Limnology (2004) 5:155–163
DOI 10.1007/s10201-004-0130-9

RESEARCH PAPER

Kazuhide Hayakawa · Mitsuru Sakamoto
Michio Kumagai · Chunmeng Jiao · Xueliang Song
Zixiong Zhang

Fluorescence spectroscopic characterization of dissolved organic matter in the waters of Lake Fuxian and adjacent rivers in Yunnan, China

Received: April 5, 2004 / Accepted: September 1, 2004

Abstract The fluorescence properties of dissolved organic matter (DOM) in the water of Lake Fuxian and its adjacent rivers on the Yunnan Plateau, southwestern China, were studied to specify the characterization of DOM in the lake and river waters. The fluorescence properties with the excitation–emission matrix in the water of Lake Fuxian are different from those in the river water. The differences in these properties between the lake and river water could arise not only from their sources but also from the reactivity of the photobleaching of DOM. In the lake, the supplying of allochthonous fluorescent materials from inflowing rivers to the fluorescent DOM is less significant than the photobleaching of fluorescent substances.

Key words Fluorescence · Excitation–emission matrix · Lake Fuxian · Dissolved organic carbon · Photobleaching

Introduction

Fluorescence spectroscopy has been preferentially applied to the study of dissolved organic matter (DOM) in natural waters (e.g., Mopper and Schultz 1993) because the technique is simple, sensitive, and effective for characterizations of the nature and sources of DOM. Recent studies of fluorescence analysis using an excitation–emission matrix (EEM) have revealed two distinct types of fluorescent substances in natural waters. One is the fluorescence originating from proteins (protein-like fluorescence) and the other is the fluorescence of humic substances supplied from terrestrial and aquatic sources (humic-like fluorescence) (Mopper and Schultz 1993; Coble 1996). Some variations in humic-like fluorescence were observed for fluorophores based on chemical structure, molecular weight, and binding with metals (Spark and Swift 1994; Nagao et al. 2003). Although aquatic humic substances of diverse origins have similar fluorescence spectra, there is a distinct difference in the spectra between marine humic fluorophores and terrestrial humic fluorophores (e.g., Coble 1996, Chen et al. 2002).

The fluorescence spectra of fulvic acids of terrestrial origin isolated from streams and rivers are different from those from lakes containing autochthonous materials from microbial sources (McKnight et al. 2001). Hayakawa et al. (2003) found that the fluorescence spectra of DOM in Lake Hovsgol, a large lake in Mongolia, are different from those of DOM in its adjacent rivers. These reports suggested the possibility that the fluorescence is diluted by underground water or changed by photobleaching within the lake. However, few evaluations have been made of the external supply and in-lake alteration of fluorescence in large lakes such as deep tectonic lakes. In general, the allochthonous input of organic matter from the watershed is lower than the autochthonous production in large lakes (Wetzel 2001). Allochthonous fluorescence was found to be susceptible to microbial and photochemical alterations in aquatic environments (Moran and Zepp 1997). To use fluorescence as a reliable characterization of DOM in lakes, we need to verify its supply and possible alteration through the watershed and within the lake. This would be useful in evaluating not only DOM characterization but also terrestrial organic matter in the carbon cycle throughout lake–watershed ecosystems.

To provide basic information on this subject, we surveyed fluorescence in the waters of oligotrophic Lake Fuxian, a large tectonic lake in Yunnan, China, and its adjacent rivers. In this paper, we characterized DOM in lake and river waters by fluorospectroscopy using EEMs and assessed both the external supply of DOM from rivers and its in-lake alteration.

K. Hayakawa (✉) · M. Kumagai · C. Jiao
Lake Biwa Research Institute, 1-10 Uchidehama, Otsu 520-0806, Japan
Tel. +81-77-526-4800; Fax +81-77-526-4803
e-mail: hayakawa@lbri.go.jp

M. Sakamoto
School of Environmental Science, The University of Shiga Prefecture, Hikone, Japan

X. Song · Z. Zhang
Yunnan Institute of Geological Science, Kunming, China

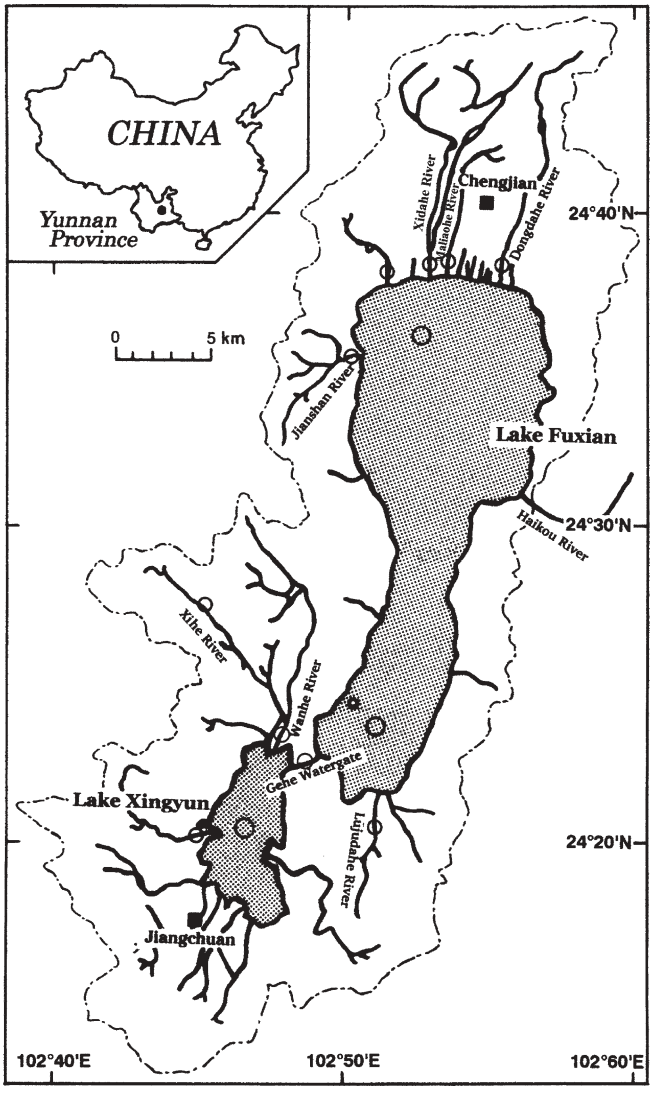

Fig. 1. Map of Lake Fuxian and its inflowing rivers. *Circles* denote sampling stations in this study

Materials and methods

Lake Fuxian is a deep tectonic lake located at an altitude of 1721 m on the Yunnan Plateau in southwestern China (Fig. 1). The lake has a surface area of 212 km^2 and a mean depth of 89.6 m. In the watershed of 1044.6 km^2, the lowland is occupied by large farms and many small towns, which have approximately 280 000 total population (Jin 1995). More than 20 rivers flow into the lake from the surrounding hills. The only outflow from the lake is via the Haikou River. While flowing through farms and towns, river waters are contaminated with domestic and industrial organic wastes. Lake Xingyun, upriver from Lake Fuxian, is seriously eutrophied and characterized by an abundance of nuisance cyanobacterial blooms. The eutrophic water of Lake Xingyun flows into oligotrophic Lake Fuxian through the Gehe Watergate. The topographic features of Lake Fuxian, which is surrounded by hills, have protected it from anthropogenic eutrophication for a long time. However, recent economic developments in Chinese society, including industrialization, agricultural cultivation, and deforestation within the watershed, have adversely affected the water quality of the lakes (Hayakawa et al. 2002).

Waters samples were collected by a Niskin water sampler from the center of the northern (24°36.43′N, 102°52.48′E) and southern (24°23.96′N, 102°51.52′E) regions of Lake Fuxian, the center of Lake Xingyun (24°21.21′N, 102°46.17′E), and their nine inflowing rivers in mid-November 2000 and mid-June 2001 (Fig. 1). Water samples from the upper reaches of the Xihe River were collected in mid-November 2000. The collected samples were filtered through precombusted glass fiber filters (Whatman GF/F, Whatman, Kent, UK) and the filter samples and filtrates were kept frozen until chemical analysis. Dissolved organic carbon (DOC) concentration in the filtrates was measured by the high-temperature catalytic-oxidation method using a total organic carbon analyzer (Shimadzu TOC-5000A, Shimadzu, Kyoto, Japan). Fluorescence spectra of the lake and river samples were analyzed using a Hitachi F-4500 spectrofluorometer (Hitachi High-Technologies, Tokyo, Japan). The band pass width was 5 nm for both excitation and emission. The scan speed was 1200 $nm \cdot min^{-1}$ and the scan range was 225–400 nm for excitation and 225–500 nm for emission. The photomultiplier voltage was set to 700 V. The fluorescence data were collected for every 1 nm of emission wavelength and for every 5 nm of excitation wavelength, resulting in an EEM (Hayakawa et al. 2003). Triplicated fluorescence data for each sample were normalized by the integrated intensities of the water Raman scattering band (Determann et al. 1994) and by subtracting the fluorescence of Milli-Q water (Millipore, Billerica, USA). The fluorescence intensity was expressed in Raman units (RU, nm^{-1}) and the fluorescence intensities of the peaks in the EEM were calculated as average intensities over 10-nm excitations and 20-nm emissions. In the EEM spectra drawn by Raman-normalized data, the maximum peak position may be shifted toward longer excitation wavelengths compared with quinine-sulfate normalization, which was used in many previous studies (Yoshioka, personal communication).

Microbial alteration of fluorescence was examined by dark incubation of river water with bacteria. The water samples were collected from a zone 1 km above the mouth of the Wanhe River in May 2002. The collected samples were filtered through GF/C and 0.2-μm Nuclepore filters (Whatman). The GF/C filtrates were kept cool (4°C) and the 0.2-μm filtrates were kept frozen until dark incubation. The 0.2-μm filtrates were sterilized at 120°C for 25 min, poured into two 2-liter glass bottles, and stirred gently. A milliliter of the GF/C filtrates containing bacteria was added to one bottle, and as a control, nothing was added to the second bottle. As the third bottle, the 0.2 μm filtrates which had not been sterilized were also poured into two 2-liter glass bottles. All bottles were capped by a 0.5-μm PTFE membrane filter (Milli Wrap, Millipore, Billerica, USA) and kept in an incubator at 20°C in the dark. After 5, 12, and 20 days, the DOC concentration, EEM, and bacterial

density of each sample were measured. The bacterial density was estimated by the DAPI staining count method (Porter and Feig 1980). A few milliliters of two subsamples from three bottles were filtered by 0.2-μm black Nuclepore filters, and bacteria on the filters were enumerated using an epifluorescent microscope with a standard B excitation system.

Photobleaching of fluorescence was examined by conducting experiments on lake and river water under sunlight exposure. The water samples were collected at a depth of 50 m in Lake Fuxian in January 2003 and from a zone 1 km above the mouth of the Wanhe River in January 2003. The collected samples were filtered through 0.2-μm Nuclepore filters (Whatman), and the filtrates were kept frozen until the photobleaching experiments. Aliquots of 100 ml of the filtrates were poured into two quartz bottles with a height of 110 mm and a diameter of 40 mm. One bottle was exposed to sunlight while the other was wrapped in aluminum foil as a dark control. The experiments were performed in an isothermal water tank at 20°C. Sunlight penetration of the water was monitored with a light-intensity recorder (MDS-MkV/L, Alec Electronics, Kobe, Japan). After sunlight exposure for a few days, the DOC and fluorescence of the samples were measured as described above. The experiments were duplicated and the data were incorporated into one dataset. Asymptotic regression lines to the experimental data were made by computer graphic software (SigmaPlot 2001, SPSS, Chicago, USA).

Results

DOC concentrations of lake and river water

The DOC concentrations in Lake Fuxian, Lake Xingyun, and their adjacent rivers in November 2000 and June 2001 ranged from 53 to 116 μMC, 629 to 658 μMC, and 37 to 428 μMC, respectively (Fig. 2). The DOC concentrations in Lake Xingyun and the Gehe Watergate were about seven times higher than those in Lake Fuxian (Fig. 2) because there was a cyanobacterial bloom in Lake Xingyun and the Gehe Watergate. The DOC concentrations in the inflowing rivers were also higher than those in Lake Fuxian (Fig. 2). Because domestic sewage systems in the watersheds of Lakes Fuxian and Xingyun were improperly installed, sewage flows directly into the rivers (Jin 1995). Most of the river water samples containing organic waste had high DOC concentrations; the upper reaches of streams without influx of sewage had low DOC concentrations (Fig. 2).

Fluorescence properties of DOM

The fluorescence spectra in all the Lake Fuxian samples were similar. The fluorescence properties of DOM in Lake Fuxian were characterized by three strong peaks in the EEM spectra (Fig. 3a). The first peak was in the range shorter than 240 nm of excitation, the second was in the range of Ex/Em 270–280 nm/310–330 nm, and the third was in the range of Ex/Em 330–350 nm/410–440 nm. These three peaks are consistent with the findings from previous studies of fluorescence in lakes and oceans (Coble 1996; McKnight et al. 2001; Hayakawa et al. 2003). In this article, we desig-

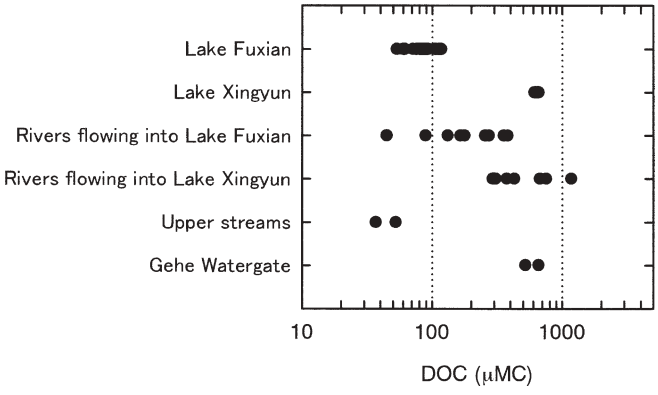

Fig. 2. Dissolved organic carbon (*DOC*) concentrations in Lake Fuxian, Lake Xingyun, and their inflowing rivers in November 2000 and June 2001. *Circles* denote data for each sample

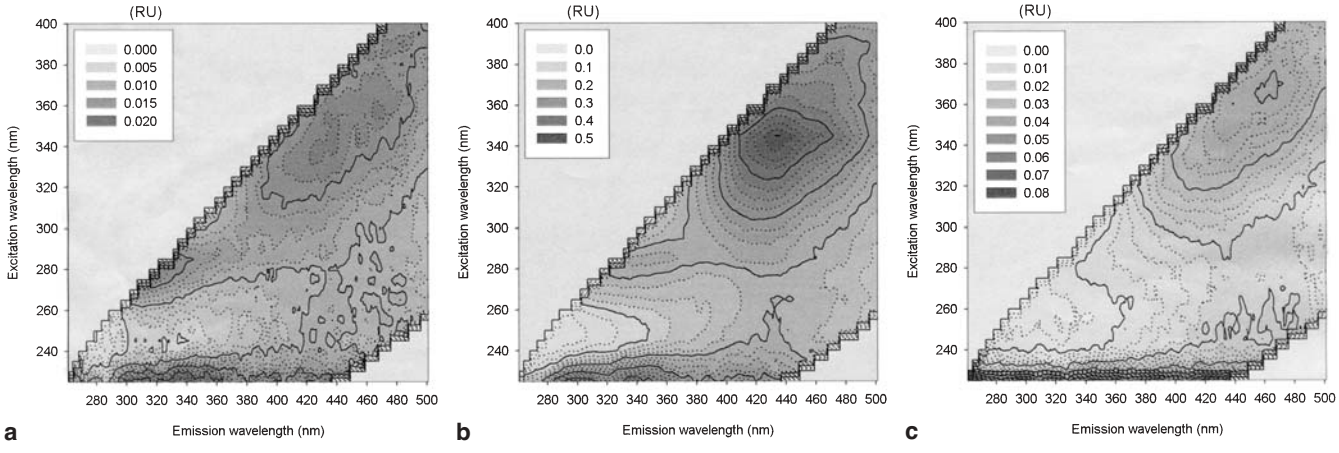

Fig. 3a–c. Fluorescence spectra with an excitation–emission matrix (EEM) of dissolved organic matter in **a** the surface water (0.5 m depth) from Lake Fuxian, **b** the Wanhe River, and **c** the upper reaches of the Xihe River in June 2001. *RU*, Raman Units

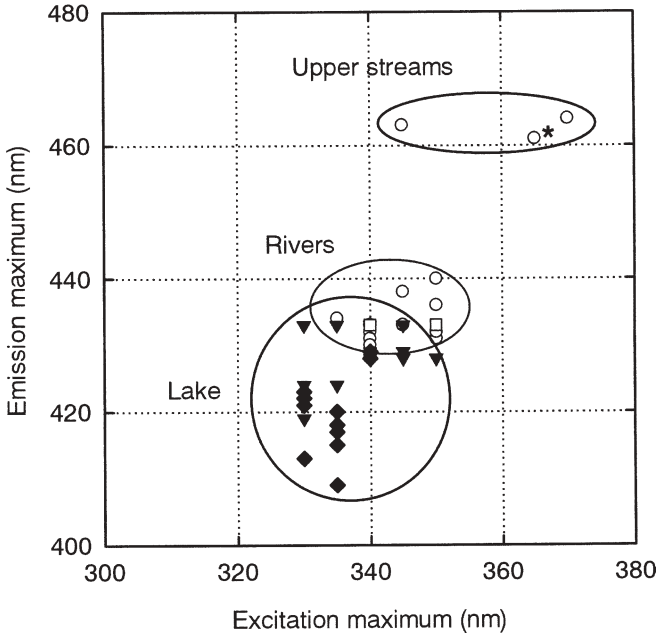

Fig. 4. Peak wavelength positions of excitation and emission wavelengths of humic-like fluorescence in all the water samples from Lake Fuxian and its inflowing rivers. *Triangles* denote lake samples collected in November. *Diamonds* denote lake samples collected in June. *Circles* denote river samples and *squares* denote samples from the Gehe Watergate. The data labeled *upper streams* are from the upper reaches of the Xihe River, except for the point indicated by an *asterisk*, which is from the lower reaches of the Xidahe River

nated the peak at Ex/Em 270–280 nm/310–330 nm as Peak A, and that at Ex/Em 330–350 nm/410–440 nm as Peak B.

The fluorescence properties of DOM in the rivers were also characterized by three strong peak ranges, i.e., under 240 nm of excitation, Ex/Em 280–290 nm/330–350 nm, and Ex/Em 330–350 nm/410–440 nm (Fig. 3b). The fluorescence spectra in all river samples were similar except for those of the reaches upper of the Xihe River and the Xidahe River (Fig. 3c). The upper reaches of the Xihe River with low DOC concentrations exhibited a Peak B in the EEM at Ex/Em 350–370 nm/450–470 nm, which differed from those of the other river samples (Fig. 4). The fluorescence properties of the Xidahe River was an exception among all river samples, being similar to those of the upper reaches of the Xihe River.

The position of Peak B in the lake samples was located at a shorter wavelength than that in the river samples (Fig. 4). The mean positions of Peak B in the lake and river samples were Ex/Em 337 nm/424 nm and Ex/Em 343 nm/434 nm, respectively. The difference was significant ($P < 0.05$). The fluorescence intensities of Peak B in the lake samples were 3–20 times lower than those in the river samples.

Vertical distributions of DOC and fluorescence in the lake

The waters of Lake Fuxian in both November 2000 and June 2001 were vertically stratified with a distinct thermocline at a depth of 20–30 m (Fig. 5a). The surface waters of the stratified lake had a higher DOC concentration than those of the deep water (Fig. 5b). The DOC concentrations in June 2001 were higher at all the depths than those in November 2000.

Fluorescence intensities of Peak A had higher values for surface water than for deep water with a profile similar to the vertical changes in DOC concentration (Fig. 5c). Higher fluorescence intensities for Peak A were observed in June 2001 than in November 2000 at all depths. A significant correlation was found between the DOC concentrations and fluorescence intensities of Peak A in the lake water ($P < 0.05$, Table 1). The intercept of the DOC axis in the graph was at about 20 μMC. The fluorescence intensities of Peak B were vertically almost constant throughout the water column of the lake (Fig. 5c), with no significant difference in the intensity between November and June. As for the position of Peak B, the results for samples collected in June were mostly located at a shorter emission wavelength than those collected in November (Fig. 4).

Distributions of DOC and fluorescence from streams to rivers

The water collected from the upper reaches of the Xihe River without influx for sewage had low DOC concentrations and low fluorescence intensities, whereas those collected downriver with influx for sewage had high DOC concentrations and high fluorescence intensities. Although we do not have any statistical data for sewage loadings in the watershed of Lake Fuxian, a river with influx of industrial sewage, the Wanhe River, and a river flowing through an urban area, the Maliaohe River, had extremely high DOC concentrations and high fluorescence intensities. It is reasonable to conclude that these high DOC and fluorescence intensities were a result of contamination of the river waters with organic waste. The fluorescence intensities of Peaks A and B in river water were linearly correlated with the DOC concentrations ($P < 0.05$, Table 1). The intercepts at the DOC axis for Peaks A and B in the graph were about 17 μMC, and −11.4 μMC, respectively.

Alteration in DOC and humic-like fluorescence of river water during bacterial growth

The DOC concentration in water that was first inoculated with bacteria decreased with time (Fig. 6a) as bacterial numbers in the bottle increased rapidly (Fig. 6b). On the other hand, no change was observed in the DOC concentration, and bacterial numbers were low in the control bottles (Fig. 6a,b). The fluorescence intensities of Peak B in all bottles were almost constant during dark incubation (Fig. 6c). The maximum peak positions of Peak B in all the bottles did not change during the experiment. These results show that the humic-like fluorescence of Peak B did not change during the decomposition of labile DOM by bacteria.

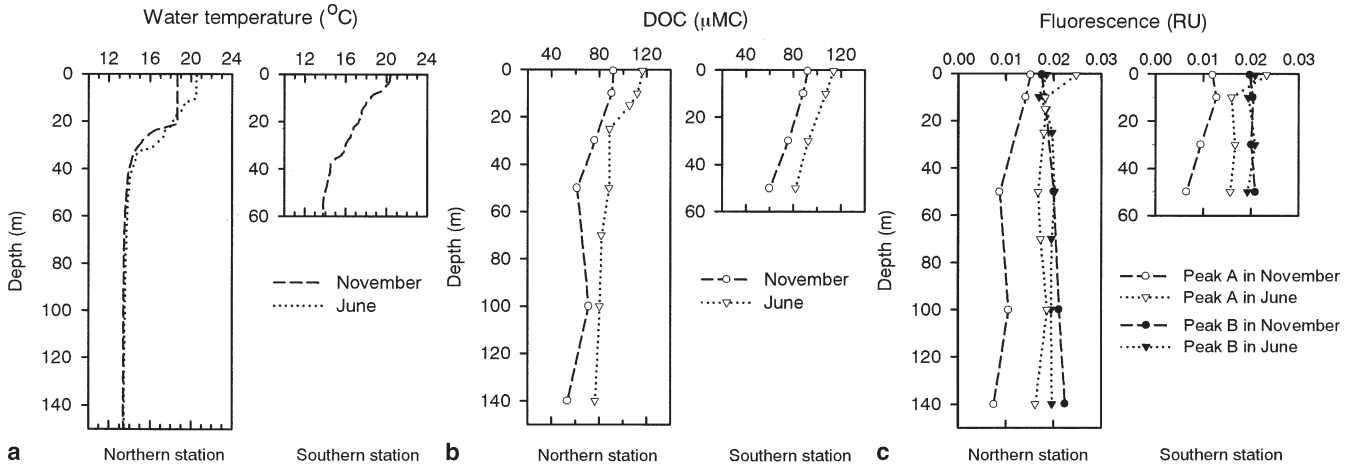

Fig. 5. Vertical distributions of water temperature (**a**), DOC concentration (**b**), and fluorescence intensities of Peak A and B extracted from the EEM spectra (**c**) for the two stations in Lake Fuxian in November 2000 and June 2001

Table 1. Regressions of fluorescence intensity of peaks in the excitation–emission matrix to dissolved organic carbon (DOC) in water of Lake Fuxian and adjacent rivers.

	Lake water[a]		River water[b]	
	Peak A	Peak B	Peak A	Peak B
Fluorescence intensity ($\times 10^{-2}$ RU)	0.97–2.16	1.80–2.44	0.90–23.1	2.90–52.5
Regression to DOC				
Slope ($\times 10^{-2}$)	0.022	−0.004	0.036	0.113
Intercept ($\times 10^{-2}$)	−0.44	2.34	−0.60	1.29
r^2	0.68	0.35	0.77	0.89

[a] n = 21, DOC = 53.5–117 μMC
[b] n = 15, DOC = 37.0–428 μMC

Sterilization of river water was thought likely to affect the DOC concentration and fluorescence, however little difference was found in DOC concentrations between sterilized (experimental water) and non-sterilized water (Fig. 6a). The fluorescence intensities of Peak B in the river water increased as a result of sterilization; however, the fluorescence intensities of Peak B during dark incubation were almost constant (Fig. 6c). This finding indicates that sterilization of the water had little effect on the results of this experiment.

Photobleaching of humic-like fluorescence in lake and river waters

Under sunlight exposure, the fluorescence intensities of Peak B in lake and river water decreased exponentially with time, whereas we observed no decrease in the control samples (Fig. 7a,b). The decreases of the DOC concentration of lake and river water during sunlight exposure were 6% and 17% of the initial concentrations, respectively (data not shown). When the integrated sunlight irradiance from the start of exposure was less than 20 MJm^{-2}, the fluorescence intensity of Peak B in lake water rapidly decreased exponentially (Fig. 7a), whereas when it was greater than 20 MJm^{-2}, the rate of decrease significantly declined. The photobleached fluorescence intensities from the Wanhe River during sunlight exposure were higher than those of the lake water; however, in the river water, the photobleached fluorescence intensities exhibited a similar decline in fluorescence to that in the lake water (Fig. 7b). Even after the integrated sunlight irradiance exceeded 20 MJm^{-2}, the fluorescence in the river water continued to decline.

The position of Peak B in the EEM of the Wanhe River water was shifted by 59 MJm^{-2} of sunlight exposure during the 6 days of the experiment (Table 2), with the emission wavelength in the river samples shifting to a shorter wavelength (Table 2), although there was little shifting of the emission position of Peak B in the lake water during the experiments.

Discussion

Relationship between DOC concentrations and fluorescence intensities in the lake water

In summer, the chemical oxygen demand of the surface water in Lake Fuxian increases (Nanjing Institute of Geography and Limnology 1990). The POC concentration

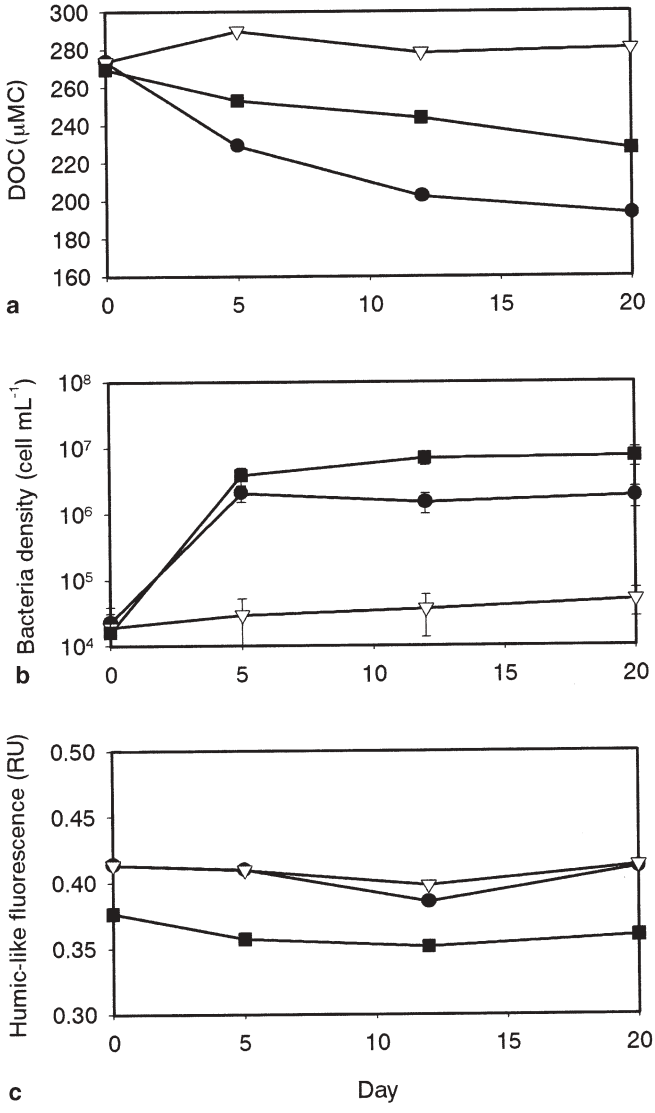

Fig. 6. Changes in **a** DOC concentration, **b** bacterial density, and **c** humic-like fluorescence intensity in the EEM during dark incubations. *Circles* denote the experimental waters which were inoculated with bacteria, *triangles* indicate bacteria-free control waters, and *Squares* denote nonsterilized water for comparison between sterilized and nonsterilized water

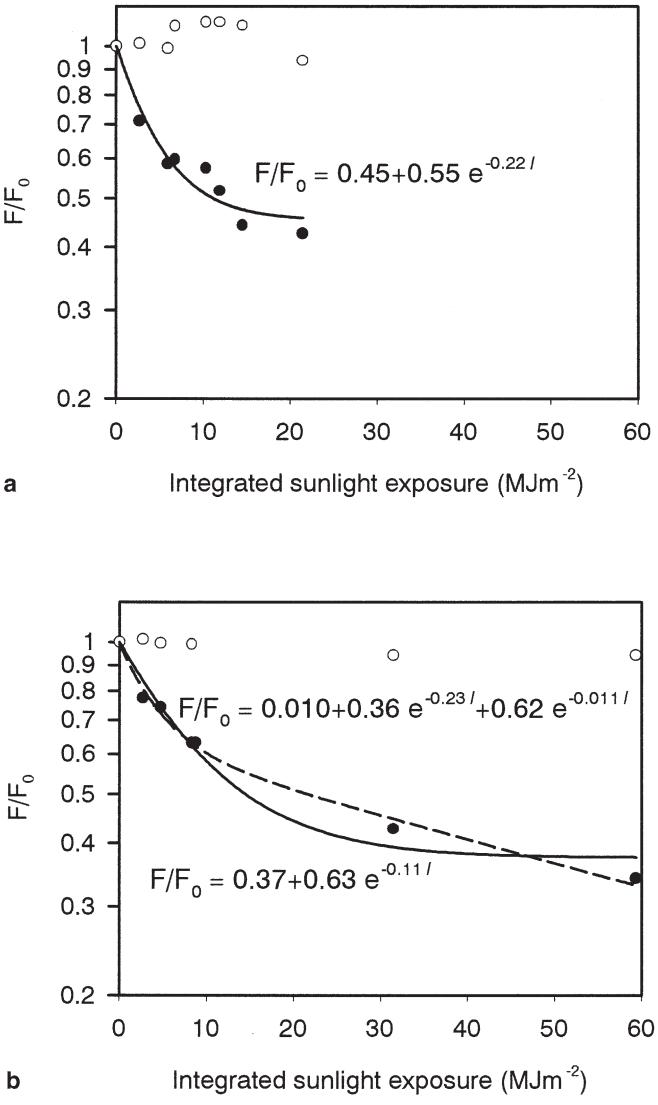

Fig. 7a,b. Changes in peak-intensity ratios (F/F_0) of Peak B in the EEM during the sunlight exposure experiment. **a** Lake water from a depth of 50 m; **b** Wanhe River water. F, fluorescence intensity after sunlight exposure; F_0, fluorescence intensity before sunlight exposure. *Solid circles* denote the sample exposed to sunlight and *open circles* denote the control sample not exposed to sunlight. *Solid lines* are drawn based on Eq. 1, and the *dotted* is based on Eq. 3

Table 2. Influence of sunlight exposure on peak positions of the humic-like fluorescence in Lake Fuxian water and Wanhe River water

	Integrated sunlight exposure		
	Level 1	Level 2	Level 3
Lake Fuxian			
ISE (MJm^{-2})	–	12	21
Ex/Em (nm/nm)	345/429	340/432	340/434
Intensity (RU)	0.025	0.013	0.012
Wanhe River			
ISE (MJm^{-2})	–	9	59
Ex/Em (nm/nm)	350/439	345/440	340/430
Intensity (RU)	0.253	0.147	0.121

ISE, integrated sunlight exposure; RU, Raman units

of the surface water was higher in June 2001 (37.5 μMC at a depth of 0.5 m) than that in November 2000 (25.8 μMC, Hayakawa et al. 2002). Hence, the high DOC concentration of the surface water might be caused by the increase in primary productivity in summer, although a further detailed study is needed to confirm this.

The position of Peak A in the EEM in this study was almost the same as those observed for protein-like fluorescence reported in the marine and freshwater DOM of previous studies (Determann et al. 1998; Yamashita and Tanoue 2003). The dissolved protein and amino acids in natural lake water could have originated from microorganisms (phytoplankton, bacteria) and zooplankton. The strong correlation observed between the DOC concentra-

tions and fluorescence intensities of Peak A indicates that the DOC variation in the lake water was mainly due to microorganisms in the lake. Furthermore, the intercept of the DOC axis in the graph was about 20 μMC (Table 1), indicating that 56%–83% of DOC was autochthonous matter. Our previous study showed that the primary productivity of Lake Fuxian was significantly greater (<13 times) than the input fluxes of organic matter from the surrounding rivers (Hayakawa et al. 2002). The predominance of autochthonous DOM in the present study is consistent with the findings in previous studies.

The position of Peak B in the EEM was almost the same as that of the humic-like fluorescence reported by Coble (1996). The correlation between the DOC concentrations and the fluorescence intensities of Peak B in the lake water was weak (Table 1), suggesting that the DOC variation in the lake water is not related to humic substances.

Relationship between DOC concentrations and fluorescence intensities in rivers

The fluorescence intensities of Peaks A and B in the river waters were linearly correlated with the DOC concentrations ($P < 0.05$, Table 1). Earlier studies of fluorescence suggested that domestic waste can produce humic-like fluorescence (Senesi et al. 1991; LeCoupannec et al. 2000), and that urban rivers contaminated by domestic waste show high intensities of humic-like fluorescence (Suzuki et al. 1998). Peaks A and B for river samples in the present study may reflect the influence of domestic and industrial organic waste sources.

The fluorescence intensities of water from the upper reaches were weak and their EEM spectra differed from those of water from the lower reaches that was contaminated with organic waste. In the upper reaches, the data probably represent humic substances derived from soil and plant material in the watershed. In the rivers receiving domestic waste, contamination of organic waste could mask the fluorescence from soil and plant material.

Photobleaching of humic-like fluorescence in the lake and rivers

Photobleaching of humic-like fluorescence has been reported for marine and lake DOM (Kramer 1979; Kauassi and Zika 1990; Skoog et al. 1996; Hayakawa et al. 2003). Kauassi and Zika (1990) and Skoog et al. (1996) proposed that there were two steps of fluorescence decay during photobleaching: the early rapid decrease and the subsequent slow (or constant) decrease in fluorescence intensity. In the present study, the rate of decrease of photobleached fluorescence also consisted of the two steps. We attempted to describe the decay course of photobleaching by the following equation after Skoog et al. (1996):

$$F = L + (F_0 - L)e^{-kI} \quad (1)$$

where F is fluorescence intensity, L is fluorescence rest intensity (assumed constant), F_0 is the initial fluorescence intensity, k is the decay rate constant, and I is the integrated sunlight irradiance. Eq. 1 can be rewritten as:

$$F/F_0 = L/F_0 + (1 - L/F_0)e^{-kI} \quad (2)$$

The solid line in Fig. 7a is the asymptote based on Eq. 2 and shows good regression matching to the data ($r^2 = 0.89$), indicating that the fluorescence of the lake DOM may contain some elements resistant to photobleaching from sunlight exposure. Experiments using several types of lake samples are needed for confirmation of this hypothesis. From Eq. 2, we estimate that the initial half-life of the fluorescence intensity in the lake water was 2.03 days. In prior studies on the photobleaching of fluorescence in marine waters, fluorescence half-life was a few hours in the Gulf of Mexico (Kauassi and Zika 1990), 0.4–4.6 days in the Baltic Sea (Skoog et al. 1996), and 2.54 days in the Mid-Atlantic Bight (Vodacek et al. 1997). The present results are consistent with these published data.

The decrease in the fluorescence intensities of the Wanhe River during photobleaching can be described by a regression curve derived from Eq. 2 (Fig. 7b). The regression line also shows good regression matching to the observed data ($r^2 = 0.93$). However, the present study shows that the fluorescence of the river water may be composed of two kinds of fluorophores, i.e., domestic waste from towns and farms and humic substances from soil and plant materials. Hence, we can describe the time course of fluorescence decay as follows:

$$F = L + (F_1 - L)e^{-k_1 I} + (F_2 - L)e^{-k_2 I} \quad (3)$$

where F_1 is the initial fluorescence intensity of the initial assumed material, k_1 is the decay rate constant of F_1, F_2 is the initial fluorescence intensity of the second assumed material, and k_2 is the decay rate constant of F_2. The asymptote of Eq. 3 is depicted by the dotted line in Fig. 6b and also fits the data well ($r^2 = 0.95$). This supports the suggestion that the fluorescence of the river water could be composed of two kinds of fluorophores, or that the photobleaching reaction may contain progressive reactions, e.g., alteration of parent species followed by further secondary reactions. At this time, because we did not identify the fluorescent substances, nothing can be said about the chemical structure of photobleached fluorescence in the river water. However, the changes in the position of Peak B in the EEM of the Wanhe River after versus before sunlight exposure (350 to 340 nm emission, Table 2) indicate a qualitative change in fluorophore.

Sources and sinks of humic-like fluorescent substances in the lake

The differences in Peak B position and the intensities in the EEM between lake and river water indicate discriminable fluorescent substances. It suggests that the origin of the humic-like fluorescence could differ between lake and river water, or that fluorescent DOM could be transformed in the lake despite the common origin of the humic-like fluores-

cence in the lake and river water. If, on the other hand, the origin of the humic-like fluorescence in the lake differs from that in the rivers, it must be produced by aquatic organisms. The aquatic origin of humic substances in lakes was reported in early studies of humic substances (e.g., Steinberg and Münster 1985). However, a quantitative assessment of the production of aquatic humic substances is difficult, because such substances are produced not only by algae as the primary producer but also by heterotrophic organisms.

It is possible, however, to quantitatively assess the inputs and outputs of fluorescent DOM in the lake. We may speculate that, if all the humic-like fluorescence of the lake water is consistent with that of the river water, the input of riverine fluorescent substances into the lake must be balanced with the output from the lake and any sinks in the lake. Here, we regard the fluorescence intensity of Peak B in the EEM as analogous to the concentration of fluorescent substances, and we examine the mass balance of the sources and sinks in Lake Fuxian under the assumption that all the fluorescent substances in the lake are terrestrial in origin.

The input of fluorescent substances from the river was calculated from the weighted mean of the water flow rate and the flux of the fluorescent substances. The flow rates of river water in a single day were based on data from the Nanjing Institute of Geography and Limnology (1990) and the weighted mean flux of the fluorescent substances was estimated using data from the same source. The input of fluorescent substances from the rivers to Lake Fuxian was estimated as $6.05 \times 10^4\,\mathrm{RU\,m^3\,day^{-1}}$. Similarly, output from the lake to the one outflouring river (the Haikou) was estimated as $0.6 \times 10^4\,\mathrm{RU\,m^3\,day^{-1}}$. The present study showed that the microbial decomposition of fluorescent substances in river water is not a major factor (Fig. 6). Photobleaching is assumed to be a sink of fluorescent substances based on observations that photobleaching is important as a sink of chromophoric DOM in the ocean (Vodacek et al. 1997; Chen et al. 2002). In lakes, photobleaching occurs only in the surface water. Considering the attenuation coefficient of ultraviolet radiation (around 330 nm as the maximum excitation) in the lake, the photobleaching of fluorescence would be restricted to the zone only a few meters below the surface (e.g., Vincent et al. 2001). Ultraviolet light penetration of the water column was calculated from observed data of photosynthetically available radiation (PAR) penetration in the lake (data not shown). Solar radiation at the air surface of Lake Fuxian is $8.2\,\mathrm{MJ\,m^{-2}\,day^{-1}}$ as an annual average (Nanjing Institute of Geography and Limnology, personal communication), and the integrated light exposure is calculated as $2.4\,\mathrm{MJ\,m^{-2}\,day^{-1}}$ in the lake water. Finally the fluorescence decay in the water column is calculated from Eq. 1 from the photobleaching experiments, indicating that the fluorescence decay in the epilimnion of Lake Fuxian could reach $3.61 \times 10^6\,\mathrm{RU\,m^3\,day^{-1}}$ when there is thorough mixing of the surface water.

Based on these estimates, the combined sink and output of fluorescent substances in the lake is more than seven times their input, leading to the conclusion that most fluorescent substances must be supplied to the lake by an autochthonous production process. That process depends not only on phytoplankton and bacteria in the water but also on macrophytes and soils in the littoral area and sediment (Wetzel 2001). The present data show that most of the fluorescent substances in the surface water of the lake is lost through photobleaching. The fluorescence decay in the epilimnion of the lake in a single year is $3.61 \times 10^8\,\mathrm{RU\,m^3\,year^{-1}}$, which is equal to 88% of the total fluorescent substances in lake ($4.11 \times 10^8\,\mathrm{RU\,m^3}$), indicating that photobleaching exerts a major influence on fluorescent substances in the lake.

Acknowledgments We wish to thank Dr. E. Tanoue for his kindness in allowing us the use of the EEM fluorometers. We are also indebted to Drs. K. Okubo, M. Sugiyama, J. Murase, and J. Li for their assistance in the collection of water samples and to Dr. K. Ohta for providing equipment for the photobleaching experiments. Dr. T. Nakajima helped with the count of bacterial numbers. Dr. T. Yoshioka and two anonymous reviewers improved the manuscript considerably. This work was supported in part by a grant from the Nihon Seimei Zaidan and by a Grant-in-Aid for Scientific Research (No. 13575034) from the Ministry of Education, Sports, Science and Technology of Japan.

References

Chen RF, Zhang Y, Vlahos P, Rudnick SM (2002) The fluorescence of dissolved organic matter in the Mid-Atlantic Bight. Deep-Sea Res II 49:4439–4459

Coble PG (1996) Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Mar Chem 51:325–346

Determann S, Reuter R, Wagner P, Willkomm R (1994) Fluorescent matter in the eastern Atlantic Ocean. Part 1: method of measurement and near-surface distribution. Deep-Sea Res I 41:659–675

Determann S, Lobbes JM, Reuter R, Rullkötter J (1998) Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. Mar Chem 62:137–156

Hayakawa K, Sakamoto M, Murase J, Song X, Zhang Z (2002) Distribution and dynamics of organic carbon in Lake Fuxian. Yunnan Geog Environ Res 14:34–40

Hayakawa K, Sekino T, Yoshioka T, Maruo M, Kumagai M (2003) Dissolved organic carbon and fluorescence in Lake Hovsgol: factors reducing humic content in lake water. Limnology 4:25–33

Jin X (1995) Lakes in China: Research of Their Environments. Vol. II. China Ocean, Beijing

Kouassi AM, Zika RG (1990) Light-induced alteration of the photophysical properties of dissolved organic matter in seawater. Part I. Photoreversible properties of natural water fluorescence. Netherlands J Sea Res 27:25–32

Kramer JM (1979) Degradation by sunlight of dissolved fluorescing substances in the upper layers of the eastern Atlantic Ocean. Netherlands J Sea Res 13:325–329

LeCoupannec F, Morin D, Sire O, Peron JJ (2000) Characterization of dissolved organic matter (DOM) in landfill leachates using fluorescence excitation–emission matrix. Environ Technol 21:515–524

McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for precursor organic material and aromaticity. Limnol Oceanogr 46:38–48

Mopper K, Schultz CA (1993) Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. Mar Chem 41:229–238

Moran MA, Zepp RG (1997) Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol Oceanogr 42:1307–1316

Nagao S, Matsunaga T, Suzuki Y, Ueno T, Amano H (2003) Characteristics of humic substances in the Kuji River waters as determined by high-performance size-exclusion chromatography with fluorescence detection. Water Res 37: 4159–4170

Nanjing Institute of Geography and Limnology (1990) Fuxian Hu Lake Fuxian (in Chinese) China Ocean, Beijing

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Senesi N, Miano TM, Provenzano MR, Brunett G (1991) Characterization, differentiation and classification of humic substances by fluorescence spectroscopy. Soil Sci 152:259–271

Skoog A, Wedborg M, Fogelqvist E (1996) Photobleaching of fluorescence and the organic carbon concentration in a coastal environment. Mar Chem 55:333–345

Spark KM, Swift RS (1994) Investigation of some of the parameters affecting the fluorescence spectra of humic substances. In: Senesi N, Miano TM (eds) Humic substances in the global environment and implications on human health. Elsevier Science, Amsterdam, pp 153–160

Steinberg CEW, Münster U (1985) Geochemistry and ecological role of humic substances in lakewater. In: Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (eds) Humic substances in soil, sediment, and water. Wiley, NewYork, pp 105–145

Suzuki Y, Nagao S, Nakaguchi Y, Matsunaga T, Muraoka S, Hiraki K (1997) Spectroscopic properties of fluorescent substances in natural waters (1) (in Japanese). Chikyukagaku (Geochemistry) 31:171–180

Suzuki Y, Nakaguchi Y, Hiraki K, Nagao S, Kudo M, Kimura M (1998) Characteristics of the fluorescent substances in the Yodo River system by three-dimensional excitation–emission matrix spectroscopy (in Japanese). Chikyukagaku (Geochemistry) 32:21–30.

Vodacek A, Blough NV, DeGrandpre MD, Peltzer ET, Nelson RK (1997) Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. Limnol Oceanogr 42:674–686

Wetzel RG (2001) Limnology. Lake and river ecosystem, 3rd Edn. Academic, San Diego

Vincent WF, Kumagai M, Belzile C, Ishikawa K, Hayakawa K (2001) Effects of seston on ultraviolet attenuation in Lake Biwa. Limnology 2:179–184

Yamashita Y, Tanoue E (2003) Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. Mar Chem 82:255–271