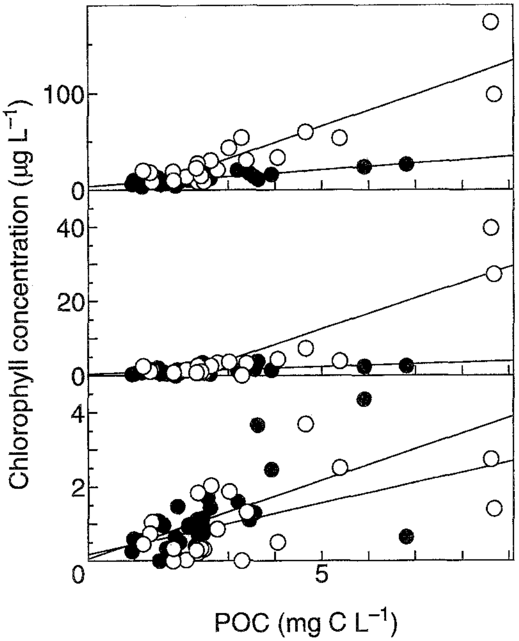

Figure 9. Chlorophyll-a (top), -b (middle) and -c (bottom) dependency on POC. Open and closed symbols indicate the seasons of high and low A_{280}/A_{335}. The dependency of chlorophyll-a and -b on POC was different according to seasons. The relation between chlorophyll-c and POC, on the other hand, was independent from the ratio

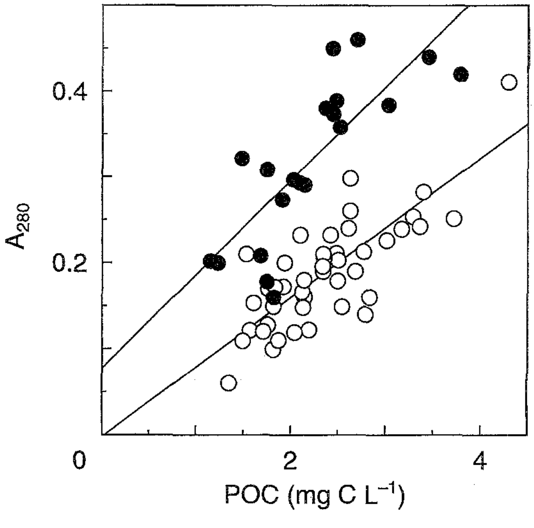

Figure 10. The absorbance of POM acid extraction at wavelength of 280 nm correlated with the POC concentration. Open and closed symbols indicate the absorbance ratio A_{280}/A_{335} greater than 0.7 or less. There was no significant difference in the slopes ($t = 1.671 < t\ (60, 0{,}05) = 2.000$) but in intercept ($t = 11.25 > t\ (60, 0.001) = 3.460$)

The optical density at $\lambda = 280$ nm was proportional to POC concentration as shown in Fig. 10. The regression depends on the A_{280}/A_{335} ratio, as

$$\mathrm{POC} = 9.15\,A_{280} - 7.06 \times 10^{-2},$$

for samples with $A_{280}/A_{335} \geq 0.7$ ($r^2 = 0.627$) and

$$\mathrm{POC} = 12.35\,A_{280} - 2.96 \times 10^{-2},$$

for samples with $A_{280}/A_{335} < 0.7$ ($r^2 = 0.689$), respectively. There was no significant difference in the slopes ($t = 1.671 < t\,(60, 0.05) = 2.000$) but in intercept ($t = 11.25 > t\,(60, 0.001) = 3.460$).

Discussion

The optical density spectrum of DOM was dependent on the wavelength λ as λ^{-4}, rather than the exponential dependency reported for yellow substances (e.g. Bricaud et al., 1981). This spectrum dependency indicates that the optical density is based primarily on scattering by dissolved molecules, since intensity of Rayleigh scattering by molecules varies with λ^{-4} in the wavelength (e.g. Jenkins and White, 1981). Hence, DOM of the bog water seems to be composed mostly of small organic molecules without complex structures such as aromatic rings containing nitrogen. There were also small absorption maxima at wavelengths of 280 nm (peak) and 320 nm (shoulder). The intensities of these small absorption peaks were on the order of one tenth of that of scattering. The absorption at 280 nm suggests the existence of aromatic compounds such as phenol, benzene or amino acids. Those absorption maxima have been shown to correspond to peaks in excitation spectra of fluorescence of acidified sea water from different depths (Coble et al., 1990). This spectral similarity suggests chemical similarity of dissolved organic matter in the bog and the seawater.

As the optical density (OD) of seawater at the wavelengths of 280 nm linearly depends on dissolved organic carbon for estuary samples (e.g. Krom and Sholkovitz, 1977; Naganuma and Seki, 1993), the OD was shown to be dependent linearly on the DOC of the bog water. The dependency was constant throughout years. It indicates that the composition of DOM varies only in the optically undetectable range.

The optical spectrum of the POM acid extract shows specific absorption at wavelength of 220 nm and 335 nm. These absorption maxima were not found in the supernatant of acid extractiones at pH 3 or above. These absorption peaks belong to the flocculates and precipitates for water samples with higher pH treatment, as confirmed by spectrometry of the re-solubilized sample of these flocculates. The absorption spectrum of acid extraction corresponds to basic substances that flocculate at pH 3 or above. These substances would be solubilized not only by protonation but also by possible hydrolysis and fragmentation during acid treatment. In natural waters with pH higher than 3, these substances could exist as suspended particles since these substances would be deprotonated and exist as larger molecules without the possible fragmentation by the acid treatment.

Although the optical absorption of acid extracts at 280 nm (A_{280}) and 335 nm (A_{335}) was proportional to each other, the ratio A_{280}/A_{335} varied with the N/C ratio of POM and of the chlorophyll fraction in POC. A correlation between A_{228}/A_{335} and the N/C ratio was observed for values of $A_{280}/A_{335} > 0.7$ and $N/C > 0.14$ ($C/N < 7$), indicating that the A_{280} component corresponds to the nitrogen-rich fraction of POM. It could be reasoned that the A_{280} component corresponds to amino acids, protein or nucleic acid. Three amino acids and protein show optical absorption maxima at 280 nm and their N/C ratio range from 0.1 to 0.5. Nucleic acids show optical absorption at 260 nm and their N/C ratio ranges from 0.2 to 0.6. The A_{280}/A_{335} ratio correlates with the composition of particulate organic substances.

The slopes of regression in POC vs chlorophyll plot were different for different A_{280}/A_{335} ratios. These slopes indicate ratio of chlorophyll fraction in POC. Hence, the A_{280}/A_{335} ratio indicates chlorophyll fraction in POC: The higher A_{280}/A_{335} ratio indicates the greater chlorophyll fraction in POC. The absorption bands at 220 nm and 335 nm correspond to the non-chlorophyll fraction of particulate organic substances. Since phytoplankton and littoral vegetations are two dominant sources of POC in the bog (Ytow et al., 1994), the A_{280}/A_{335} ratio also indicates the contribution from these sources to POC.

The A_{280}/A_{335} ratio showed a minimum during autumn. During this season, the littoral vegetations are under gradual degradation (Ytow et al., 1994). Therefore, the optical absorptions at wavelength of 220 nm and 335 nm should correspond to the microbial degradation products of littoral vegetations. The basic nature of the absorption component (or flocculate) and dependency on N/C ratio suggest the incorporation of disintegration products from dead plants into microbial cell components such as peptide glycans or the direct release of alkaloids from littoral vegetations. The C/N ratio of decomposing phytoplankton (Otsuki and Hanya, 1967) which increased from 5 to 7 during progress of decomposition and sustained the ratio of 7 suggests the incorporation into microbial cell components.

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