Limnol. Oceanogr., 46(1), 2001, 38–48

Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity

Diane M. McKnight
University of Colorado, Institute of Arctic and Alpine Research, 1560 30th Street, Boulder, Colorado 80309

Elizabeth W. Boyer
State University of New York, College of Environmental Science and Forestry, Syracuse, New York 13210

Paul K. Westerhoff
Arizona State University, Department of Civil Engineering, Tempe, Arizona

Peter T. Doran
University of Illinois, Department of Earth Environmental Sciences, Chicago, Illinois 60607

Thomas Kulbe
Alfred-Wegener Institute for Polar and Marine Research, Telegrafenberg A43, 14473 Potsdam, Germany

Dale T. Andersen
McGill University, Department of Geography, Montreal, QC H3A 2K6, Canada

Abstract

We studied the fluorescence properties of fulvic acids isolated from streams and rivers receiving predominantly terrestrial sources of organic material and from lakes with microbial sources of organic material. Microbially derived fulvic acids have fluorophores with a more sharply defined emission peak occurring at lower wavelengths than fluorophores in terrestrially derived fulvic acids. We show that the ratio of the emission intensity at a wavelength of 450 nm to that at 500 nm, obtained with an excitation of 370 nm, can serve as a simple index to distinguish sources of isolated aquatic fulvic acids. In our study, this index has a value of ~1.9 for microbially derived fulvic acids and a value of ~1.4 for terrestrially derived fulvic acids. Fulvic acids isolated from four large rivers in the United States have fluorescence index values of 1.4–1.5, consistent with predominantly terrestrial sources. For fulvic acid samples isolated from a river, lakes, and groundwaters in a forested watershed, the fluorescence index varied in a manner suggesting different sources for the seepage and streamfed lakes. Furthermore, we identified these distinctive fluorophores in filtered whole water samples from lakes in a desert oasis in Antarctica and in filtered whole water samples collected during snowmelt from a Rocky Mountain stream. The fluorescence index measurement in filtered whole water samples in field studies may augment the interpretation of dissolved organic carbon sources for understanding carbon cycling in aquatic ecosystems.

Aquatic fulvic acid is a major fraction of dissolved organic matter (DOM) in natural waters. Aquatic fulvic acids are heterogeneous, moderate molecular weight, yellow-colored organic acids. Examples of processes controlled by fulvic acid are (1) chemical speciation and transport of trace metals through complexation reactions (e.g., Breault et al. 1996); (2) formation of trihalomethanes in drinking water by interactions between chlorine and fulvic acid during water treatment (Reckhow et al. 1990); and (3) absorption of visible and UV light and generation of photoproducts (Scully and Lean 1994; Laurion et al. 1997).

Variations in chemical characteristics of fulvic acids in surface waters are related to differences in precursor organic material (McKnight et al. 1994) and to geochemical processes acting on fulvic acids (McKnight et al. 1992). Aquatic fulvic acids derived from plant litter and soils generally contain a significant content of aromatic carbon (25%–30% of total carbon), reflecting the contribution of lignin degradation to their formation (Malcolm 1990). The aromaticity of microbially derived fulvic acids is lower (12%–17% of total carbon), reflecting the lower content of moieties containing sp^2-hybridized carbon in cell wall material and other components of microbial cells (McKnight et al. 1994). These chemical differences influence the ecological role of fulvic acids. For example, because of their lower aromaticity, microbially derived fulvic acids absorb less visible and ultra-

Acknowledgments

We thank K. Cunningham for help with spectroscopic methods and G. Aiken, J. Debroux, P. Coble, and four anonymous reviewers for valuable comments on the manuscript. This research was supported by the National Science Foundation's Office of Polar Programs (OPP 88-17113 and 92-11773) and Hydrology Program (EAR-9304794) and by the NASA Exobiology Program (NAGW 1947). This is contribution 1029 of the Alfred-Wegener Institute for Polar and Marine Research.

violet light than plant- or soil-derived fulvic acid (McKnight et al. 1994), potentially resulting in a deeper euphotic zone or a greater depth of harmful UV for similar plant- or soil-derived fulvic acid concentrations (Laurion et al. 1997).

Our current understanding of the chemistry of aquatic fulvic acids is largely based on studies that used preparative quantities of fulvic acid isolated from various field sites (e.g., Malcolm 1990). The isolation procedures to obtain a sufficient sample for chemical characterization require processing of large volumes of water and are labor intensive. These methods are not practical in studies involving many sampling sites or many time points. Therefore, simple characterizations of fulvic acid that could be carried out rapidly on small volumes of filtered water samples would be useful.

Because fulvic acid is the major light-absorbing solute in the range of 800–200 nm in many natural waters (Stewart and Wetzel 1980, 1981), spectroscopic techniques have the potential to meet this need. The UV/visible absorption spectra of aquatic fulvic acids are relatively featureless, with a steady increase in absorbance with decreasing wavelengths from 600 to 200 nm. Chin et al. (1994) have shown that the molar absorptivity at 280 nm of aquatic fulvic acid increases with molecular weight and aromaticity and that these properties can be estimated from molar absorptivity if the fulvic acid concentration is known independently.

Numerous studies have shown that the fluorescence of dissolved fulvic acids accounts for a significant portion of the fluorescence in natural water samples (Stewart and Wetzel 1980, 1981; Lochmuller and Saavedra 1986; Ewald and Belin 1987; Goldberg and Weiner 1989; Coble et al. 1990; Green et al. 1992; De Souza Sierra et al. 1994; Green and Blough 1994; Coble 1996). Fluorescence characterization has proven to be a useful means of characterizing oceanic water masses (e.g., Hayase et al. 1988; Coble et al. 1990; Mopper and Schultz 1993; Coble 1996). In a study of a freshwater lake, Stewart and Wetzel (1980) showed that larger molecular weight aquatic humic fractions had a greater absorbance but lower fluorescence than smaller molecular weight fractions. Furthermore, absorbance and fluorescence results were interpreted as showing that in calcium-rich waters, the higher molecular weight humic fractions were removed (Stewart and Wetzel 1981).

Unlike the UV/visible absorption spectra, the fluorescence spectra have characteristic maxima that may vary between environments (Ewald et al. 1983; Coble et al. 1990; De Souza Sierra et al. 1994; Coble 1996; Mobed et al. 1996). In scans of emission over a range of excitation wavelengths (excitation-emission matrices [EEMs]), Goldberg and Weiner (1989) identified two fluorophores in fulvic acid isolated from the Suwannee River (the standard aquatic fulvic acid of the International Humic Substances Society [IHSS]). The presence of two fluorophores in humic substances has been confirmed (Green et al. 1992; Coble 1996; Mobed et al. 1996). Mobed et al. (1996) showed that significant differences in EEMs occurred among soil and wetland humic and fulvic acids. Coble (1996) showed that the position of one of the humic fluorophores in EEMs of natural waters was shifted to lower excitation and emission wavelengths for marine samples, compared with riverine and coastal samples. These results suggest that differences in fluorescence properties are related, in a consistent way, to structural characteristics of aquatic fulvic acids and therefore may be indicative of source organic material.

Our first objective was to evaluate fluorescence spectra of fulvic acids isolated from water samples collected in end-member environments representative of the range of precursor sources of organic matter. These end members are areas in which the DOM is (1) microbially derived, resulting from such processes as extracellular release and leachate of algae and bacteria, and (2) terrestrially derived (and transported to the lake or stream from its drainage basin), originating from decomposition and leaching of plant and soil organic matter. The microbially derived end member is represented by fulvic acids from lakes and ponds in Antarctica where DOM is autochthonous (Lake Hoare, Lake Fryxell, and Pony Lake in the McMurdo Sound region). The terrestrially derived end member is represented by fulvic acids from streams and rivers in the United States where terrestrial plant and soil organic matter are the dominant precursor sources of DOM (the Suwannee River, which drains a large wetland in Georgia, and Deer Creek, Snake River, and Coal Creek, all of which drain forested catchments in Colorado). Structural properties of these fulvic acids have been well characterized (Leenheer et al. 1989; McKnight et al. 1991, 1992, 1994; Aiken et al. 1996). We also compare spectra for fulvic acids isolated from four large river basins in the United States and from natural waters in a temperate forest in Minnesota.

Our second objective was to examine fluorescence spectra of filtered whole water samples from end-member source areas, to see whether the characteristics of the fluorescence spectra for fulvic acids from different sources were discernible. The microbially derived end member is represented by whole water samples collected from lakes and ponds in another Antarctic desert oasis, the Bunger Hills, and the terrestrially derived end member is represented by whole water samples collected during snowmelt in Deer Creek. Our goal was to develop a fluorescence index that can be readily measured in filtered whole water samples to indicate precursor source and chemical properties of dissolved fulvic acid..

Site descriptions and methods

Table 1 summarizes the site locations that are included in this study. Dissolved organic carbon (DOC) analyses were done by use of a Dohrmann carbon analyzer that employs a high temperature combustion method or an Oceanography International Model 700 total organic carbon analyzer that employs a UV-persulfate oxidation method. DOC concentrations (Table 2) ranged from 110 mg C L^{-1} for Pony Lake to 1.5 mg C L^{-1} for Lake Hoare. Whole water samples were filtered through precombusted Whatman GF/C glass fiber filters into precombusted 125-ml glass bottles and were acidified with phosphoric acid. All isolated fulvic acids were obtained by use of preparative scale-column chromatography with XAD-8 resin, following the method of Thurman and Malcolm (1981).

Desert oases in Antarctica: microbially derived DOM end members—Antarctic desert oases provide an end-member environment in which DOM in lakes and ponds is derived

Table 1. Summary of sampling sites and data sets in this paper.

Site name	Description	Abbreviation
Bunger Hills (e.g., 6 m lake, BW-5)	Samples collected from 56 lakes and ponds in the Bunger Hills region of Antarctica	BH
Coal Creek	Forested catchment (65 km^2) draining the Flat Tops Wilderness Area, Colorado	CC
Deer Creek	Small headwater catchment (10.5 km^2) in Rocky Mountains, Montezuma, Colorado	DC
Lake Fryxell	Oligotrophic lake (7.0×10^6 m^2) in the McMurdo Dry Valleys region of western Antarctica	LF
Lake Hoare	Oligotrophic lake (2.90×10^6 m^2) in the McMurdo Dry Valleys region of western Antarctica	LH
Missouri River	Major river draining north central U.S.; sampled at Sioux City, Iowa	MR
Ogeechee River	Large river draining piedmont; sampled at Grange, Georgia	OgR
Ohio River	Major river draining east central U.S.; sampled at Cincinnati, Ohio	OhR
Pony Lake	Hypereutrophic, coastal pond (8.0×10^3 m^2) in the Cape Royds area, east of McMurdo Sound, Antarctica	PL
Shingobee Lake	Lake (6.56×10^5 m^2) draining Shingobee River headwaters area, north-central Minnesota	ShL
Shingobee River	River inlet to Shingobee Lake, located in north-central Minnesota	ShR
Snake River	Small headwater catchment (11.8 km^2) in Rocky Mountains, Montezuma, Colorado	SnR
Suwanee River	River which drains the Okeefenokee Swamp, a cypress wetland in southern Georgia	SuR
Williams Lake	Lake with no channelized surface-water inflow or outflow, in north-central Minnesota	WL
Yakima River	Large river draining northwestern U.S.; sampled at CleElum and Kiona, Washington	YR
Blank	Sample blank of Milli-Q deionized water	DI

by autochthonous microbial processes. Terrestrial vegetation is sparse, with only mosses, algae, and lichens growing near snowfields and streams; algal and microbial material in the water column or littoral zone are the main source of precursor material for DOM. We analyzed fulvic acids isolated from Lake Fryxell and Lake Hoare, two permanently ice-covered, oligotrophic lakes in the McMurdo Dry Valleys (McKnight et al. 1991), and from Pony Lake, a shallow hypereutrophic pond on Cape Royds, east of McMurdo Sound (McKnight et al. 1994).

We measured fluorescence in filtered whole water samples from lakes in the Bunger Hills Oasis of east Antarctica, an area of low-lying, rocky rolling hills and vault valleys (Klokov et al. 1990; Verkulich and Hiller 1994). Lakes in the center of the oasis are ice free in summer, and lakes in contact with glaciers are permanently ice covered. Lakes on the eastern oasis were sampled in 1992, and lakes in the northwestern and southern regions were sampled in 1993 and 1994 (Melles 1994; Melles et al. 1994). The samples were acidified with phosphoric acid to pH 2 on collection or just prior to DOC analysis.

Catchments in the United States: terrestrially derived DOM end members—In many catchments, terrestrial plant and soil organic matter are the dominant sources of DOM to streamflow. We chose four catchments to typify this end member: the Suwanee River, Coal Creek, Deer Creek, and Snake River. For the Suwannee River, the sources of organic material are vegetation and decaying peat in the Okeefenokee Swamp (Malcolm et al. 1989). The Suwanee River fulvic acid, isolated in 1983, is a standard reference of the IHSS and has been well characterized (Averett et al. 1989).

In the three mountain catchments, DOC concentrations rise during snowmelt, as a result of flushing of organic-rich waters from upper soil horizons (Hornberger et al. 1994; Boyer et al. 1997). The rapid transit times minimize the influence of in-stream microbial degradation (McKnight et al. 1993). Deer Creek and Snake River are neighboring, headwater catchments with only ~30% forest cover and willows and grasses in the alpine areas. Because of weathering of disseminated pyrite in the bedrock, the Snake River is acidic, metal-enriched, whereas Deer Creek is circumneutral (Theobald et al. 1963).

Lakes and rivers in the United States: diverse environments—We analyzed isolated fulvic acids from natural waters of varying size, land use, and location (*see* Table 1) for comparison with fulvic acids from the end-member environments. We included fulvic acids isolated from four major river basins: the Ogeechee River (at Grange, Georgia), the Yakima River (at two locations: CleElum and Kiona, Washington), the Missouri River (at Sioux City, Iowa), and the Ohio River (at Cincinnati, Ohio)(Aiken and Leenheer 1993; Westerhoff 1995). Because these basins have extremely large contributing areas, we expect that most of the fulvic acids in these rivers will be terrestrially derived.

We studied fulvic acids isolated from the Shingobee River catchment in north-central Minnesota (Table 2). Shingobee Lake has a relatively short residence time (~7 months), whereas nearby Williams Lake has a residence time of ~4 yr (Carter et al. 1993; Rosenberry et al. 1993). Thus, we expect the fluorescence characteristics of these isolated fulvic acids to fall somewhere between the two end members, with Williams Lake resembling Antarctic lakes/ponds and Shingobee Lake resembling the Colorado catchments.

Fluorescence measurements—Fluorescence measurements were made for solutions of isolated fulvic acid and filtered whole water samples. Fulvic acid solutions were prepared by dissolving H^+-saturated fulvic acid in distilled water; one set of fulvic acid solutions had concentrations of 20–50 mg C L^{-1} at pH 2, and a second set had concentra-

Table 2. Chemical characteristics of fulvic acid isolated from surface water samples.

Site	Location and sampling date (and depth, if applicable)*	DOC† of natural water	pH‡ of analyte solution	Aromaticity§	Maximum intensity/ DOC**	Peak wavelength††	Fluorescence index‡‡
Desert oases in Antarctica: microbially derived DOM (McKnight et al. 1991, 1994)							
	Lake Fryxell, 5.5 m, Dec 87	3.3	2.0	—	26.5	445	1.9
	Lake Fryxell, 7.5 m, Dec 87§§	7.0	2.0	11.9	31.3	442	1.8
	Lake Fryxell, 7.5 m, Dec 87	7.0	7.5	13.1	—	448	1.9
	Lake Fryxell, 18 m, Dec 87	30.0	2.0	—	47.9	442	2.0
	Lake Hoare, 5.6 m, Dec 87	1.3	2.0	—	18.0	446	1.8
	Lake Hoare, 12.5 m, Dec 87	3.0	2.0	11.7	20.8	446	1.8
	Pony lake, 28 Jan 92	95.0	2.0	16.5	28.5	446	1.7
	Pony Lake, 27 Jan 94§§	110.0	2.0	—	33.5	446	1.7
Catchments in the U.S.: terrestrially derived DOM (McKnight et al. 1992, and 1994; Westerhoff 1995)							
	Suwanee River, GA, 83	—	2.0	28.0	37.6	460	1.3
	Suwanee River, GA, 83	38.2	7.5	28.0	—	461	1.4
	Deer Creek, CO, 30 Oct 79	1.1	2.0	—	—	460	1.3
	Deer Creek, CO, 28 May 85§§	3.6	2.0	30.0	45.4	457	1.4
	Deer Creek, CO, 21 Jun 94	2.5	2.0	—	43.1	458	1.3
	Snake River, CO, 28 May 85§§	1.8	2.0	25.0	43.5	458	1.3
	Coal Creek, CO, Jun 82	—	7.5	27.4	—	458	1.4
U.S. rivers and lakes (Westerhoff 1995)							
	Missouri River, IA, Aug 81	3.4	7.5	20.4	—	453	1.5
	Ohio River, OH, Jun 81	3.7	7.5	24.3	—	454	1.5
	Ogeechee River, GA, May 82	—	7.5	24.8	—	457	1.4
	Yakima River, Kiona, WA, Jul 87	—	7.5	25.3	—	451	1.5
	Yakima River, CleElum, WA, Jun 82	—	7.5	26.6	—	460	1.4
	Williams Lake groundwater, Sep 92	—	7.5	11.9	—	450	1.9
	Williams Lake, MN, Jun 92	—	7.5	10.4	—	450	1.7
	Shingobee Lake, MN	—	7.5	18.5	—	453	1.6
	Shingobee River, MN, Jun 92	—	7.5	20.4	—	455	1.5
	Shingobee River, MN, Jun 93	—	7.5	24.5	—	456	1.5

* One sample for each location and date.
† Dissolved organic carbon (DOC) concentrations in mg C L^{-1}.
‡ Measurements were made on fulvic acid solutions adjusted to two different pH values (see text).
§ Relative peak area as % for 110–160 ppm in 13C NMR spectra which includes sp^2-hybridized carbon atoms.
** Peak emission intensity at 370-nm excitation, normalized for DOC concentration of analyte.
†† Wavelength (nm) of peak emission intensity at 370-nm excitation.
‡‡ Fluorescence index is ratio of emission intensity (450 nm/500 nm) at 370 nm-excitation.
§§ Samples on which 3-D fluorescence scans were conducted.

tions of 2.0–3.0 mg C L^{-1} at pH 7.5 (Westerhoff 1995). Solutions of Lake Fryxell and Suwannee River fulvic acids were analyzed in both sets. The pH of the filtered whole water samples was adjusted to 2 with concentrated phosphoric or hydrochloric acid. Low pH has been used elsewhere by Ewald et al. (1983), although Mobed et al. (1996) have shown that variations in pH between 2 and 10 can alter EEMs of Suwannee River humic acids and soil fulvic acid. At low pH, most metal complexes disassociate, which should minimize the quenching of fluorescence due to metal complexation. At neutral pH, fluorescence quenching could vary among natural waters because of the wide range of iron, copper, zinc, and other metals that form strong complexes with fulvic acids (Green et al. 1992).

Fluorescence was measured using a Hitachi F-3010 multiwavelength fluorescence spectrophotometer with a xenon lamp. Measurements were made over a broad range of emission and excitation values for four fulvic acid solutions and a sample of Milli-Q deionized water acidified with HCl (DOC $<$ 0.2 mg C L^{-1}); these spectral data are referred to as EEMs. The fluorescence intensity was measured at excitation wavelengths ranging from 250 to 400 nm at 10-nm increments and at emission wavelengths ranging between 400 and 550 nm at 2-nm increments. Additional measurements were made on 25 solutions of isolated fulvic acids and 26 filtered whole water samples at a fixed excitation of 370 nm; these spectral data are referred to as ''370 nm scans.'' For these samples, the fluorescence intensity was measured at emission wavelengths ranging from 400 to 700 nm with a 10-nm bandpass. For all fluorescence measurements, the fluorometer was set at a scan speed of 120 nm min^{-1} and a response time of 2 s.

The EEMs were processed as follows. To account for the absorbance by the fulvic acid of light from the lamp and emitted light, an inner-filter correction (Mobed et al. 1996) was done with use of data for the specific absorptivity of the fulvic acids from McKnight et al. (1994). We assumed that the emission spectrometer views only a small illuminated volume in the center of the 1-cm cell and that the effective pathlength of excitation light is 0.5 cm; the pathlength of the fluorescence light going through the sample to the emission monochromator is also 0.5 cm. The absorbance of excitation light (A_{excit}) is thus calculated as the measured specific absorptivity at a given wavelength multiplied by the DOC of the solution and by 0.5 cm; similarly, the absorbance of emitted light (A_{emit}) is calculated. With A(total) defined as the sum of A_{excit} and A_{emit}, then $10^{-A(total)}$ is the factor by which the real emission intensity of the solution is decreased because light is absorbed in both the excitation and emission light path. Thus, each point of the observed EEM plot was divided by $10^{-A(total)}$, to account for the inner-filter effect. The magnitude of this adjustment factor ranged between 0.40 and 0.98.

The fluorometer used in our study has a xenon lamp that emits less light at lower wavelengths; it was necessary to correct our EEM data for lamp spectral properties (note: some flourometer models have a reference-beam channel for correcting fluorescence intensity for lamp intensity—ours did not). This correction was determined by use of a solution of rhodamine, as described in the instrument manual. The lamp spectral correction was also applied to the blank data.

After correcting for the inner-filter effect and the lamp spectral correction, we subtracted the corrected sample blank values from corrected data for the fulvic acid solutions. Subtraction of the blank removes most of the effects due to Raman scattering. Any negative values in the data were set equal to zero before the data were normalized to maximal intensity. Contour plots of the EEM data were made from 0 to 1 with a contour interval of 0.025 by use of Stanford Graphics software.

The intensity values from the 370-nm scans were adjusted by subtracting the intensity of the blank. The 370-nm fixed excitation wavelength chosen for our measurements is greater than the excitation wavelength of 320 nm, which yields the maximum emission for most fulvic acid solutions. The absorbance of light by fulvic acid and the instrument blank are minimal at an excitation of 370 nm. The lower absorbance of light by fulvic acid reduces the inner-filter effect and allows for direct comparison of filtered water samples with a range of DOC concentrations. Another approach would be to dilute samples and use a lower excitation wavelength, which would require prior measurement of either DOC concentration or the absorbance of the samples. The inner-filter effect is a more important consideration in freshwaters than in ocean water because of the greater DOC concentrations and greater aromaticity of the fulvic acid in freshwaters.

Results and discussion

Table 2 presents selected chemical characteristics of the fulvic acids for which fluorescence spectra were obtained. The microbially derived fulvic acids from the lakes and pond in Antarctica have contents of sp^2-hybridized carbon atoms $<$20%, which include aromatic and olefinic carbons (McKnight et al. 1981), and the average molecular weights are $<$500 Da (Aiken et al. 1996). For the terrestrially derived fulvic acids, the sp^2-hybridized carbon atoms are primarily aromatic (Leenheer et al. 1989). The Suwanee River fulvic acid is more aromatic (28%) and has a higher molecular weight (800 Da) (Leenheer et al. 1989) than the microbially derived fulvic acids. The Deer Creek fulvic acid is comparable in molecular weight (750 Da) and in aromaticity (30%) to the Suwannee River fulvic acid. The Deer Creek and Coal Creek samples are generally similar to other fulvic acids from forested catchments. The Snake River sample has a somewhat lower aromaticity of 25%, because more aromatic fulvic acid molecules are removed by sorption onto hydrous iron oxides on the streambed (McKnight et al. 1992). Furthermore, the Snake River fulvic acid could be influenced by photochemical processes associated with photoreduction of iron oxides on the streambed (McKnight et al. 1992). The samples from the Missouri, Ogeechee, Ohio, and Yakima Rivers have aromaticities less than that of the Suwanee River sample and may reflect terrestrial inputs, as well as inputs from algal growth in reservoirs. The aromaticities of the samples from the surface waters in the Shingobee Lake area are variable, suggesting a range of sources. These samples

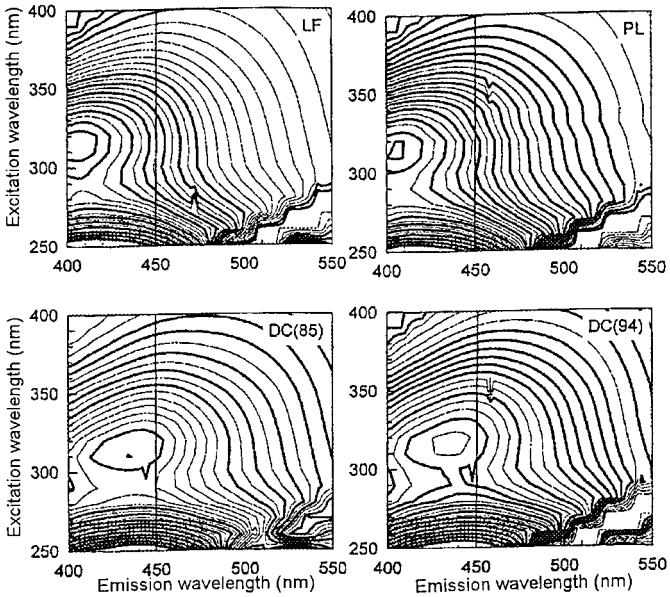

Fig. 1. Three-dimensional excitation-emission matrices (EEMs) for four isolated fulvic acid samples: Lake Fryxell fulvic acid (LF), Pony Lake fulvic acid (PL), and Deer Creek fulvic acid collected during snowmelt in 1985 (DC85) and in 1994 (DC94). A line for the emission at 450 nm is shown.

represent a range of chemical characteristics of fulvic acids in headwater catchments that could result from differences in source and from geochemical controls on solubility.

Fluorescence excitation-emission matrices of isolated fulvic acids—The EEMs for four fulvic acids are shown in Fig. 1. All of our EEMs reveal two fluorophores, as observed in previous studies of humic substances (e.g., Goldberg and Weiner 1989), although the peak of the first fluorophore occurs at a lower excitation wavelength than is shown in the figure. The two microbially derived fulvic acids from Lake Fryxell and Pony Lake have very similar spectra. One fluorophore had an emission peak at 406 nm for an excitation of 320 nm, and another had an emission peak at 412 nm for an excitation of 230 nm (not shown in Fig. 1). The intensity of the emission peak for the first fluorophore is approximately threefold smaller than that for the second fluorophore.

The spectra for the terrestrially derived fulvic acids from Deer Creek sampled in 1985 and in 1994 are very similar to each other and are clearly different from the those of the microbially derived fulvic acids. The peaks for both fluorophores (peak for the first fluorophore is shown in Fig. 1) have maximum values at the same excitation wavelengths as was observed for the Lake Fryxell and Pony Lake fulvic acids: 320 and 230 nm for the first and second fluorophores, respectively. However, the emission maximum associated with these peak values occurred at much longer wavelengths than in Lake Fryxell and Pony Lake; the emission peak for the first fluorophore is at 440 nm, and that for the second fluorophore 426 nm. The peak for the first fluorophore is broader in the Deer Creek fulvic acids than in Lake Fryxell and Pony Lake, as indicated by greater spacing between intensity contours (Fig. 1). The position of the second fluorophore relative to the scattering signal makes this peak less readily detected. For this second peak, there is also more of a possibility that absorbance of light in that wavelength range by other solutes, such as nitrate, would cause an interference.

The differences in the EEMs for microbially and terrestrially derived fulvic acids match well with differences observed by Coble (1996) between EEMs for marine and freshwater samples. Therefore, our results support the hypothesis put forward by Coble (1996) that new phytoplankton production accounts for the differences in fluorescence between the marine and freshwaters sampled in that study. Although the Antarctic freshwaters were chosen as being true end members, where DOM and the associated fulvic acids are microbially derived, they are not likely to be unique. The EEMs for the Lake Fryxell and Pony Lake fulvic acids may be representative of the many eutrophic freshwaters in temperate regions, where algal biomass may be a dominant source of DOM.

Fluorescence spectra with a 370 nm excitation: isolated fulvic acids—The results from the EEMs suggest that the first fluorophore could be characterized at a single excitation wavelength. We evaluated this possibility by examining more aquatic fulvic acids isolated from diverse environments (Tables 1 and 2).

Figure 2 presents the fluorescence spectra at an excitation of 370 nm for selected fulvic acid solutions from the end-member environments. The peak emission intensity occurs at a lower wavelength for the seven samples in which the DOM is microbially derived (Fig. 2a) than the four samples in which the fulvic acids originate from plants and soils of the landscape (Fig. 2b). We can compare the spectra by dividing the peak intensity (shown in Fig. 2) by the DOC concentration of the solutions (see Table 2). For the fulvic acid isolates, at an excitation of 370 nm the maximal emission intensity per unit carbon ranged from 18.0 to 47.9, with the highest and lowest values both being for microbially derived fulvic acids. The wide, overlapping range suggests that the intensity of fluorescence per mg carbon will not be a reliable stand alone diagnostic for chemical characteristics.

The finding that the microbially derived fulvic acids had emission maxima at lower wavelengths than terrestrially derived fulvic acids was confirmed (Table 2). The eight fulvic acid samples from the microbially derived end-member environments had emission maxima between 442 and 448 nm. The spectra from the seven samples from terrestrially derived end-member environments had emission maxima occurring at higher wavelengths between 457 and 461 nm. The five fulvic acid samples from large rivers had emission maxima between 451 and 460 nm, consistent with the expected dominance of terrestrial sources of dissolved fulvic acid in large rivers. The fulvic acids from the Shingobee area had emission maxima ranging from 450 nm for the groundwater to 455 nm for the Shingobee River during snowmelt.

As indicated in the EEMs, these spectra also show a relatively broad emission peak for the terrestrially derived fulvic acids. The differences in the breadth of the peak can be represented by taking the ratio of the emission intensity at

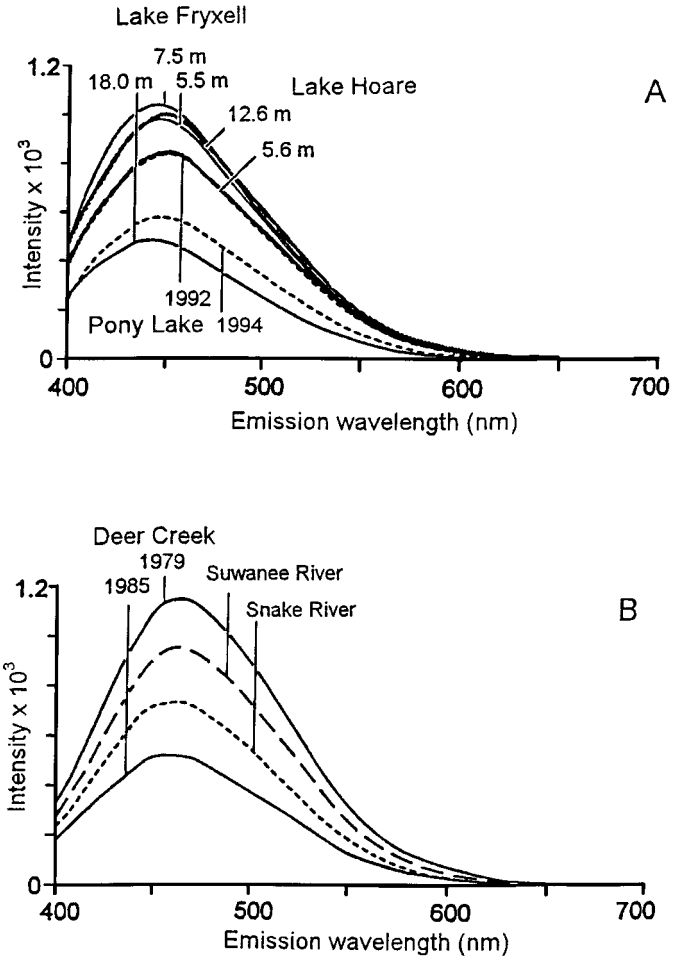

Fig. 2. 370-nm spectra for fulvic acid samples. (A) Algal derived fulvic acids and (B) plant/soil derived fulvic acids.

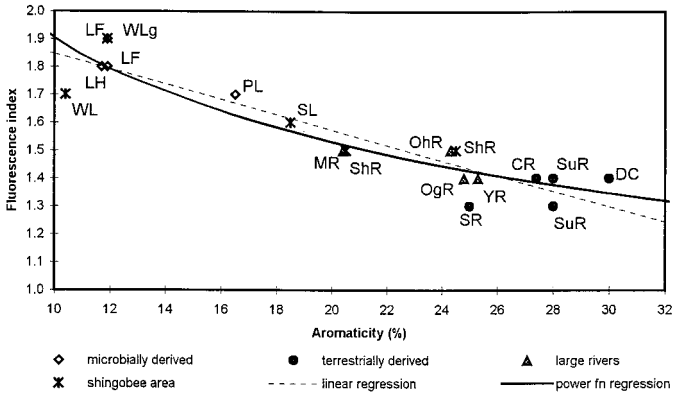

Fig. 3. Plot of fluorescence index vs. aromaticity for isolated fulvic acid samples, which was calculated as the ratio of the area of aromatic carbon region to the total area of the ^{13}C-NMR spectrum, as described by McKnight et al. (1994) (see Table 1 for site abbreviations). The linear regression equation is $y = -0.027x + 2.1$ ($r^2 = 0.85$), and the power function regression is $y = 3.94x^{-0.316}$, where x is aromaticity and y is fluoersence index.

two different wavelengths. We chose 450 nm as a wavelength intermediate between the emission maxima for the Lake Fryxell and Suwannee River fulvic acids and 500 nm as a wavelength at which the emission intensity was approximately half of the maximum intensity for the microbially derived samples. The ratio of emission intensity at 450–500 nm is ~1.9 for the microbially derived samples but ~1.4 for the terrestrially derived samples (Table 2). This ratio is a simple index that represents the greater decline in emission with increasing wavelengths for the microbially derived samples and is referred to as the ''fluorescence index'' in the following discussion. The fluorescence index values determined for the Lake Fryxell and Suwannee River fulvic acids run at pH 2 and 7.5 were similar.

The four large river samples had similar fluorescence index values (1.4–1.5), which were consistent with predominantly terrestrial sources. The five fulvic acids from the Shingobee area had a range of values for fluorescence index; the value for the groundwater sample was 1.9, and the value for the Shingobee River during snowmelt was 1.4. This variation in fluorescence index matches the variation in emission maxima, suggesting a microbial source for the groundwater fulvic acid and a plant/soil source in the river during snowmelt.

A plot of the fluorescence index versus aromaticity (percent sp^2-hybridized carbon as determined by ^{13}C-NMR [nuclear magnetic resonance]) is shown in Fig. 3. The microbially derived fulvic acids have aromaticities of 12%–17 % and fluorescence indices of 1.7–2.0. For the terrestrially derived end member, as represented by fulvic acids from mountain streams and the Suwannee River, the aromaticities range from 25% to 30%, and the fluorescence indices range from 1.3 to 1.4. Compared with the terrestrially derived end-member samples, the large river fulvic acids have slightly lower aromaticities (20.5%–25.3%) and slightly higher fluorescence indices (1.4–1.5). However, aromaticity should not be estimated based only on a fluorescence measurement without other supporting data such as molar absorptivity at 280 or 254 nm (Chin et al. 1994). First, aromaticity may be altered by geochemical processes such, as sorption to mineral surfaces and photobleaching, without causing a change in the fluorescence index. As shown in Fig. 3, the fulvic acid sample isolated from the acidic Snake River has a lower aromaticity because of removal of more aromatic fulvic acids by iron oxide sorption (McKnight et al. 1992), but the sample retained a ''terrestrial'' fluorescence index. Secondly, although the linear regression between aromaticity and fluorescence index has an R^2 of 0.85, a linear relationship could extrapolate to negative aromaticities if some microbially derived fulvic acids had fluorescence indices >2.1. The relationship may be more complicated than a simple linear relationship, possibly because of the contribution of olefinic carbons to the aromaticity of microbially derived fulvic acids as measured by ^{13}C-NMR (McKnight et al. 1991). Nonetheless, the reasonable correspondence between aromaticity and fluorescence index suggests that this ratio may serve as a surrogate for general structural features of the carbon skeleton which are related to source organic material.

The variation within the fulvic acids from the Shingobee area fell along the line defined by the end-member fulvic

acids. The sample from groundwater seeping into Williams Lake had the lowest aromaticity and the highest fluorescence index. The fulvic acid from the Shingobee River during snowmelt had the greatest aromaticity and the lowest fluorescence index. In the summer, the fulvic acids in Williams and Shingobee Lakes have characteristics intermediate between those of the groundwater fulvic acid and the fulvic acid from the river at snowmelt. Both lower flows and higher algal productivity in summer would tend to enhance the relative contribution of microbially derived fulvic acid. Thus, the fluorescence results for these samples suggest temporal and spatial variations in fulvic acid sources for natural waters in this area.

Fluorescence spectra with a 370-nm excitation: filtered whole water samples—The utility of fluorescence measurements in identifying characteristics and sources of the fulvic acid fraction of the DOM was investigated for lakes and ponds in the Bunger Hills, where we expected that the fulvic acids would be derived from algal material. DOC concentrations are higher in lakes that are ice free in summer than in glacial-contact, perennially ice-covered lakes (such as Ice River). In the ice-free lakes, DOC concentrations are either uniform with depth or increase with depth, similar to trends in lakes in the McMurdo Dry Valleys. The lower DOC concentrations in the ice-covered lakes probably are caused by low algal productivity due to the light attenuation and by dilution by glacial meltwater (Doran et al. 1996). In some dilute ice-covered lakes, the surface waters had greater DOC concentrations than bottom waters.

Fluorescence spectra were obtained for 19 filtered whole water samples with DOC concentrations >2.0 mg C L^{-1} (Fig. 4 and Table 3). Of these spectra, 14 were similar to those for microbially derived fulvic acids, with fluorescence indexes of 2.2–1.7 and wavelength of the emission maxima of 437–445 nm. We can use these results to infer that fulvic acids in most lakes in the Bunger Hills are similar to those in the McMurdo Sound region and have similar chemical characteristics, such as lower aromaticity and absorptivity and higher nitrogen content, compared with terrestrially derived fulvic acids.

Five Bunger Hills samples had fluorescence spectra showing greater fluorescence intensity at lower wavelengths than the microbially derived fulvic acids, with the emission peak occurring at wavelengths 10 nm lower and higher fluorescence indices. Three of these samples were from surface waters (BW-5, 0 m; BW-40, 0 m; and BW-43, 0 m). Furthermore, in lakes BW-40 and BW-43, the fluorescence index decreased with depth, whereas the wavelength of the emission peak increased. Both values at depth were within the normal range for microbially derived fulvic acids. These lakes have ice-free conditions similar to Pony Lake. Therefore, we do not expect the exposure to high light intensity at the surface to have caused a shift in the fluorescence properties of the fulvic acid (McKnight et al. 1994). Rather, the greater fluorescence at lower wavelengths may be associated with contributions to the fluorescence spectra by nonhumic organic compounds directly released by growing algae, as proposed by Coble et al. (1990) and Mopper and Schultz (1993). Thus, a downward shift in the emission peak and

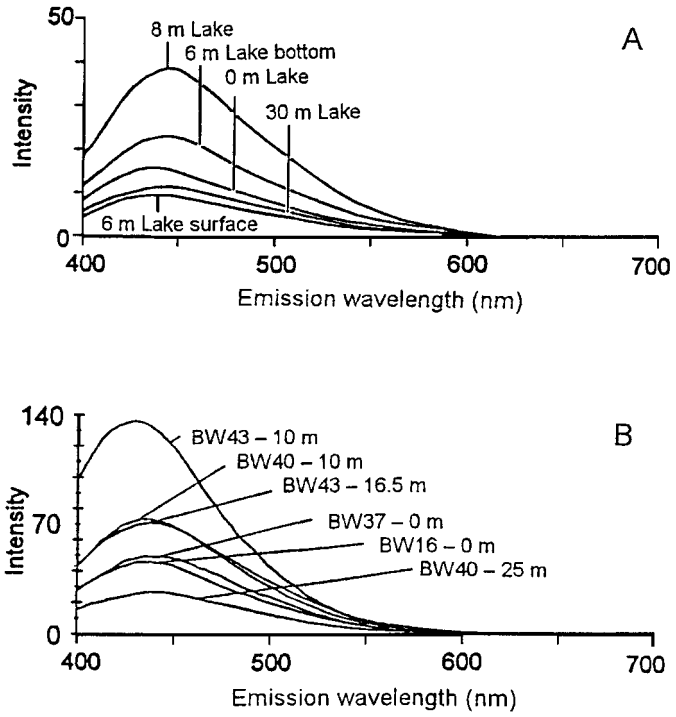

Fig. 4. 370-nm spectra for filtered whole water samples from the Bunger Hills region of Antarctica. (A) 1991/1992 expedition, (B) 1993/1994 expedition.

fluorescence indices >2 might identify samples for which this approach would not be applicable, and the fluorescence index would not accurately identify source material.

The fluorescence spectra for a time series of filtered whole water samples from Deer Creek collected during the spring snowmelt are shown in Fig. 5, and the emission maxima and fluorescence index are shown in Table 3. DOC concentrations and absorbances at 254 nm increased by a factor of ~2 as snowmelt flushed organic-rich water from upper soil horizons. Because the location of melting snow progresses up valley to higher elevations and from forest to alpine tundra, it is possible that the characteristics of the fulvic acids could change during snowmelt. The fluorescence data, however, show that no consistent shift occurred in the spectra during the snowmelt period. The constancy of the fluorescence spectra could be used to justify an assumption that there were no major shifts in the chemical properties of the fulvic acid in the stream, despite changes in hydrologic source areas. The slightly lower wavelengths for the emission maxima (453 vs. 458 nm) for the filtered whole water samples from Deer Creek, compared with the isolated fulvic acids, could be attributed to other nonhumic fluorophores in the filtered whole water samples. However, the general similarity of the results for the filtered whole water samples and the isolated fulvic acids suggests that the XAD-8 isolation method yielded a fulvic acid sample representative of the dominant fluorophore. Green and Blough (1994) emphasized the potential influence of isolation method in interpreting fluorescence of natural waters.

Table 3. Chemical characteristics of filtered whole water samples (used for 370 nm scans).

Site	Location and sampling date (and depth, if applicable)	DOC of natural water (mg C L^{-1})	Peak emission wavelength (nm)	Fluorescence index†
Antarctic Lakes: Bunger Hills (Melles et al. 1994; Doran et al. 1996)				
	0 m lake, 07 Mar 92	16.3	444	1.96
	8 m lake, shore, 07 Mar 92	20.3	444	1.90
	6 m lake, 0 m, 14 Feb 92	2.5	444	1.93
	6 m lake, 5.5 m, 14 Feb 92	22.6	444	1.80
	30 m lake, shore, 06 Mar 92	6.4	444	2.06
	BW-5, 0 m‡	4.6	429	2.82
	BW-53, 2 m	12.0	445	1.71
	BW-16, 0 m	11.7	439	2.06
	BW-33, 0 m	7.2	439	2.12
	BW-33, 52 m	11.1	438	2.16
	BW-37, 0 m	12.2	440	2.01
	BW-40, 1 m	4.4	436	2.31
	BW-40, 10 m	15.2	438	2.21
	BW-40, 25 m	7.0	442	2.05
	BW-43, 0 m	6.2	431	2.71
	BW-43, 3 m	5.0	436	2.46
	BW-43, 10 m	9.0	432	2.43
	BW-43, 16.5 m	7.9	440	2.01
	BW-50, 0.5 m	9.6	444	1.96
	BW-50, 35 m	10.3	437	2.12
U.S. streams: Colorado Rocky Mountains (McKnight et al. 1992, 1994)				
	Deer Creek, 26 Apr 94	2.9	454	1.40
	Deer Creek, 06 May 94	3.2	454	1.48
	Deer Creek, 14 May 94	3.8	454	1.44
	Deer Creek, 20 May 94	4.1	454	1.40
	Deer Creek, 06 Jun 94	2.8	454	1.44
	Deer Creek, 21 Jun 94	2.5	453	1.40
	Deer Creek, 21 Sep 94	1.3	452	1.46

* Wavelength (nm) of peak emission intensity at 370 nm excitation.
† Fluorescence index is ratio of emission intensity (450 nm/500 nm) at 370 nm excitation.
‡ All samples with BW- in the title were collected during the 1993/94 season.

Potential applications

There is now a recognition that DOM plays an important role in structuring freshwater ecosystems. Because of the inherent chemical complexity of DOM, there are few simple chemical analyses to readily characterize DOM in meaningful ways in limnological studies. The measurement of fluorescence as described here could be used as an indicator of the general source of the dissolved fulvic acid in a lake, stream, or river, which may also be related to the aromaticity of the dissolved fulvic acid. In this context, it is important to recall that although the Snake River fulvic acid had a lower aromaticity because of fractionation by sorption on iron oxides (McKnight et al. 1992) and probable attack by hydroxyl radical from photoreduction, it retained a clear, terrestrial signature.

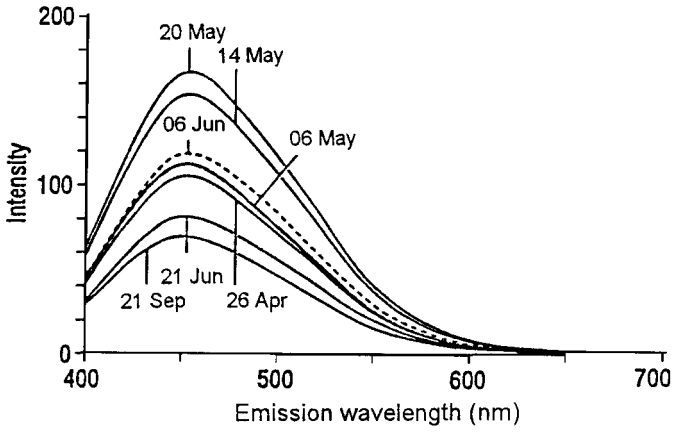

Fig. 5. 370-nm spectra for filtered whole water samples from Deer Creek, Colorado.

A measurement indicative of fulvic acid source could provide useful ancillary or supporting data in determining a carbon budget for a freshwater ecosystem. For example, fluorescence characteristics intermediate between those of the two end-member fulvic acids may indicate that both microbially and terrestrially derived organic material are significant sources of fulvic acid. The intermediate fluorescence indices of Williams and Shingobee Lake suggest more than one fulvic acid source (Table 2). The quantitative interpretation of variation in the fluorescence index will depend on the analytical instruments, the DOC concentrations, and the number of replicate samples and sampling sites in a study.

With these considerations in mind, in general, a difference in the fluorescence index of at least 0.1 may be indicative of a difference in source of fulvic acid. Interpretation of small differences in fluorescence index could be supported by also determining differences in the peak emission wavelength, which can be determined very accurately (± 1 nm) with most fluorometers. Increases in fluorescence index should be correlated with decreases in peak emission wavelength.

However, the quantitative use of the fluorescence index to assign proportions of precursor organic material is significantly constrained by the variation in fluorescence intensity per mg C of the different fulvic acids. For example, the fluorescence intensity as a function of DOC concentration varied by a factor of >2 for both sets of end-member fulvic acids. Thus, calculation of the relative proportion of each source from the fluorescence spectra with an intermediate fluorescence index is not recommended, although this issue could be addressed in future laboratory experiments. A second important caution is fluorescence data should be properly corrected for instrumental biases—otherwise, the fluorescence index may be slightly dependent on fluorometer configuration because of the influence of the emission monochromater grating and the light detector efficiency. A calibration using fulvic acids of known origin, such as the IHSS Suwannee River fulvic acid, is recommended. Finally, the fluorescence index may not be indicative of the source of nonhumic components of the DOM.

The fluorescence spectra or the fluorescence index could be used in combination with other limnological and water-quality data to infer fulvic acid characteristics relevant to specific biogeochemical cycles and ecological processes in lakes and streams. In a study of the Surumoni River, a blackwater tributary of the Orinoco River in Venezuela, Battin (1998) used the fluorescence index, along with measurements of absorbance and DOC, to infer that the downriver reaches of the Surunomi River received autochthonous sources of DOC from near-channel water bodies. Another potential application would be in selecting parameters to be used in spectral models for visible and UV light attenuation in lakes, which have been shown to be influenced by DOC quality as well as DOC concentration (Laurion et al. 1997). In modeling the light regime of a lake under different conditions, DOC-related parameters could be chosen on the basis of a measurement of fluorescence index. Further, it has been found that the metal-binding properties of fulvic acid can vary significantly with source (Breault et al. 1996), and fluorescence may be a useful tool in refining metal-fulvic acid formation constants for application of metal-ion binding models to field studies (Benedetti et al. 1996).

This method may be useful for sorting large sample sets to be tested by other characterization methods or for characterization of archived samples. For example, Donahue et al. (1998) characterized archived samples from experimentally acidified lakes in the Experimental Lakes Area, in northwestern Ontario, to show that acidification caused a change in the quality of the DOC to a more ''autochthonous-like'' (or microbially derived) quality, compared with the allochthonous (or terrestrially derived) quality characteristic of control lakes, and that the DOC quality recovered after acidification.

References

AIKEN, G. R., AND J. A. LEENHEER. 1993. Isolation and chemical characterization of dissolved and colloidal organic matter. Chem. Ecol. **8:** 135–151.

———, D. MCKNIGHT, R. HARNISH, AND R. WERSHAW. 1996. Geochemistry of aquatic humic substances in the Lake Fryxell Basin, Antarctica. Biogeochemistry **34:** 157–188.

AVERETT, R. C., J. A. LEENHEER, D. M. MCKNIGHT, AND K. A. THORN [EDS.]. 1989. Humic substances in the Suwanee River, Georgia: Interactions, properties, and proposed structures. U.S. Geological Survey open-file report 87-557.

BATTIN, T. J. 1998. Dissolved organic matter in a blackwater tributary of the upper Orinoco River, Venezuela. Org. Geochem. **28:** 561–569.

BENDETTI, M. F., W. H. VANRIEMSDIJK, L. K. KOOPAL, D. G. KINNIBURGH, D. C. GOODDY, AND C. J. MILNE. 1996. Metal ion binding by natural organic matter: From the model to the field. Geochim. Cosmochim. Acta **60:** 2503–2513.

BOYER, E. W., G. W. HORNBERGER, K. E. BENCALA, AND D. M. MCKNIGHT. 1997. Response characterisitics of DOC flushing in an alpine catchment. Hydro. Process. **11:** 1635–1647.

BREAULT, R. F., J. A. COLMAN, G. R. AIKEN, AND D. M. MCKNIGHT. 1996. Copper speciation and binding by organic matter in stream water. Environ. Sci. Technol. **30:** 3477–3486.

CARTER, V., P. T. GAMMON, D. O. ROSENBERRY, AND M. TURTORA. 1993. Aquatic macrophytes and selected physical properties of Shingobee and Williams Lakes, Minnesota, 1991–92. U.S. Geological Survey open-file report 93-143.

CHIN, Y., G. R. AIKEN, AND E. O'LOUGHLIN. 1994. Molecular weight, polydispersity, and spectroscopic properties of auatic humic substances. Environ. Sci. Technol. **28:** 1853–1858.

COBLE, P. G. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. Mar. Chem. **51:** 325–346.

———, S. A. GREEN, N. V. BLOUGH, AND R. B. GAGOSIAN. 1990. Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. Nature **348:** 432–435.

DE SOUZA SIERRA, M. M., O. F. X. DONARD, M. LAMOTTE, C. BELIN, AND M. EWALD. 1994. Fluorescence spectroscopy of coastal and marine waters. Mar. Chem. **47:** 127–144.

DONAHUE, W. F., D. W. SCHINDLER, S. J. PAGE, AND M. P. STAINTON. 1998. Acid-induced changes in DOC quality in an experimental whole-lake manipulation. Environ. Sci. Technol. **32:** 2954–2960.

DORAN, P., C. P. MCKAY, M. A. MEYER, D. T. ANDERSEN, R. A. WHARTON, JR., AND J. T. HASTINGS. 1996. Climatology and implications for perennial lake ice occurrence at Bunger Hills Oasis, East Antarctica. Antarct. Sci. **8:** 289–296.

EWALD, M., AND C. BELIN. 1987. Fluorescence from photic zone water in the Atlantic Ocean. J. Sci. Total Environm. **62:**149–155.

———, ———, AND P. BERGER. 1983. Spectrofluorimetry of humic substances from estuarine waters: Progress of the technique, p. 461–466. *In* R. F. Christman and E. T. Gjessing [eds.], Aquatic and terrestrial humic materials. Ann Arbor Science, Ann Arbor, Michigan.

GOLDBERG, M. C., AND E. R. WEINER. 1989. Fluorescence measurements of the volume, shape, and fluorophore composition of fulvic acid from the Suwanee River, p. 179–204. *In* Humic substances in the Suwanee River, Georgia: Interactions, prop-

erties, and proposed structures. U.S. Geological Survey open-file report 87-557.

GREEN, S. A., AND N. V. BLOUGH. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. Limnol. Oceanogr. **39:** 1903–1916.

———, F. M. M. MOREL, AND N. V. BLOUGH. 1992. Investigation of the electrostatic properties of humic substances by fluorescence quenching. Environ. Sci. Technol. **26:** 294.

HAYASE, K., H. TSUBOTA, AND I. SUNADA. 1988. Vertical distribution of fluorescent organic matter in the North Pacific. Mar. Chem. **25:** 373–381.

HORNBERGER, G. M., K. E. BENCALA, AND D. M. MCKNIGHT. 1994. Hydrological controls on dissolved organic carbon during snowmelt in the Snake River near Montezuma, Colorado. Biogeochemistry **25:** 147–165.

KLOKOV, V., E. KAUP, R. ZIERATH, AND D. HAENDEL. 1990. Lakes of the Bungar Hills (East Antarctica): chemical and ecological properties. Pol. Polar Res. **11:** 147–159.

LAURION, I., W. F. VINCENT, AND D. R. S. LEAN. 1997. Underwater ultraviolet radiation: Development of spectral models for the northern high latitude lakes. Photochem. Photobiol. **65:** 107–114.

LEENHEER, J. A., D. M. MCKNIGHT, E. M. THURMAN, AND P. MACCARTHY. 1989. Structural components and proposed structural models of fulvic acid from the Suwannee River, p. 331–360. *In* R. C. Averett, J. A. Leenheer, D. M. McKnight, and K. A. Thorn [eds.], Humic substances in the Suwanee River, Georgia: Interactions, properties, and proposed structures. U.S. Geological Survey open-file report 87-557.

LOCHMULLER, C. H., AND S. S. SAAVEDRA. 1986. Conformational cahnges in a soil fulvic acid measured by time-dependent fluorescence depolarization. Anal. Chem. **58:** 1978–1981.

MALCOLM, R. 1990. The uniqueness of humic substances in each of soil, stream, and marine environments. Anal. Chim. Acta **232:** 19–30.

———, D. M. MCKNIGHT, AND R. C. AVERETT. 1989. History and description of the Okefenokee Swamp—origin of the Suwannee River, p. 1–22. *In* R. C. Averett, J. A. Leenheer, D. M. McKnight, and K. A. Thorn [eds.], Humic substances in the Suwanee River, Georgia: Interactions, properties, and proposed structures. U.S. Geological Survey open-file report 87-557.

MCKNIGHT, D. M., G. R. AIKEN, AND R. L. SMITH. 1991. Aquatic fulvic acids in microbially based ecosystems: Results from two desert lakes in Antarctica. Limnol. Oceanogr. **36:** 998–1006.

———, E. D. ANDREWS, S. A. SPAULDING, AND G. R. AIKEN. 1994. Aquatic fulvic acids in algal-rich Antarctic ponds. Limnol. Oceanogr. **39:** 1972–1979.

———, K. E BENCALA, G. W. ZELLWEGER, G. R. AIKEN, G. L. FEDER, AND K. A.THORN. 1992. Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. Environ. Sci. Technol. **26:** 1388–1396.

———, R. L. SMITH, R. A. HARNISH, C. L. MILLER, AND K. E. BENCALA. 1993. Seasonal relationships between planktonic microorganisms and dissolved organic material in an alpine stream. Biogeochemistry **21:** 39–59.

MELLES, M. [ED.]. 1994. Reports on polar research: The expeditions NORILSK/TAYMYR 1993 and BUNGAR OASIS 1993/94 of the AWI research unit Potsdam. Ber. Polarforsch. 148.

———, T. KULBE, P. P. OVERDUIN, AND S. VERKULICH. 1994. The expedition BUNGAR OASIS 1993/94 of the AWI Research Unit Potsdam. *In* M. Melles [ed.], Reports on polar research: The expeditions NORILSK/TAYMYR 1993 and BUNGAR OASIS 1993/94 of the AWI research unit Potsdam, p. 29–80. Ber. Polarforsch. 148.

MOBED, J. J., S. L. HEMMINGSEN, J. L. AUTRY, AND L. B. MCGOWN. 1996. Fluorescence characterization of IHSS humic substances: Total luminescence spectra with absorbance correction. Environ. Sci. Technol. **30:** 3061–3065.

MOPPER, K., AND C. A. SCHULTZ. 1993. Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. Mar. Chem. **41:** 229–238.

RECKHOW, D. A., P. C. SINGER, AND R. L. MALCOLM. 1990. Chlorination of humic materials: Byproduct formation and chemical interpretations. Environm. Sci. Technol. **24:** 1655–1664.

ROSENBERRY, D. O., J. W. LABAUGH, T. M. MCCONNAUGHEY, R. G. STRIEGL, AND T. C. WINTER. 1993. The interdisciplinary research initiative: Hydrologic research in the Shingobee headwaters area, Minnesota. U.S. Geological Survey open-file report 93–446.

SCULLY, N. M., AND D. R. S. LEAN. 1994. The attenuation of ultraviolet light in temperate lakes. Arch. Hydrobiol. **43:** 135–144.

STEWART, A. J., AND R. G. WETZEL. 1980. Fluorescence : absorbance ratios—a molecular-weight tracer of dissolved organic matter. Limnol. Oceanogr. **25:** 559–564.

———, AND ———. 1981. Asymetrical relationships between absorbance, fluorescence, and dissolved organic carbon. Limnol. Oceanogr. **26:** 590–597.

THEOBALD, P. K., H. W. LAKIN, AND D. B. HAWKINS. 1963. The precipitation of aluminum, iron, and manganese at the junction of Deer Creek with the Snake River in Summit County, Colorado. Geochim. Cosmochim. Acta **27:** 121–132.

THURMAN, E. M., AND R. L. MALCOLM. 1981. Preparative isolation of aquatic humic substances. Environ. Sci. Technol. **15:** 463–466.

VERKULICH, S. R., AND A. HILLER. 1994. Holocene deglaciation of Bungar Hills revealed by ^{14}C measurements on stomach oil deposits in snow petrel colonies. Antarct. Sci. **6:** 395–400.

WESTERHOFF, P. 1995. Ozone oxidation of bromide and natural organic matter. Ph.D. dissertation, Department of Civil, Environmental, and Architectural Engineering, University of Colorado.

Received: 26 May 2000
Amended: 19 September 2000
Accepted: 26 September 2000