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Author(s)	Hafuka, Akira; Ding, Qing; Yamamura, Hiroshi; Yamada, Koji; Satoh, Hisashi
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3 **Interactions of dissolved humic substances with oppositely charged fluorescent**
4 **dyes for tracer techniques**

5

6 Akira Hafuka ^{a,*}, Qing Ding ^b, Hiroshi Yamamura ^a, Koji Yamada ^c, Hisashi Satoh ^d

7

8 ^a Department of Integrated Science and Engineering for Sustainable Society, Faculty of
9 Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo
10 112-8551, Japan

11 ^b Division of Civil and Environmental Engineering, Graduate School of Science and
12 Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan

13 ^c Division of Environmental Materials Science, Graduate School of Environmental
14 Science, Hokkaido University, North-10, West-5, Sapporo 060-0810, Japan

15 ^d Division of Environmental Engineering, Graduate School of Engineering, Hokkaido
16 University, North-13, West-8, Sapporo 060-8628, Japan

17

18 E-mail address:

19 Akira Hafuka: hafuka.14p@g.chuo-u.ac.jp

20 Qing Ding: dingqing1988@gmail.com

21 Hiroshi Yamamura: yamamura.10x@g.chuo-u.ac.jp

22 Koji Yamada: yamada@ees.hokudai.ac.jp

23 Hisashi Satoh: qsatoh@eng.hokudai.ac.jp

24

25 * *Corresponding author.* Akira Hafuka

26 Tel. & fax: +81-3-3817-7283

27 E-mail: hafuka.14p@g.chuo-u.ac.jp

28

29 **Abstract**

30

31 To investigate interactions between oppositely charged fluorescent dyes and dissolved
32 humic substances, fluorescence quenching of fluorescein and rhodamine 6G with
33 dissolved humic substances was performed. Binding coefficients were obtained by the
34 Stern–Volmer equation. The fluorescence of rhodamine 6G was largely quenched by the
35 addition of humic acid and a non-linear Stern–Volmer plot was obtained. This strong
36 quenching may be caused by the electrostatic interaction between cationic rhodamine
37 6G and humic acid and strengthened by the hydrophobic repulsion. In contrast, the
38 quenching and interactive effects of dissolved humic substances for fluorescein were
39 relatively weak.

40

41 **Keywords:** Tracer, Stern–Volmer plot, Fluorescein, Rhodamine 6G

42

43 **1. Introduction**

44

45 Fluorescent dyes have been widely employed in various technologies such as dye
46 tracing, bio-imaging, chemical sensing, and dye lasers (Yuan et al., 2013;

47 Basabe-Desmonts et al., 2007; Li and Psaltis, 2008; Harden et al., 2008). Dye tracer
48 techniques are powerful tools that enable better understanding of solute transport in the
49 liquid phase under a variety of hydrological conditions by tracking natural tracers and/or
50 spiked artificial tracers. In the field of water and environmental engineering, tracers
51 have been applied to investigate water flow in surface and ground water systems, as
52 well as in laboratory experiments (Goppert and Goldscheider, 2008; Jones and Smith,
53 2005; Sabaliunas et al., 2003). To be effective, tracers must be absent from (or present
54 in very low concentrations) natural aquatic environments, have high water solubility and
55 low sorptiivity to soil and sediment, be safe, highly detectable, and chemically,
56 physically, and biologically stable. Among a variety of tracers, fluorescent dyes
57 composed of synthetic organic compounds possess most of these characteristics, are
58 cost effective and easy to use, and can be easily identified on-site using a portable
59 fluorometer. Fluorescein is a popular fluorescent dye tracer commonly used in aquatic
60 systems (Sabaliunas et al., 2003; Harden et al., 2008). Rhodamine 6G is also sometimes
61 used as a fluorescent dye tracer (Kwak et al., 2013); however, this compound has been
62 shown to have toxic effects and is therefore generally limited to use in laboratory
63 experiments (Alford et al., 2009). The difference of two dyes is that fluorescein is
64 anionic and rhodamine 6G is cationic.

65

66 Smart and Laidlaw (1977) investigated the sensitivity, detectability, effect of water
67 chemistry, decay rates, adsorption resistance, toxicity, and cost of eight fluorescent dye
68 tracers, including fluorescein. Since then, ecotoxicological assessments (Behrens et al.,
69 2001), photobleaching (Larsen and Crimaldi, 2006), and effects of solvents and pH
70 (Saini et al., 2009; Oba and Poulson, 2012) of fluorescent dye tracers have been
71 investigated. However, no studies have focused on the effects of dissolved humic
72 substances (DHS) on fluorescent dyes. Although fluorescent dyes can be used for
73 qualitative analysis, the interactions might inhibit the optical detection of fluorescent
74 dyes due to the potential quenching caused by the presence of DHS. DHS is known to
75 interact with organic compounds such as herbicides, pesticides, and polycyclic aromatic
76 hydrocarbons (PAHs) in surface water (Ravacha and Rebhun, 1992; Schlautman and
77 Morgan, 1993; Hesketh et al., 1996; Spark and Swift, 2002), and to affect their fate and
78 transport. Various methods such as simple adsorption experiments, dialysis,
79 microcalorimetry, reverse phase separation, solubility enhancement, and fluorescence
80 quenching have been used to investigate the interactions between DHS and these
81 organic compounds (Hesketh et al., 1996; Spark and Swift, 2002; Raber et al., 1998;
82 Yamamoto et al., 2003; Schlautman and Morgan, 1993). Fluorescence quenching is a

83 simple and useful method commonly employed to investigate interactions of DHS with
84 PAHs because some PAHs show fluorescence and DHS acts as a quencher. The Stern–
85 Volmer equation (also known as the fluorescence static quenching equation) can be
86 plotted by conducting fluorescence quenching experiments (Gauthier et al., 1986). The
87 binding coefficient can be obtained easily by determining the slope of the Stern–Volmer
88 plot. In this study, the fluorescence quenching method was applied to analyze
89 interactions of oppositely charged fluorescent dyes, fluorescein and rhodamine 6G, with
90 DHS to investigate its effects on the dyes. This paper would contribute to the practice of
91 selecting suitable fluorescent dye tracers in aquatic and hydrology studies.

92

93 **2. Materials and methods**

94

95 *2.1 Fluorescent dyes*

96

97 The chemical structures of fluorescein, rhodamine 6G, and rhodamine B are shown in
98 Fig. 1. All fluorescent dyes are xanthene derivatives with many valuable characteristics,
99 including sharp and intense absorption and fluorescence bands, high fluorescence
100 quantum yields, high molar absorption coefficients, good photo-chemical stability,

101 relatively long emission wavelength, and good water solubility (Chen et al., 2012).
102 Fluorescein sodium salt (MW: 376.27), rhodamine 6G (MW: 479.01), and rhodamine B
103 (MW: 479.01) were purchased from Sigma-Aldrich (St. Louis, United States) and used
104 without further purification.

105

106 *2.2 Sample preparation and fluorescence quenching experiments*

107

108 Fluorescence quenching experiments were conducted in batch experiments. Stock
109 solutions of the fluorescent dyes (330 μM) were prepared by dissolving the dyes
110 separately in Milli-Q water (18.25 $\text{M}\Omega\text{cm}$). IHSS Suwannee River Humic Acid
111 Standard II (2S101H) and Fulvic Acid Standard II (2S101F) were used as humic
112 substances standards. The humic substances were dissolved in Milli-Q water, filtered
113 through a 0.45- μm -pore-size membrane (A045A047A; Toyo Roshi Kaisya, Ltd., Tokyo,
114 Japan) and stored separately as DHS stock solutions. Dissolved organic carbon (DOC)
115 concentrations of the stock solutions were determined using a total organic carbon
116 analyzer (TOC-L CSH; Shimadzu Corporation, Kyoto, Japan). Test solutions were
117 prepared by adding 30 μL of the dye stock solution and an appropriate volume of the
118 DHS stock solution into 200 μL of sodium phosphate buffer (10 mM, pH 6.9) in a

119 10-mL volumetric flask. The mixture was then diluted in Milli-Q water and mixed
120 gently. Final concentrations of the dye and phosphate were 1 μM (0.38 mg/L for
121 fluorescein and 0.48 mg/L for rhodamine 6G and rhodamine B) and 0.2 mM (19
122 mg- PO_4^{3-} /L), respectively. The linearity of fluorescence intensities in these dye
123 concentrations was confirmed (see figure S1 in supplementary data). The test solutions
124 were incubated at room temperature under dark conditions for 1 h. The pH of the
125 solutions was maintained after the incubation. The fluorescence and absorption spectra
126 were measured using a fluorescence spectrophotometer (F-2700; Hitachi
127 High-Technologies Corporation, Tokyo, Japan) and UV-Vis spectrophotometer
128 (UV-1800; Shimadzu Corporation, Kyoto, Japan), respectively. Both the excitation and
129 fluorescence slit widths were 5.0 nm. All experiments were performed in triplicate.

130

131 For the inner filter effect, measured fluorescence spectra were corrected. When DHS
132 absorbed the excitation and emission light, the measured fluorescence spectra were
133 corrected by equation 1 (Lakowicz, 2006):

$$F_{corr} = F_{obs} \text{antilog} \left(\frac{A_{ex} + A_{em}}{2} \right), \quad (1)$$

134 where F_{corr} and F_{obs} are the corrected and observed fluorescence intensity, respectively,
135 and A_{ex} and A_{em} are the absorbance of the test solution at excitation and emission

136 wavelengths, respectively.

137

138 The apparent fluorescence quantum yields were obtained by comparing the area under
139 the observed fluorescence spectrum of the test solution with that of the solution of
140 fluorescein in 0.1 M NaOH or rhodamine 6G in ethanol, which have a reported
141 fluorescence quantum yield (Φ_R) of 0.91 and 0.95, respectively (Valeur and
142 Berberan-Santos, 2012). The fluorescence quantum yield (Φ_S) for each test solution was
143 calculated using equation 2 (Lakowicz, 2006):

$$\Phi_S = \Phi_R \times \frac{S_S}{S_R} \times \frac{A_R}{A_S} \times \left(\frac{\eta_S}{\eta_R} \right)^2, \quad (2)$$

144 where Φ is the quantum yield, S is the integrated area of the corresponding fluorescence
145 spectrum, A is the absorbance at the excitation wavelength, η is the refractive index of
146 the solvent used, and S and R refer to the sample and the reference fluorescent dyes,
147 respectively.

148

149 *2.3 Binding analysis*

150

151 We analyzed the binding interaction of fluorescent dyes and DHS using equation 3 and
152 7. The Stern–Volmer equation is expressed as (Gauthier et al., 1986):

$$\frac{F_0}{F} = 1 + K_{doc}[DOC], \quad (3)$$

153 where F_0 and F are the fluorescence intensity of the fluorescent dye in the absence (F_0)
154 and presence (F) of DHS, respectively, and K_{doc} is the binding coefficient. Plotting F_0/F
155 against the DHS concentration ($[DOC]$) revealed a linear relationship between these
156 values with an intercept of 1 and a slope that represents the value of K_{doc} . Pan et al.
157 (2007) demonstrated the limitations of the Stern-Volmer plot for obtaining the binding
158 coefficients between phenanthrene and DOM because the binding coefficients can vary
159 with the concentrations of free phenanthrene as well as different DOM concentrations.
160 Other studies reported non-linear binding interactions between PAHs and DOM and
161 their binding coefficients depends on free solute concentration (Laor and Rebhun, 2002;
162 Borisover et al., 2006). Laor et al. (2002) suggested the Freundlich-type equation to
163 explain the non-linear binding. Considering these limitations, we evaluated the binding
164 coefficients of two dyes with humic and fulvic acid under same experimental condition
165 in which the total dye concentrations were 1 μ M and the range of DHS concentrations
166 were 0 – 5 mg-C/L. These DOC concentrations were used considering natural river
167 water in Japan. If non-linear binding is expected, equation 4 is applied:

$$C_S = K_F C_{free}^n, \quad (4)$$

168 where C_S is the DHS-bound fluorescent dye concentration (w/w), C_{free} is the free dye

169 concentration (w/v), and K_F is the Freundlich coefficient. Equation 4 can be expressed
170 as:

$$C_S = \frac{C_{bound}}{[DOC]} = \frac{C_{total} - C_{free}}{[DOC]} = K_F C_{free}^n, \quad (5)$$

171 where C_{bound} and C_{total} are the bound and total dye concentrations (w/v), respectively.

172 Equation 5 can be rearranged as:

$$\frac{C_{total}}{C_{free}} = 1 + K_F [DOC] C_{free}^{n-1}. \quad (6)$$

173 The fluorescent intensity of a dye is proportional to its free concentration. Therefore,

174 equation 6 can be rearranged as follows:

$$\frac{F_0}{F} = 1 + K_F' [DOC] F^{n-1}. \quad (7).$$

175 We calculated the values of K_F' and n and obtained a non-linear model by Origin Pro

176 9.1J software. K_F' was modified to K_F by a factor of f^{n-1} . The f value is the specific

177 fluorescence activity of a fluorescent dye, which was given by fluorescence intensity

178 divided by its concentration.

179

180 **3. Results and discussion**

181 Figure 2 shows the absorption spectra of fluorescein and rhodamine 6G in the presence

182 of humic acid or fulvic acid. Fluorescein and rhodamine 6G exhibited strong absorption

183 bands with large molar absorption coefficients around 489 nm ($\epsilon = 5.3 \times 10^4 \text{ L mol}^{-1}$)

184 cm^{-1}) and 526 nm ($\epsilon = 8.5 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$), respectively. In the wavelength region
185 below 450 nm, absorbance gradually increased with increasing DOC concentrations due
186 to chromogenic dissolved organic matter (CDOM; Zhang et al., 2009). Interestingly, the
187 absorption peak of rhodamine 6G at 526 nm shifted significantly to 536 nm upon
188 addition of humic acid (Fig. 2-(c)). This peak shift indicates that the mechanism of
189 binding interaction between rhodamine 6G and humic acid is different from that of
190 fluorescein-DHS complexes and rhodamine 6G-fulvic acid complex.

191

192 The fluorescence spectra of fluorescein and rhodamine 6G in the presence of humic acid
193 or fulvic acid are shown in Fig. 3. The inner filter effect correction factors were around
194 1.1 for all test solutions (data not shown). This value indicated that the inner filter
195 effects of DHS were negligible because the excitation and emission bands of DHS did
196 not overlap with those of fluorescent dyes (Henderson et al., 2009), and we prepared the
197 test solutions of the dyes with the absorbance under 0.1. As shown in Fig. 3, fluorescein
198 and rhodamine 6G produced sharp fluorescence bands with high fluorescence quantum
199 yields around 512 nm ($\Phi = 0.82$) and 551 nm ($\Phi = 0.86$), respectively. Fluorescence
200 quenching was observed with increasing DHS concentrations, with the fluorescence of
201 rhodamine 6G strongly decreasing upon addition of humic acid (Fig. 3-(c)). Apparent

202 fluorescence quantum yields of fluorescein and rhodamine 6G in the presence of humic
203 acid or fulvic acid (5 mg-C/L) are listed in Table 1. Fluorescence quantum yields also
204 decreased in response to the addition of DHS. Among them, the quantum yield of
205 rhodamine 6G decreased significantly from 0.86 to 0.10 in response to the addition of
206 humic acid. These results also indicate a strong fluorescence quenching effect of humic
207 acid on rhodamine 6G.

208

209 The Stern–Volmer plots of the fluorescent dyes are shown in Fig. 4. Linear relationships
210 were observed for fluorescein with humic and fulvic acids and rhodamine 6G with
211 fulvic acid. The K_{doc} values are summarized in Table 2. The K_{doc} value of rhodamine
212 6G with fulvic acid (1.34×10^{-1} L/mg-C) is slightly larger than that of fluorescein with
213 humic and fulvic acids (7.57×10^{-2} L/mg-C and 1.00×10^{-1} L/mg-C, respectively).
214 Conversely, a non-linear curved plot towards the y-axis was obtained for rhodamine 6G
215 with humic acid. Different concentration of rhodamine 6G also resulted in the
216 non-linearity (see figure S2 in supplementary data). Some studies have attributed the
217 non-linear binding to different causes. For example, combination of static and dynamic
218 quenching could result in the non-linear plot (Pan et al., 2007). Another study also
219 attributed the non-linear Stern-Volmer plot to the inherent heterogeneity of DOM (Laor

220 and Rebhun, 2002). We tried to evaluate the Stern-Volmer constant (K_{SV}) and the
221 stability constant (K_S) by using the equation considering static and dynamic quenching.
222 However, we could not get those values from the equation (see figure S3 in
223 supplementary data). Therefore, we concluded that static quenching was the primary
224 mechanism and we applied the Freundlich-type equation to explain the non-linear
225 binding. Previous researchers also often excluded dynamic quenching. The Freundlich
226 coefficient (K_F) of rhodamine 6G with humic acid was 7.84×10^{-1} L/mg-C and the n
227 value was 0.67. This strong quenching of rhodamine 6G with humic acid may indicate
228 the electrostatic interaction between cationic rhodamine 6G and anionic groups of
229 humic acid. Furthermore, this interaction could be strengthened by the hydrophobic
230 repulsion of less polar moieties from aqueous bulk. As compared with fulvic acid, the
231 increased interaction between rhodamine 6G and humic acid is in well agreement with
232 the significance of less hydrated hydrophobic moieties in its components.

233

234 Rhodamine B was also used for fluorescence quenching experiments with DHS. Figure
235 5 shows the Stern–Volmer plot of rhodamine B with DHS. The K_{doc} values for humic
236 and fulvic acids were 4.11×10^{-2} L/mg-C and 1.12×10^{-2} L/mg-C, respectively.
237 Interestingly, the non-linear binding was observed for rhodamine 6G but not for

238 rhodamine B. As compared with rhodamine 6G, rhodamine B has carboxylic group. The
239 carboxylic group was supposed to be ionized under the experimental pH condition and
240 the presence of ionized carboxylic group resulted in a greater aqueous solubility of the
241 dye as well as in an enhanced electrostatic rejection from DHS. In addition, the fact that
242 rhodamine B does not donate H-bond from its diethyl amino groups may also reduce the
243 binding affinity to DHS compared with rhodamine 6G.

244

245 **4. Conclusions**

246

247 In this study, the binding interactions between oppositely charged fluorescent dyes
248 (fluorescein and rhodamine 6G) and DHS (dissolved humic and fulvic acid) were
249 investigated by the fluorescence quenching method. The fluorescent intensities of dyes
250 decreased in response to the addition of DHS, and the binding coefficients were
251 obtained from the Stern–Volmer plots. Among them, the fluorescence of rhodamine 6G
252 was strongly quenched by humic acid and the non-linear Stern–Volmer plot was
253 obtained. This strong quenching may be caused by the electrostatic interaction between
254 cationic rhodamine 6G and anionic groups of humic acid. Then, the interaction could be
255 strengthened by the hydrophobic repulsion of less polar moieties from aqueous bulk. As

256 compared with rhodamine 6G, the quenching effects and binding coefficients of
257 fluorescein with dissolved humic substances were relatively weak. These results would
258 contribute to the practice of selecting suitable fluorescent dye tracers in aquatic and
259 hydrology studies.

260

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265

266 **References**

- 267 Alford, R., Simpson, H.M., Duberman, J., Hill, G.C., Ogawa, M., Regino, C.,
268 Kobayashi, H., Choyke, P.L., 2009. Toxicity of organic fluorophores used in
269 molecular imaging: literature review. *Molecular Imaging* 8 (6), 341-354.
- 270 Basabe-Desmonts, L., Reinhoudt, D.N., Crego-Calama, M., 2007. Design of fluorescent
271 materials for chemical sensing. *Chemical Society Reviews* 36 (6), 993-1017.
- 272 Behrens, H., Beims, U., Dieter, H., Dietze, G., Eikmann, T., Grummt, T., Hanisch, H.,
273 Henseling, H., Kass, W., Kerndorff, H., Leibundgut, C., Muller-Wegener, U.,

274 Ronnefahrt, I., Scharenberg, B., Schleyer, R., Schloz, W., Tilkes, F., 2001.
275 Toxicological and ecotoxicological assessment of water tracers. *Hydrogeology*
276 *Journal* 9 (3), 321-325.

277 Borisover, M., Laor, Y., Bukhanovsky, N., Saadi, I., 2006. Fluorescence-based evidence
278 for adsorptive binding of pyrene to effluent dissolved organic matter. *Chemosphere*
279 65 (11), 1925-1934.

280 Chen, X.Q., Pradhan, T., Wang, F., Kim, J.S., Yoon, J., 2012. Fluorescent chemosensors
281 based on spiroring-opening of xanthenes and related derivatives. *Chemical Reviews*
282 112 (3), 1910-1956.

283 Gauthier, T.D., Shane, E.C., Guerin, W.F., Seitz, W.R., Grant, C.L., 1986. Fluorescence
284 quenching method for determining equilibrium-constants for polycyclic
285 aromatic-hydrocarbons binding to dissolved humic materials. *Environmental*
286 *Science & Technology* 20 (11), 1162-1166.

287 Goppert, N., Goldscheider, N., 2008. Solute and colloid transport in karst conduits
288 under low- and high-flow conditions. *Ground Water* 46 (1), 61-68.

289 Harden, H.S., Roeder, E., Hooks, M., Chanton, J.P., 2008. Evaluation of onsite sewage
290 treatment and disposal systems in shallow karst terrain. *Water Research* 42 (10–11),
291 2585-2597.

292 Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., Khan, S.J., 2009.
293 Fluorescence as a potential monitoring tool for recycled water systems: a review.
294 Water Research 43 (4), 863-881.

295 Hesketh, N., Jones, M.N., Tipping, E., 1996. The interaction of some pesticides and
296 herbicides with humic substances. *Analytica Chimica Acta* 327 (3), 191-201.

297 Jones, E.H., Smith, C.C., 2005. Non-equilibrium partitioning tracer transport in porous
298 media: 2-D physical modelling and imaging using a partitioning fluorescent dye.
299 Water Research 39 (20), 5099-5111.

300 Kwak, R., Guan, G.F., Peng, W.K., Han, J.Y., 2013. Microscale electro dialysis:
301 concentration profiling and vortex visualization. *Desalination* 308, 138-146.

302 Lakowicz, J.R., 2006. Principles of fluorescence spectroscopy, third ed. pp54-56 and
303 Springer, New York.

304 Laor, Y., Rebhun, M., 2002. Evidence for nonlinear binding of PAHs to dissolved humic
305 acids. *Environmental Science & Technology* 36 (5), 955-961.

306 Larsen, L.G., Crimaldi, J.P., 2006. The effect of photobleaching on PLIF. *Experiments*
307 in Fluids 41 (5), 803-812.

308 Li, Z.Y., Psaltis, D., 2008. Optofluidic dye lasers. *Microfluidics and Nanofluidics* 4
309 (1-2), 145-158.

310 Oba, Y., Poulson, S.R., 2012. Octanol-water partition coefficients (K_{ow}) vs. pH for
311 fluorescent dye tracers (fluorescein, eosin Y), and implications for hydrologic tracer
312 tests. *Geochemical Journal* 46 (6), 517-520.

313 Pan, B., Ghosh, S., Xing, B.S., 2007. Nonideal binding between dissolved humic acids
314 and polyaromatic hydrocarbons. *Environmental Science & Technology* 41 (18),
315 6472-6478.

316 Raber, B., Kogel-Knabner, I., Stein, C., Klem, D., 1998. Partitioning of polycyclic
317 aromatic hydrocarbons to dissolved organic matter from different soils.
318 *Chemosphere* 36 (1), 79-97.

319 Ravacha, C., Rebhun, M., 1992. Binding of organic solutes to dissolved humic
320 substances and its effects on adsorption and transport in the aquatic environment.
321 *Water Research* 26 (12), 1645-1654.

322 Sabaliunas, D., Webb, S.F., Hauk, A., Jacob, M., Eckhoff, W.S., 2003. Environmental
323 fate of Triclosan in the River Aire Basin, UK. *Water Research* 37 (13), 3145-3154.

324 Saini, G.S.S., Sharma, A., Kaur, S., Bindra, K.S., Sathe, V., Tripathi, S.K., Mhahajan,
325 C.G., 2009. Rhodamine 6G interaction with solvents studied by vibrational
326 spectroscopy and density functional theory. *Journal of Molecular Structure* 931
327 (1-3), 10-19.

328 Schlautman, M.A., Morgan, J.J., 1993. Effects of aqueous chemistry on the binding of
329 polycyclic aromatic-hydrocarbons by dissolved humic materials. *Environmental*
330 *Science & Technology* 27 (5), 961-969.

331 Smart, P.L., Laidlaw, M.S., 1977. An evaluation of some fluorescent dyes for water
332 tracing. *Water Resources Research* 13, 15-33.

333 Spark, K.M., Swift, R.S., 2002. Effect of soil composition and dissolved organic matter
334 on pesticide sorption. *Science of the Total Environment* 298 (1–3), 147-161.

335 Valeur, B., Berberan-Santos. M.N., 2012. *Molecular fluorescence: principles and*
336 *applications*, second ed. p272, Wiley-VCH, Weinheim.

337 Yamamoto, H., Liljestr and, H.M., Shimizu, Y., Morita, M., 2003. Effects of
338 physical-chemical characteristics on the sorption of selected endocrine disruptors by
339 dissolved organic matter surrogates. *Environmental Science & Technology* 37 (12),
340 2646-2657.

341 Yuan, L., Lin, W.Y., Zheng, K.B., He, L.W., Huang, W.M., 2013. Far-red to near
342 infrared analyte-responsive fluorescent probes based on organic fluorophore
343 platforms for fluorescence imaging. *Chemical Society Reviews* 42 (2), 622-661.

344 Zhang, Y.L., van Dijk, M.A., Liu, M.L., Zhu, G.W., Qin, B.Q., 2009. The contribution
345 of phytoplankton degradation to chromophoric dissolved organic matter (CDOM) in

346 eutrophic shallow lakes: field and experimental evidence. *Water Research* 43 (18),

347 4685-4697.

348

349

350 **Figure and table captions**

351

352 Fig. 1 Chemical structures of fluorescein, rhodamine 6G, and rhodamine B.

353

354 Fig. 2 Absorption spectra of fluorescein (a, b) and rhodamine 6G (c, d) with increasing
355 DOC concentrations of humic acid or fulvic acid. The spectra were taken at DOC
356 concentrations of 0, 0.5, 1, 2, and 5 mg-C/L. The concentration of the fluorescent dye
357 was 1.0 μM (0.38 mg/L for fluorescein and 0.48 mg/L for rhodamine 6G).

358

359 Fig. 3 Fluorescence spectra of fluorescein (a, b) and rhodamine 6G (c, d) with
360 increasing DOC concentrations of humic acid or fulvic acid. The spectra were taken at
361 DOC concentrations of 0, 0.5, 1, 2, and 5 mg-C/L. The concentration of the fluorescent
362 dye was 1.0 μM (0.38 mg/L for fluorescein and 0.48 mg/L for rhodamine 6G). The
363 excitation wavelengths of fluorescein and rhodamine 6G were 480 nm and 520 nm,
364 respectively.

365

366 Fig. 4 Stern-Volmer plots for fluorescein (a) and rhodamine 6G (b) with increasing
367 DOC concentrations of humic acid or fulvic acid. Fluorescence intensities of fluorescein

368 and rhodamine 6G were plotted at 512 nm and 551 nm, respectively. Solid lines
369 represent linear relationships. A non-linear relationship is shown as a dashed line.

370

371 Fig. 5 Stern–Volmer plots for rhodamine B with increasing DOC concentrations of
372 humic acid or fulvic acid. Fluorescence intensities of rhodamine B were plotted at 574
373 nm.

374

375 Table 1 Apparent fluorescent quantum yields of fluorescein and rhodamine 6G in the
376 presence of DHS (5 mg-C/L).

377

378 Table 2 K_{doc} , Intercept, K_F , n , and r^2 value obtained in this study.

379

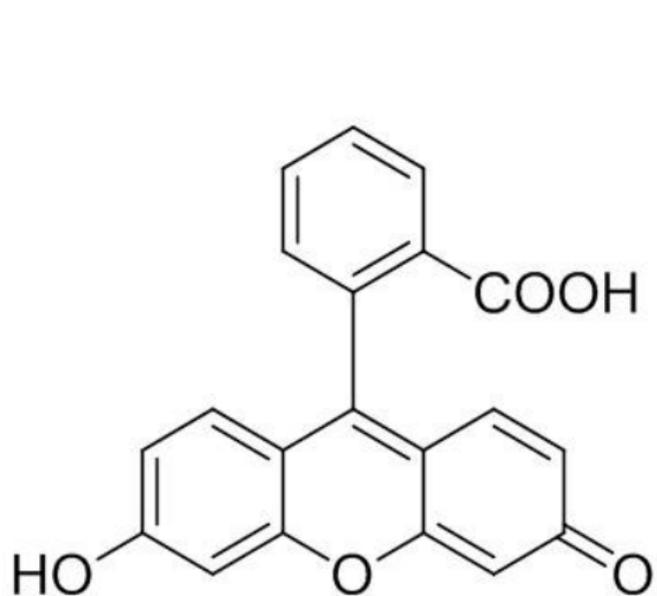
1 Table 1 Apparent fluorescent quantum yields of fluorescein and rhodamine 6G in the
2 presence of DHS (5 mg-C/L).

3

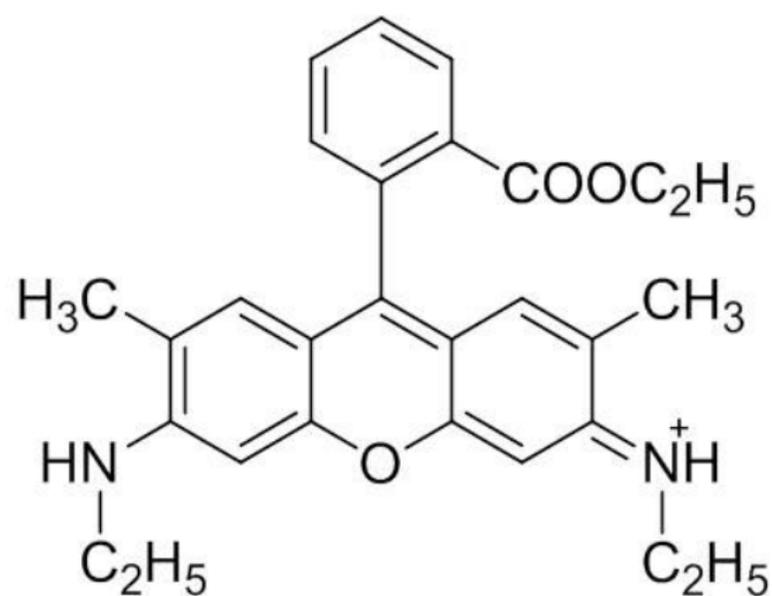
Fluorescent dye	DHS	Φ
Fluorescein	–	0.82 ± 0.01
	Humic acid	0.45 ± 0.02
	Fulvic acid	0.55 ± 0.02
Rhodamine 6G	–	0.86 ± 0.01
	Humic acid	0.10 ± 0.00
	Fulvic acid	0.58 ± 0.02

Table 2 K_{doc} , Intercept, K_{F} , n, and r^2 value obtained in this study.

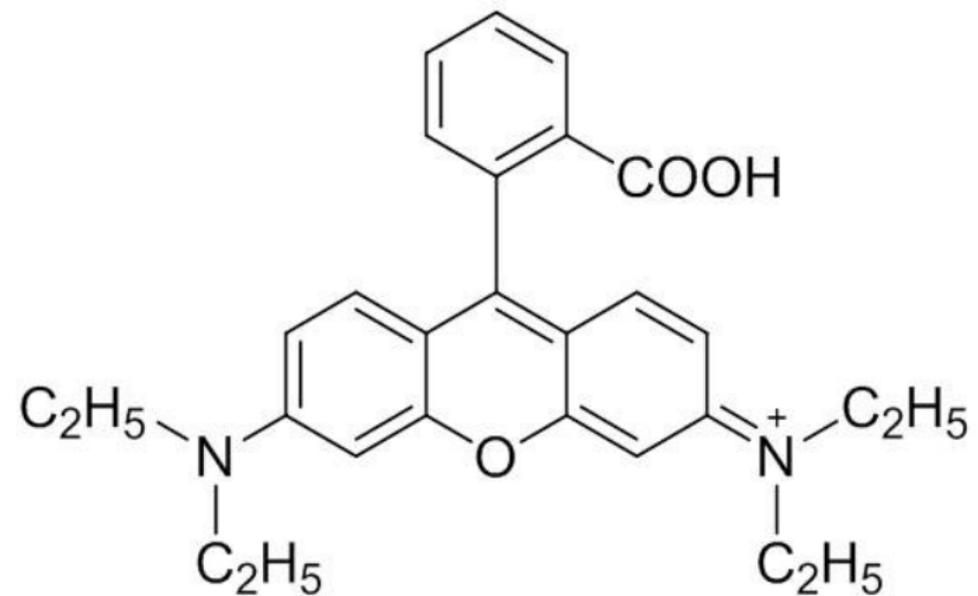
Fluorescent dye	DHS	K_{doc} (L/mg-C) (= Slope)	Intercept	K_{F} (L/mg-C)	n	r^2
Fluorescein	Humic acid	$7.57 \times 10^{-2} (\pm 1.60 \times 10^{-3})$	$1.01 (\pm 3.41 \times 10^{-2})$	–	–	0.992
	Fulvic acid	$1.00 \times 10^{-1} (\pm 9.90 \times 10^{-3})$	$1.00 (\pm 2.29 \times 10^{-2})$	–	–	0.994
Rhodamine 6G	Humic acid	–	–	$7.84 \times 10^{-1} (\pm 1.92 \times 10^{-1})$	$0.67 (\pm 0.090)$	0.999
	Fulvic acid	$1.34 \times 10^{-1} (\pm 3.78 \times 10^{-2})$	$1.02 (\pm 2.08 \times 10^{-2})$	–	–	0.998



Fluorescein



Rhodamine 6G



Rhodamine B

Fig. 1 Chemical structures of fluorescein, rhodamine 6G, and rhodamine B.

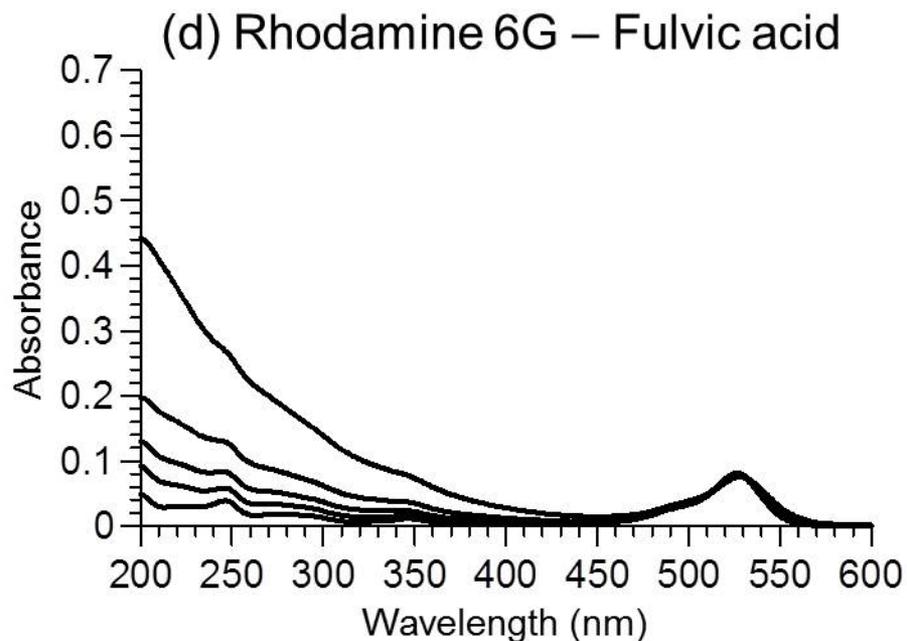
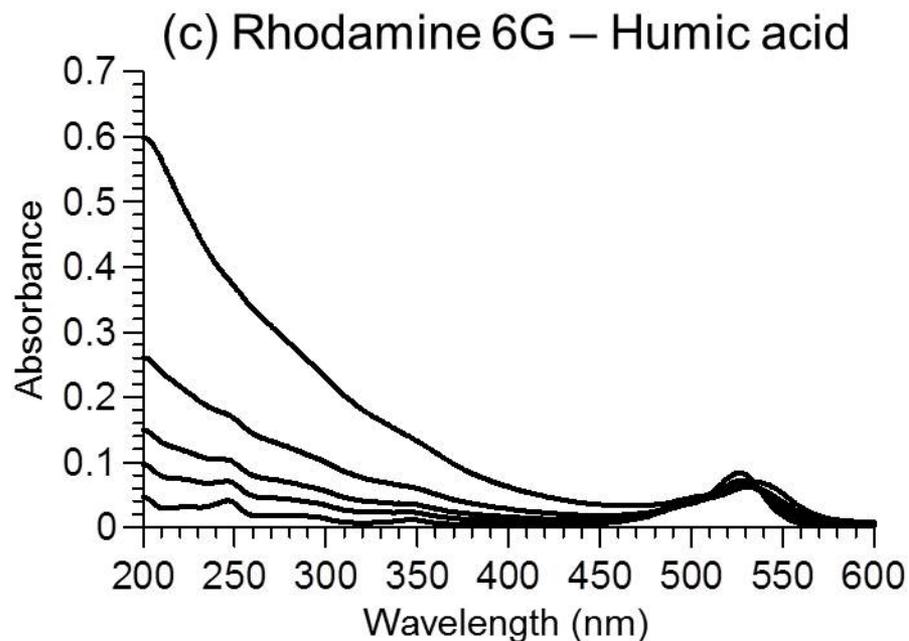
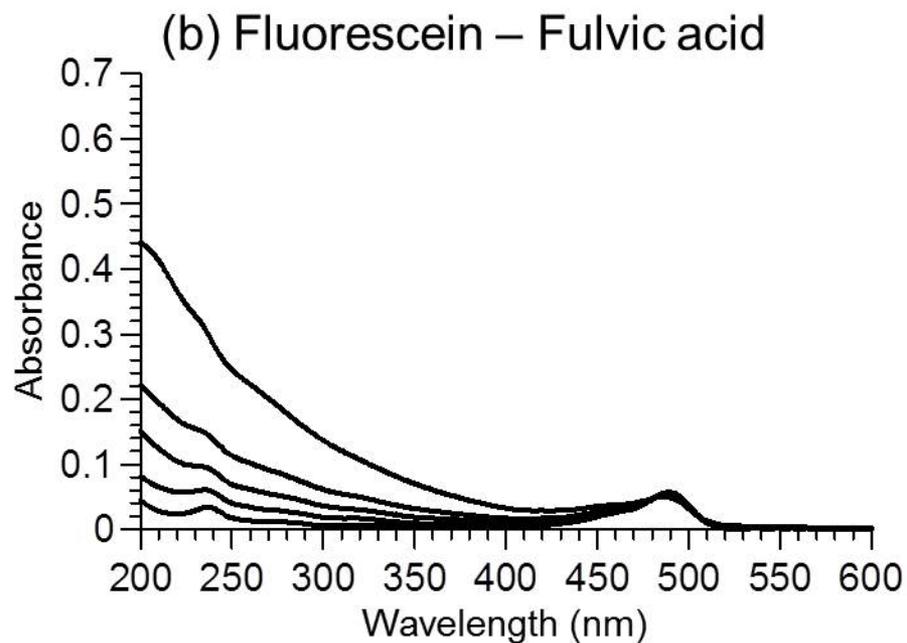
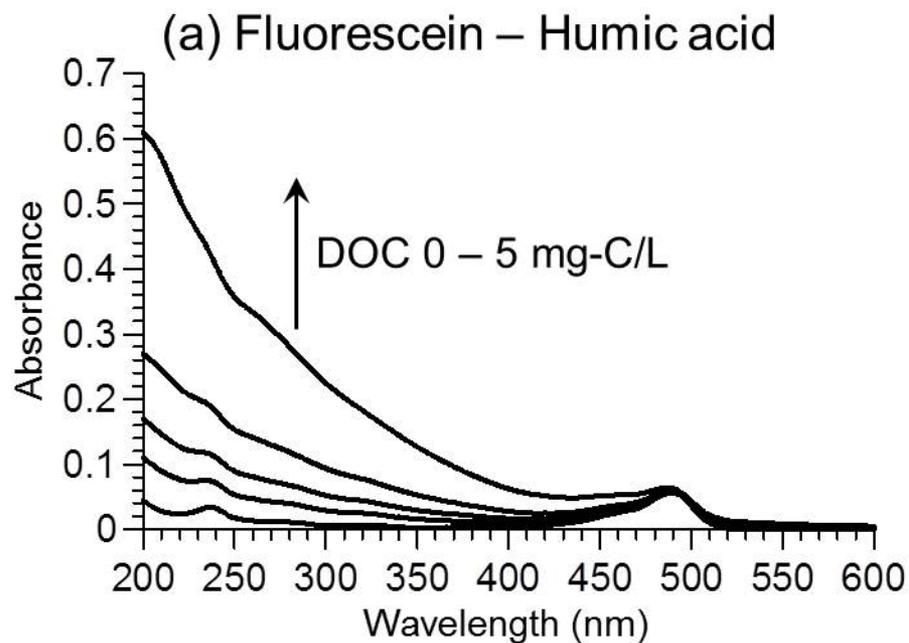


Fig. 2 Absorption spectra of fluorescein (a, b) and rhodamine 6G (c, d) with increasing DOC concentrations of humic acid or fulvic acid. The spectra were taken at DOC concentrations of 0, 0.5, 1, 2, and 5 mg-C/L. The concentration of the fluorescent dye was 1.0 μM (0.38 mg/L for fluorescein and 0.48 mg/L for rhodamine 6G).

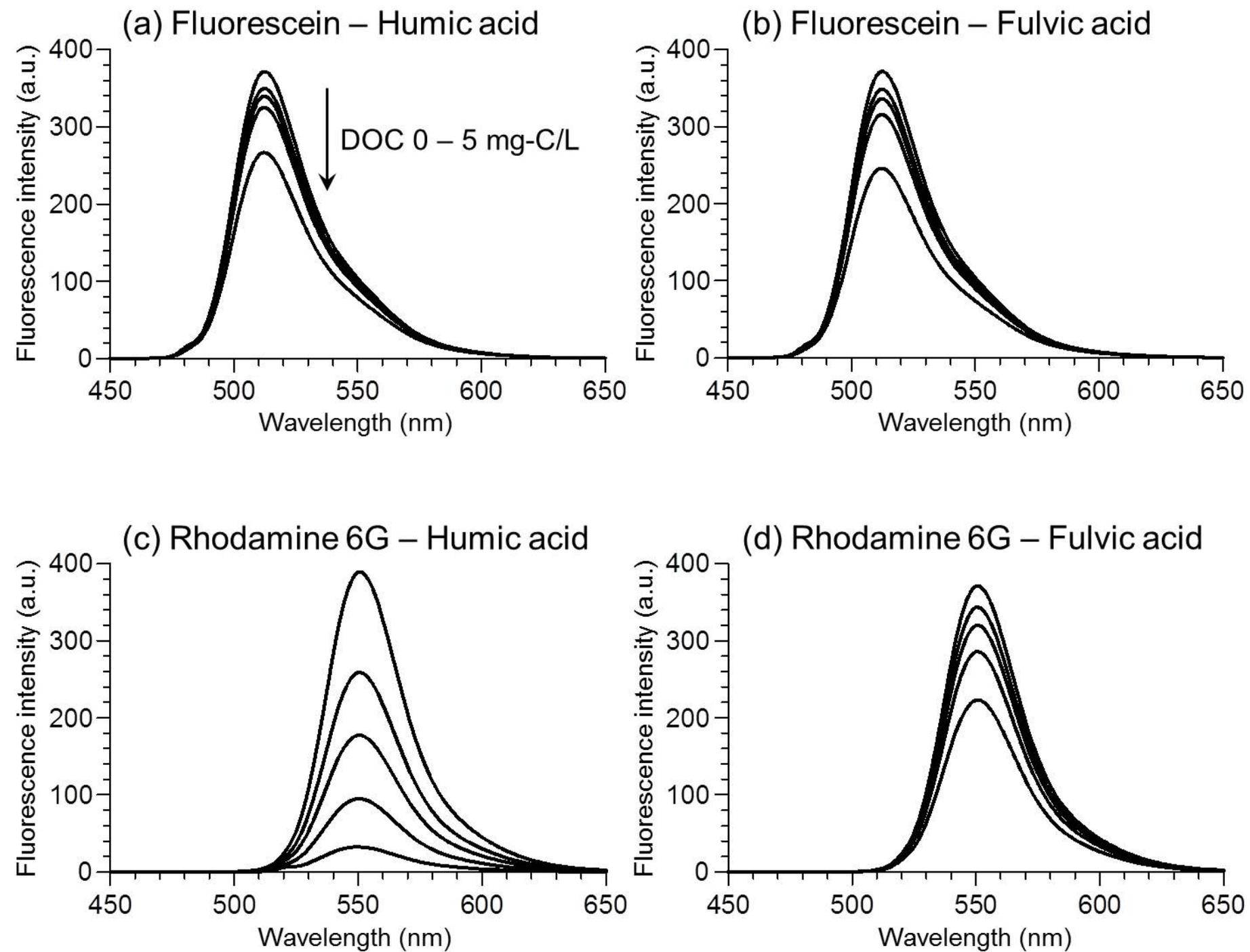


Fig. 3 Fluorescence spectra of fluorescein (a, b) and rhodamine 6G (c, d) with increasing DOC concentrations of humic acid or fulvic acid. The spectra were taken at DOC concentrations of 0, 0.5, 1, 2, and 5 mg-C/L. The concentration of the fluorescent dye was 1.0 μ M (0.38 mg/L for fluorescein and 0.48 mg/L for rhodamine 6G). The excitation wavelengths of fluorescein and rhodamine 6G were 480 nm and 520 nm, respectively.

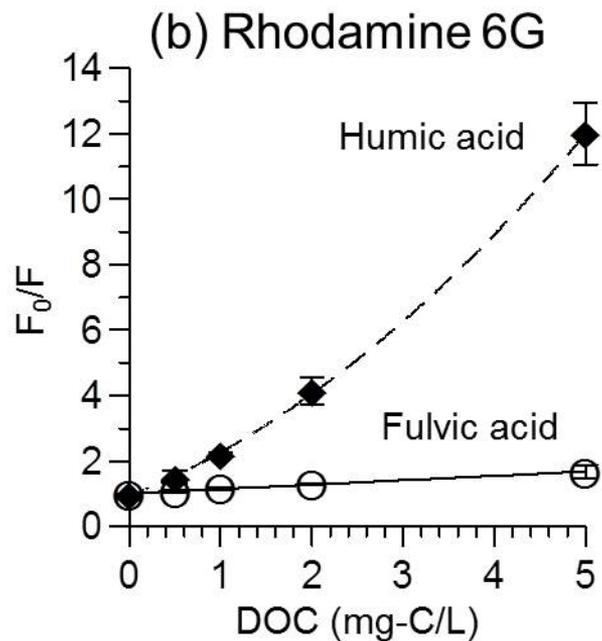
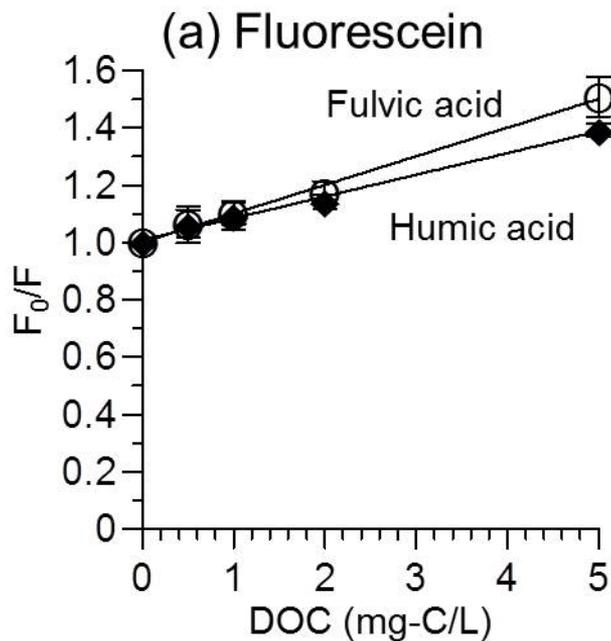


Fig. 4 Stern-Volmer plots for fluorescein (a) and rhodamine 6G (b) with increasing DOC concentrations of humic acid or fulvic acid. Fluorescence intensities of fluorescein and rhodamine 6G were plotted at 512 nm and 551 nm, respectively. Solid lines represent linear relationships. A non-linear relationship is shown as a dashed line.

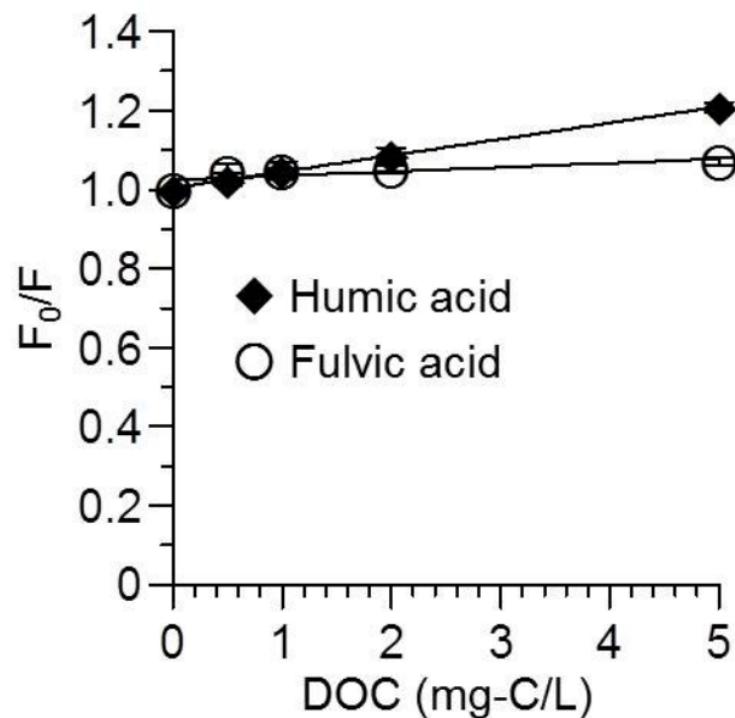


Fig. 5 Stern–Volmer plots for rhodamine B with increasing DOC concentrations of humic acid or fulvic acid. Fluorescence intensities of rhodamine B were plotted at 574 nm.