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Amounts and Spectral Properties of Dissolved Organic Compounds from some Freshwaters of the Southeastern U. S.

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With 4 figures and 1 table in the text

Abstract

Collections of stream, river, and lake water taken from the coastal plain area of South Carolina were analyzed for dissolved organic carbon using gas chromatography. Complete absorbance spectra between 200 and 950 nm were also obtained on many of the samples so that statistical tests could be made of the intensity and form of the absorbance-organic carbon relationship at various wavelengths. Dissolved organic carbon levels averaged about 7 ppm in the predominately dark-water areas covered by the survey, and ranged between 3 and 16 ppm. Correlations between organic carbon and absorbance reached a maximum of about 0.92 between 300 and 360 nm. The upper end of this range, specifically 360 nm, offers maximum sensitivity consistent with minimum relative variance as determined by the analysis of regression slopes at all wavelengths. Change in the intercept of the dissolved carbon-absorbance relation at various wavelengths is apparently related to the presence of three or more groups of compounds with differing optical properties. The data are used to derive an equation that accurately predicts dissolved organic carbon from absorbance at any wavelength between 260 and 360 nm. The precision of this prediction and its possible general applicability are discussed.

Introduction

Dissolved organic compounds have attracted an increasing amount of attention in the past decade due to their intimate relationships with optical properties of water and chemical-biological processes in aquatic environments. Despite the comparatively early recognition of the importance of these compounds in aquatic systems (e.g., BIRGE & JUDAY 1934), a poverty of adequate methods and instrumentation has until recently rendered many critical lines of inquiry impractical. The invention of a reliable technique for measuring dissolved organic carbon (MENZEL & VACCARO 1964) has of course been particularly stimulating. A comprehensive view of the distribution of dissolved organics in nature will nevertheless be slow to emerge unless present analytical methods can be simplified. This paper is intended

to contribute regional information on the dissolved organic content of several habitats in the coastal plain of the southeastern U. S. and to determine the optimum means by which comparable information could have been obtained using simple spectrophotometry instead of gas chromatography.

The streams of the southeastern coastal plain flow from sandy soils and are typically acid and highly colored with organic compounds. The surface waters, including vast swamps, are generally poor in electrolytes and markedly oligotrophic in their natural condition. Many habitats of the coastal plain region are remarkably similar to the dark-water areas of the Amazon Basin described by SIOLI (1954, 1955). The distinctive dark coloration of these waters together with their low conductance suggests that biological effects of dissolved organic compounds may be exceptionally significant.

Numerous investigators have commented on or explored the possibility of using simple optical methods to measure the concentration of dissolved organic compounds. The two methods that offer promise are fluorescence in the UV region and absorbance at a specific wavelength, usually toward the UV end of the spectrum. The fluorescence method, which is reviewed by GOLTERMAN (1969), appears to be insensitive, nonlinear, and requires special equipment. Using absorbance rather than fluorescence, OGURA & HANYA (1966, 1967) have recently shown that organic matter in sea water can be predicted from absorbance at 220 nm if nitrate and bromide concentrations are known. FORSBERG (1967) reports a moderately high correlation between organic carbon and extinction at 420 nm in Swedish lakes, as does BANOUB (1973) at 260 nm for Lake Constance. It seems probable that for any specific habitat, a significant correlation could be obtained between dissolved organic carbon and absorbance at any wavelength between 200 nm and the middle of the visible range. The following treatment of regional data attempts to establish (1) the wavelength range that provides optimum predictability of dissolved organic carbon from absorbance, and (2) the reliability of the optimum prediction for a set of regional samples from several habitats.

Methods and Study Sites

Samples were taken from three different streams, from the Savannah River, and from the epilimnion of Par Pond, a 1130-ha impoundment within the study area (Fig. 1). The streams are all highly colored due to the presence of natural organic compounds, as is the Savannah River. None of the stream sites except site 4 (Fig. 1) receives any domestic or industrial effluent. Site 4 receives industrial cooling water from the Savannah River and is essentially equivalent to heated but otherwise unaltered Savannah River water. Water from the Savannah

River is polluted with city sewage ($\text{NO}_3\text{-N}$, 1700 ppb; $\text{PO}_4\text{-P}$, 350 ppb; LEWIS, unpublished). The water of the Par Pond epilimnion is not darkly colored as is that of the streams and the Savannah River. The waters tested thus vary greatly in composition, although they share some regional characteristics such as low calcium.

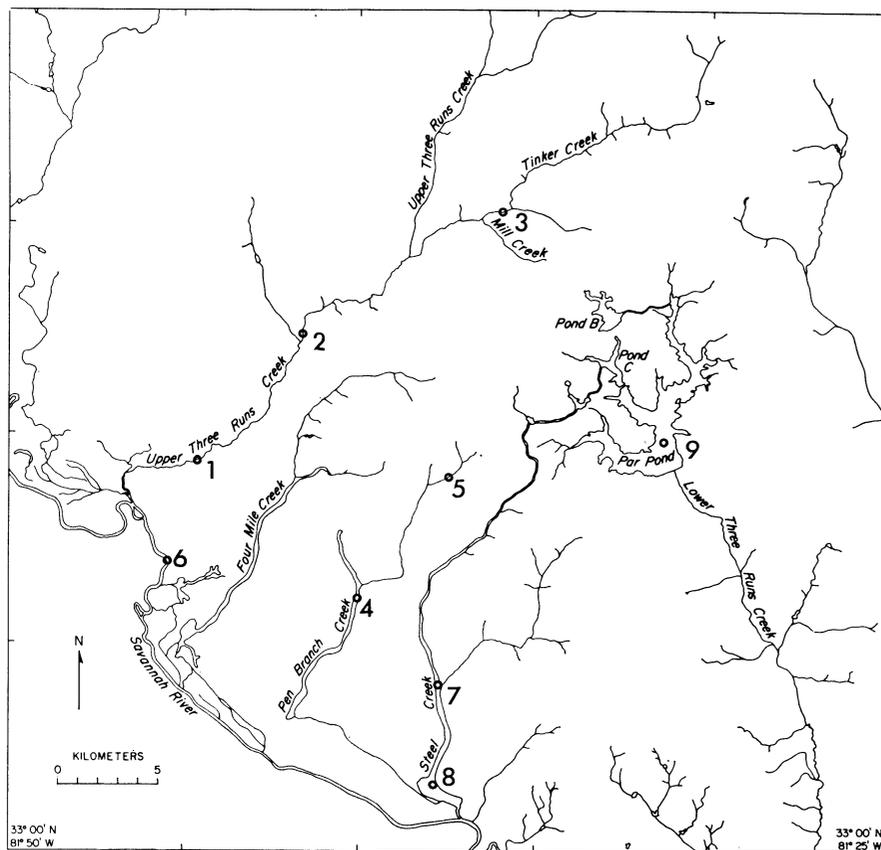


Fig. 1. Surface water in the study area. Collection points marked by numbers are referred to in Table 1.

Samples were collected with a VAN DORN bottle and transported in clean glass bottles to the laboratory. Aliquots of each sample were analyzed with a Beckman Model 915 total carbon analyzer after filtration through washed Millipore HA filters (0.45μ pore size). A comparison of filtered and unfiltered blanks showed that no significant amounts of dissolved organic carbon were released from the filters after rinsing. Dissolved CO_2 was removed prior to analysis by acidification and bubbling with nitrogen. Potassium phthalate was used to calibrate the carbon analyzer.

A second portion of the filtrate from each sample was used to obtain optical data by means of a Bausch and Lomb Spectronic 700 spectrophotometer. This

portion was added to a 10-cm quartz cell and its absorbance measured against an empty cell at intervals of 10 nm between 200 and 950 nm. Total analysis of each sample thus yielded the amount of dissolved organic carbon (DOC) and an essentially continuous absorbance curve for wavelength range 200—950 nm.

Results and Discussion

Table 1 lists the dissolved organic carbon content and pH of water samples from 9 sites on two different dates. Less extensive data sets for other dates during the summer are generally similar to those tabulated. Dissolved organic carbon was variable, even between two reaches of the same stream. Readings over the entire summer ranged between 3 and 16 ppm and averaged about 7 ppm. There was some indication of a positive relationship between stream flow and DOC levels. Stream flow on 10 July, for example, exceeded stream flow on 12 August by a factor of approximately 1.5 (U.S.G.S., unpubl. data), and DOC was higher at all sites on the first date by an average factor of 1.6.

Table 1. Amounts of dissolved organic carbon (DOC) and pH of water from various locations within the study area (Fig. 1).

| Location | July 10 | | August 12 | |
|----------|---------|---------|-----------|---------|
| | pH | ppm DOC | pH | ppm DOC |
| 1 | 5.82 | 12.7 | 6.33 | 4.5 |
| 2 | 6.55 | 5.0 | 5.88 | 3.3 |
| 3 | 6.24 | 8.7 | 6.31 | 4.5 |
| 4 | 7.22 | 7.1 | 7.32 | 3.5 |
| 5 | 5.78 | 15.7 | 6.30 | 8.8 |
| 6 | 6.68 | 8.0 | 6.88 | 5.7 |
| 7 | 7.01 | 7.5 | 7.03 | 5.3 |
| 8 | 6.98 | 7.1 | 6.80 | 4.5 |
| 9 | — | — | 7.73 | 4.8 |

The spectra of individual samples need not be reproduced here, since it is well known that absorbance by natural organics increases steadily toward the ultraviolet end of the spectrum. Data on the shortest wavelengths (200—250 nm) were not included in the analysis due to the complicating absorbance by nitrate in the short UV range. Wavelengths above 460 nm were also excluded at the outset due to the insensitivity of absorbance to changes in DOC at longer wavelengths. Statistical comparisons of absorbance and DOC data are thus limited to the 260—460 nm range.

If absorbance at a specific wavelength and DOC can be assumed to conform approximately to bivariate normality, it is possible to submit all of the data to correlation-regression analysis as a means of determining the optimum wavelength range for DOC prediction. Figure 2 shows the

change in the PEARSON product-moment correlation coefficients (r) and the coefficients of determination (r^2) over the wavelength range selected for analysis. All of the correlations at intervals of 10 nm between 260 and 460 nm are significant at $\alpha = 0.05$, but the correlation is higher in the middle of the wavelength range than at either extreme. The optimum correlation of about 0.92 is obtained between 300 and 360 nm. There is a steady decline in the correlation at higher wavelengths that is probably due to the decreasing sensitivity of absorbance to the presence of organic compounds. Drastic decline in r below 280 nm is probably due to an increasing amount of interference from inorganic substances or to differential absorbance by organic compounds of different classes. It thus appears that DOC is most closely related to absorbance in the wavelength range 300–360 nm, and that about 85% of the variance in absorbance can be accounted for by variation in DOC within this range.

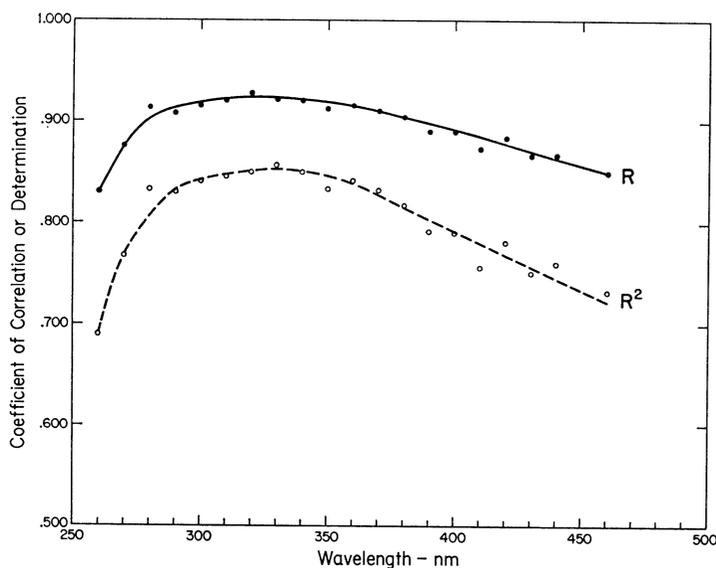


Fig. 2. Pearson product-moment correlation coefficients (r) and coefficients of determination (r^2) indicating the intensity of association between absorbance and dissolved organic carbon as a function of wavelength. Each point was obtained by correlation-regression analysis of organic carbon and absorbance data for 17 samples at the indicated wavelength. The 17 samples were taken from several different habitats as explained in the text.

The slopes of the regressions of DOC (Y) against absorbance (X) give additional information about the relationship between these two variables. These slopes are plotted in Fig. 3 as a function of wavelength. There is a marked increase in the slopes of regression lines at higher wavelengths,

which indicates a declining sensitivity of absorbance to the presence of organic compounds. If this were the only trend to be considered, prediction of DOC would of course be best accomplished on the basis of absorbances at the shortest wavelengths, since this is the region of greatest sensitivity. Figure 3 shows, however, that the variance of the slope changes little with wavelength and is only slightly greater in the region of lower sensitivity than in the region of higher sensitivity. It is thus obvious that the variance of the slope expressed as a fraction of the slope is much higher in the region of high sensitivity. Considering the combined criteria of sensitivity and variance, the optimum predictive wavelength range is intermediate, or very near 360 nm. The correlation coefficients and slopes thus both suggest that the ideal analytical wavelength is between 300 and 360 nm, and the slope analysis would argue for the higher portion of this range. The wavelength 360 nm confers the additional advantage that no UV light source

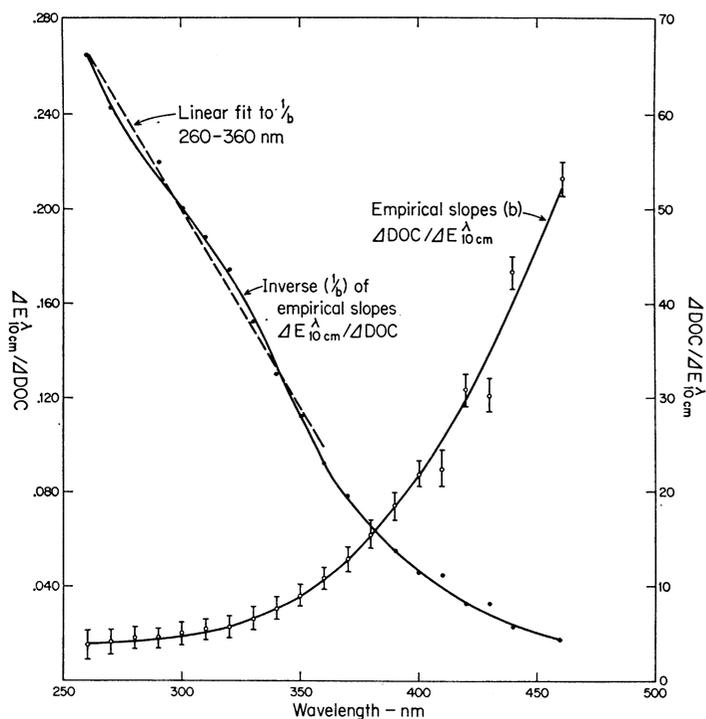


Fig. 3. Trend with increasing wavelength of the slopes (b) for the linear relationship between dissolved organic carbon (DOC) and absorbance in a 10-cm quartz cell. One standard error is marked above and below each slope. The inverse of the slope is also plotted for each wavelength to indicate the linearity of the inverse slope-wavelength relationship between 260 and 360 nm. The regression line fitting the inverse slopes is superimposed on the points.

is required for analysis, so 360 nm could be considered an ideal analytical wavelength. At 360 nm the change in absorbance (10-cm cell) per unit change in DOC (ppm) is 0.094 with 95 % confidence limits 0.081—0.103.

The inverses of the slopes of the regressions of DOC on absorbance are also plotted in Fig. 3 as a function of wavelength. The slope of this curve is essentially linear between 260 and 360 nm, which suggests a simple means of expressing slope as a function of wavelength for the wavelength range of interest. A linear fit to the points between 260 and 360 nm gives

$$1/b = (-1.66 \lambda + 697) \times 10^{-3} \quad (1)$$

where b is the slope of the linear relation between DOC (Y , ppm) and absorbance (X , 10-cm cell) at wavelength λ . Thus

$$b = (1/(697 - 1.66 \lambda)) \times 10^3. \quad (2)$$

The DOC can thus be predicted simply from absorbance and wavelength by the equation

$$\text{DOC (ppm)} = (1/(697 - 1.66 \lambda)) \times 10^3 \times E_{10}^{\lambda} \text{ cm} + a \quad (3)$$

where a is the Y intercept of the regression. The Y intercept, however, varies with λ , so that it is necessary to express a in terms of λ .

The variation of the Y intercept of regressions of DOC on absorbance is plotted in Fig. 4 as a function of wavelength. All of the Y intercepts are significantly greater than zero. This must be interpreted to mean that at all of the wavelengths at and above 260 nm, some compounds amounting to at least 15 % of the total carbon are non-absorbing. The proportion of non-absorbing compounds increases with wavelength as might be expected due to the known absorbance peak at short wavelengths. The exact nature of the increase in a is in fact quite interesting, in that there is an abrupt increase in a between 280 and 310 nm but a much steadier value of a outside this range. The transition zone for a can be reasonably accounted for as the optical junction between two major groups of compounds causing absorbance at and above 260 nm, one of which becomes optically inactive above the 280—320 nm range. There would thus appear to be at least 3 groups of compounds in all: (1) compounds not absorbing significantly above 260 nm, accounting for about 15 % of DOC, (2) compounds distinct from the first group but not absorbing significantly above 320 nm, accounting for approximately 20 % of the DOC, and (3) compounds absorbing evenly between 320 and 370 nm and gradually becoming inactive above this range, accounting for approximately 65 % of the DOC.

Due to the abrupt change in a over a short range of wavelengths, the variation in a with wavelength is well fitted by a logistic curve. The least

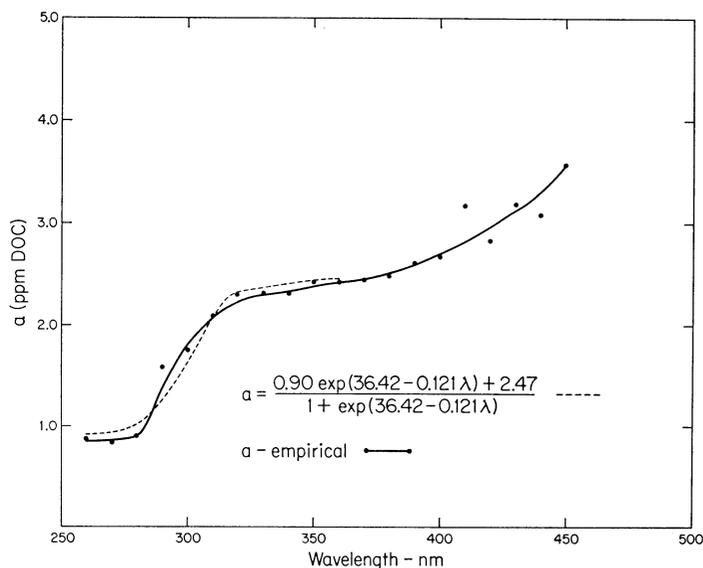


Fig. 4. Variation with wavelength in the constant term (a) of the linear regression equations relating dissolved organic carbon (DOC, ppm) to absorbance in a 10-cm quartz cell. The logistic equation fitted by least squares to the empirical curve between 260 and 360 nm is shown as a dashed line.

squares fit and the corresponding equation are shown in Fig. 4. It is thus possible to complete equation 3 in terms of wavelength and absorbance:

$$\text{DOC (ppm)} = (1/697 - 1.66 \lambda) \times 10^3 \times E_{10}^{\lambda} \text{ cm} + \frac{0.90 \exp(36.4 - 0.121 \lambda) + 2.47}{1 + \exp(36.4 - 0.121 \lambda)} \quad (4)$$

Equation 4 can be used to predict DOC from optical data alone. The inaccuracy deriving from the equation itself is negligible compared to the imprecision of DOC analysis by the method used in this study. If the optical data for 360 nm are used in the equation, the predicted average DOC is only 3.8% below the expected as determined by DOC analysis. The difference between observed and predicted in fact is not statistically significant according to the nonparametric signs test ($N = 17$). The standard deviation of the difference between predicted and measured DOC is nevertheless about 19% of the mean for average DOC levels (7 ppm). Four separate absorbance observations on a single habitat would thus be required to reduce the standard error of the estimated DOC to 10% of the mean DOC. Narrower confidence intervals around the prediction could be established by refinement of DOC measurement on which the DOC prediction is based.

It remains to be seen whether the relationship derived from the data on hand is applicable outside the geographic region in which these samples were taken. The results are encouraging insofar as the samples were drawn from numerous sites, including three habitat types, yet the absorbance-DOC relationship is sufficiently clear to permit the derivation of a reasonably simple equation that predicts DOC with excellent accuracy and tolerable precision over a range of wavelengths.

Acknowledgements

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Summary

Dissolved organic carbon was measured at 9 different locations in the southeastern U.S. during summer 1973, including 3 dark-water streams, the Savannah River, and a reservoir. Absorbance spectra were obtained for the wavelength range 200—950 nm on each sample. The dissolved organic carbon levels were between 3 and 16 ppm and appeared to be inversely related to volume of flow in the running water environments.

Absorbance data were statistically compared to the amounts of dissolved organic carbon in the samples. Correlation coefficients of DOC and absorbance reached a maximum of about 0.92 in the wavelength range 300—360 nm. Maximum sensitivity consistent with low relative variation of slopes for linear DOC-absorbance relationships was also maximum in the upper part of this wavelength range. The 300—360 nm range, particularly the 360 nm wavelength, is thus optimal for predicting DOC from absorbance data alone. An equation was developed on the basis of the empirical relationships between DOC and absorbance. The equation allows a DOC prediction of high accuracy to be made from absorbance data for any wavelength in the 260—360 nm range. Possibilities that this or analogous relationships can be used in other geographic regions seem promising, since a variety of water types were used in the derivation.

Zusammenfassung

Im Sommer 1973 wurde gelöster organischer Kohlenstoff an 9 verschiedenen Orten im Südosten der USA gemessen. Die Proben stammen aus 3 Schwarzwasserflüssen, dem Savannah River und aus einem Reservoir. Absorptionsspektren wurden für jede Probe in dem Wellenbereich 200—950 nm bestimmt. Die Konzentrationen von gelöstem Kohlenstoff lagen zwischen 3 und 16 mg/l und schienen in umgekehrtem Verhältnis zur Abflußmenge der Flüsse zu stehen.

Die Absorptionswerte wurden mit den Konzentrationen von gelöstem organischem Kohlenstoff statistisch verglichen. Die Korrelationskoeffizienten für gelösten organischen Kohlenstoff und Absorption erreichten ein Maximum von ungefähr 0.92 in dem Wellenlängenbereich 300—360 nm. Die maximale Empfindlichkeit, verbunden mit niedriger Relativvariation des Verhältnisses von linearem Kohlenstoff und Absorption, lag am oberen Ende dieses Wellenlängenbereichs. Der 300—360 nm-Bereich, vor allem die 360 nm-Wellenlänge, erwies

sich also als optimal für eine Bestimmung des gelösten organischen Kohlenstoffes, die nur auf Absorptionwerten beruht. Auf Grundlage der empirischen Verhältnisse zwischen gelöstem organischem Kohlenstoff und Absorption wurde eine Gleichung abgeleitet. Die Gleichung ermöglicht eine genaue Vorausbestimmung des gelösten organischen Kohlenstoffs mit Hilfe der Absorptionen bei irgendeiner Wellenlänge im Bereich von 260—360 nm. Da verschiedenartige Wassertypen für die empirische Ableitung verwendet wurden, scheint eine Übertragung dieser oder analoger Verhältnisse auf andere geographische Gebiete möglich zu sein.

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