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DOI:

[10.1016/j.watres.2014.05.013](https://doi.org/10.1016/j.watres.2014.05.013)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Carstea, EM, Baker, A, Bieroza, M, Reynolds, DM & Bridgeman, J 2014, 'Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching', *Water Research*, vol. 61, pp. 152–161. <https://doi.org/10.1016/j.watres.2014.05.013>

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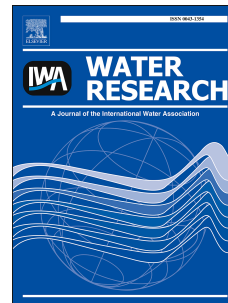
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Accepted Manuscript

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PII: S0043-1354(14)00358-3

DOI: [10.1016/j.watres.2014.05.013](https://doi.org/10.1016/j.watres.2014.05.013)

Reference: WR 10667

To appear in: *Water Research*

Received Date: 3 March 2014

Revised Date: 26 April 2014

Accepted Date: 10 May 2014

Please cite this article as: Carstea, E.M., Baker, A., Bieroza, M., Reynolds, D.M., Bridgeman, J., Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching, *Water Research* (2014), doi: 10.1016/j.watres.2014.05.013.

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1 **Characterisation of dissolved organic matter fluorescence**
2 **properties by PARAFAC analysis and thermal quenching**

3

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17

18 **Abstract**

19 The fluorescence intensity of dissolved organic matter
20 (DOM) in aqueous samples is known to be highly influenced
21 by temperature. Although several studies have demonstrated
22 the effect of thermal quenching on the fluorescence of DOM,
23 no research has been undertaken to assess the effects of
24 temperature by combining fluorescence excitation – emission
25 matrices (EEM) and parallel factor analysis (PARAFAC)
26 modelling. This study further extends previous research on
27 thermal quenching by evaluating the impact of temperature on

28 the fluorescence of DOM from a wide range of environmental
29 samples, in the range 20° C - 0° C. Fluorescence intensity
30 increased linearly with respect to temperature decrease at all
31 temperatures down to 0° C. Results showed that temperature
32 affected the PARAFAC components associated with humic-like
33 and tryptophan-like components of DOM differently,
34 depending on the water type. The terrestrial humic-like
35 components, C1 and C2 presented the highest thermal
36 quenching in rural water samples and the lowest in urban water
37 samples, while C3, the tryptophan-like component, and C4, a
38 reprocessed humic-like component, showed opposite results.
39 These results were attributed to the availability and abundance
40 of the components or to the degree of exposure to the heat
41 source. The variable thermal quenching of the humic-like
42 components also indicated that although the PARAFAC model
43 generated the same components across sites, the DOM
44 composition of each component differed between them. This
45 study has shown that thermal quenching can provide additional
46 information on the characteristics and composition of DOM
47 and highlighted the importance of correcting fluorescence data
48 collected *in situ*.

49

50 **Keywords:** fluorescence spectroscopy; thermal
51 quenching; dissolved organic matter; parallel factor analysis;
52 temperature correction

53

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57

58 1. Introduction

59 In recent years, fluorescence spectroscopy has been
60 increasingly applied to the analysis of aqueous dissolved
61 organic matter (DOM). The effectiveness of this technique in
62 water quality analysis has been proven by studies on numerous
63 types of water systems (Drozdowska, 2007; Kelton et al., 2007;
64 Murphy et al., 2008; Ghervase et al., 2012; Kothawala et al.,
65 2012; Carstea et al., 2014). Fluorescence has been correlated
66 with standard parameters such as biological oxygen demand
67 (Reynolds and Ahmad, 1997; Hudson et al., 2008; Hur and
68 Kong, 2008), total organic carbon (Vodacek et al., 1995),
69 nitrogen and chemical oxygen demand (Hur and Cho, 2012;
70 Bridgeman et al., 2013). Due to its potential, researchers have
71 applied fluorescence spectroscopy in studies such as the
72 monitoring of riverine DOM and diesel pollution (Spencer et
73 al., 2007; Carstea et al., 2010), analysis of recycled waters
74 (Henderson et al., 2009), evaluation of drinking water treatment
75 processes (Bieroza et al., 2009; Shutova et al., 2014),
76 monitoring of viral abundance in wastewater (Pollard, 2012),
77 quantification of pesticides (Ferretto et al., in press) or testing
78 of potable waters microbial quality (Cumberland et al., 2012).

79 The intensive use of fluorescence spectroscopy in water quality
80 analyses arises from its advantages, which include high
81 sensitivity, small quantities of sample needed, very little or no
82 sample preparation and short measuring time (Coble, 1996;
83 Birdwell and Valsargis, 2010). However, the fluorescence
84 signal can be affected by so-called “matrix effects” which
85 include inner filter effects and fluorescence quenching
86 (Lakowicz, 2006; Henderson et al., 2009; Korak et al., 2014).
87 With regard to fluorescence quenching, it has been shown that
88 fluorescence spectroscopy is highly sensitive to temperature
89 variations. An increase in temperature increases the probability
90 of the excited electrons returning to ground state through
91 radiationless decay. Baker (2005) studied temperature
92 quenching on several types of water samples and observed a
93 decrease in fluorescence intensity ranging from 16 % to 48 %,
94 depending on the samples and DOM component analysed.
95 Elliott et al. (2006) observed a decrease in fluorescence of more
96 than 40 % for fluorophores produced by bacterial cultures
97 isolated from river samples and Seredynska-Sobecka et al.
98 (2007) studied thermal quenching on colloids obtaining similar
99 results. However, in each case the researchers did not study the
100 impact of temperature on DOM fluorescence below 10° C, due
101 to condensation which could form on the cuvette walls.
102 Patsayeva et al. (2004) and, more recently, Watras et al. (2011)
103 have analysed thermal quenching to almost 5° C and developed

104 a correction method for fluorescence spectra but both research
105 teams concentrated only on marine water samples.
106 Consequently, no research has been made, so far, to study
107 fluorescence thermal quenching below 5° C on water samples
108 from a wide range of different sources.

109 This study seeks to characterise the fluorescence
110 properties of DOM, from water samples with different sources,
111 using thermal quenching and the combination of excitation –
112 emission matrices (EEM) and parallel factor analysis
113 (PARAFAC). Several studies have shown that PARAFAC is a
114 powerful tool in separating and analysing DOM components
115 (Ohno et al., 2008; Yamashita and Jaffe, 2008; Gueguen et al.,
116 2011; Meng et al., 2013; Murphy et al., 2014; Sanchez et al.,
117 2014; Yang et al., 2014). Specifically, the aims of this study
118 were: (1) to investigate the response of DOM, from different
119 sources (urban and rural areas), at low temperatures for a better
120 understanding of DOM characteristics; (2) to evaluate the
121 impact of temperature on the most labile fractions of DOM; (3)
122 to assess the potential of applying the Watras et al. (2011)
123 correction tools at temperatures below 5° C; (4) to investigate
124 the use of EEM-PARAFAC tool combined with thermal
125 quenching to improve our understanding of DOM character. To
126 date, EEM-PARAFAC has not been applied to the investigation
127 of thermal quenching of DOM components from water samples
128 and could provide a better understanding of DOM properties.

129

130 **2. Materials and Methods**131 **2.1 Sample preparation and analysis**

132 Samples were collected from two areas: Birmingham and
133 Buxton, located in the Midlands area, UK (Fig. 1). The
134 sampling sites, with different characteristics, were selected to
135 reflect a gradient from rural to urban areas. In Birmingham, 5
136 types of water were sampled, hereafter named: brook (Sutton
137 Park), lake (Sutton Park), pond (Edgbaston pond), surface
138 runoff from storm sewers (University of Birmingham campus)
139 and canal (Worcester and Birmingham Canal). Brook and lake
140 samples were collected from Sutton Park, which is a National
141 Nature Reserve and presents a relatively rural, pristine
142 character (<http://www.birmingham.gov.uk/suttonpark>). Canal,
143 storm sewer and pond samples were collected from an urban
144 zone; however, the pond was located in a small park with lower
145 anthropogenic activity compared to canal and storm sewer.
146 From Buxton, a river water sample was collected. Buxton town
147 is located along the Wye River, within The Peak District
148 National Park, having low anthropogenic impact, according to
149 the Environment Agency
(<http://www.peakdistrict.gov.uk/microsites/sopr/landscape/river>
150 -quality).
151

152 Water was sampled in polypropylene bottles, cleaned with
153 10 % HCl and thoroughly rinsed with deionised water prior to

154 use. All measurements were performed within 24h from
155 collection. The samples were measured for conductivity, pH,
156 dissolved organic carbon (DOC) and absorbance, from 200 nm
157 to 700 nm. Conductivity and pH were measured using a Myron
158 meter, absorbance measurements were made with a WPA
159 lightwave UV-VIS diode-array S2000 spectrophotometer and
160 DOC with a Shimadzu TOC-Vcpn analyzer.

161 Fluorescence EEMs were recorded using a Varian Cary
162 Eclipse spectrofluorometer, with the following parameters:
163 excitation wavelength domain 200 – 400 nm, emission
164 wavelength domain 280 – 500 nm, steps of 5 nm and 2 nm for
165 excitation and emission, respectively, and slits of 5 nm. The
166 instrument stability was checked by recording the Raman
167 values (at excitation wavelength 348 nm and emission
168 wavelength 395 nm) before each set of measurements. The
169 average Raman value was 24.38 a.u. with a standard deviation
170 of 0.58. The fluorescence intensity of all spectra were
171 normalized to a maximum value of 1000 a.u. and corrected to
172 the average Raman value. Every set of measurements was made
173 in triplicate in order to check the instrument reproducibility (\pm
174 5%).

175 The temperature was decreased gradually from 20⁰ C to
176 0⁰ C, by the use of a Peltier temperature controller, recording
177 EEMs at every 0.5⁰ C. Each set of measurements lasted for 90
178 min, to ensure gentle cooling of the sample. Below 6⁰ C,

179 condensation usually forms on the cuvette outer walls, but, in
180 this study, it was eliminated by inserting dessicant bags inside
181 the sample chamber. The reduction in condensation was
182 checked by recording fluorescence spectra at periodic time
183 intervals and at the established temperature range. The
184 conditions with no condensation were obtained when silica gel
185 bags had been kept in the sample chamber for 26 hrs.
186 Throughout the experimental period, the dessicant bags were
187 periodically replaced. All samples were filtered with 0.7 μm
188 Whatman GF/C paper filters prior to cooling and analysis.

189 **2.2 PARAFAC analysis**

190 PARAFAC was performed on a set of 697 EEMs
191 (including triplicates) for varying temperatures for the six water
192 sources described above. Although only 6 different water
193 sources have been used in PARAFAC modelling, they provide
194 a good variation in terms of spectral properties and a large
195 number of samples helped to avoid any potential
196 autocorrelation effects during the split-half validation. Prior to
197 modeling, EEMs were pre-processed in Matlab using custom-
198 written functions to remove redundant spectral areas ($\lambda_{\text{ex}} < 220$
199 nm, $\lambda_{\text{ex}} > \lambda_{\text{em}}$, $2 \lambda_{\text{ex}} < \lambda_{\text{em}}$, Raman and Rayleigh scatter)
200 (Bierozza et al., 2011). Pre-processed EEMs were normalized to
201 the Raman scatter peak of water using procedure described in
202 Lawaetz and Stedmon (2009). The PARAFAC model was
203 fitted and validated using the DOMFluor toolbox for Matlab

204 (Stedmon and Bro, 2008). The final four-component model was
205 chosen based on the percentage of variance explained, core-
206 consistency diagnostic (Bro and Kiers, 2003), the results of the
207 split-half analysis and visual inspection of the excitation and
208 emission loadings (Table 1).

209

210 **3. Results and Discussion**

211 **3.1 Fluorescence properties of DOM**

212 The four fluorescence components identified in the water
213 samples are shown in Fig. 2. Component 1 ($\lambda_{\text{ex}} \sim 225$ nm and
214 ~ 330 nm, $\lambda_{\text{em}} \sim 460$ nm) is associated with terrestrial humic
215 substances, being similar to the PARAFAC components found
216 by Stedmon and Markager (2005), Murphy et al. (2008; 2011;
217 2014), Kowalczyk et al. (2009), Williams et al. (2010),
218 Baghoth et al. (2011), Yamashita et al. (2011), Ishii and Boyer
219 (2012), Kothawala et al. (2012), Maie et al. (2012) and
220 Yamashita et al. (2013). These studies have shown that this
221 component is ubiquitous in water systems, having a primary
222 terrestrial source and a secondary microbial source of DOM. In
223 addition, C1 is dominated by biological production and is
224 partially degraded. According to Fellman et al. (2010) and Ishii
225 and Boyer (2012), C1 has high molecular weight (>1000 Da)
226 and presents a high degree of hydrophobicity and aromaticity.

227 Component 2 (C2), found at $\lambda_{\text{ex}} \sim 225$ nm and ~ 330 nm,
228 $\lambda_{\text{em}} \sim 410$ nm, belongs to the group of humic fluorophores,

229 based on the studies of Stedmon and Markager (2005), Murphy
230 et al. (2008; 2014), Williams et al. (2010), Yamashita et al.
231 (2011), Ishii and Boyer (2012), Maie et al. (2012). These
232 studies show that C2 is found mostly in DOM dominated by
233 terrestrial sources and is photochemically produced. C2
234 presents minimal biodegradation and, according to Ohno et al.
235 (2010), has low molecular weight (<665 Da).

236 The third component, C3, λ_{ex} ~225 and ~275 nm, λ_{em}
237 ~350 nm, indicated the presence of a tryptophan-like fraction,
238 in accordance with the results of Stedmon and Markager
239 (2005), Williams et al. (2010), Murphy et al. (2011; 2014),
240 Maie et al. (2012), Yamashita et al. (2013), Shutova et al.
241 (2014). Furthermore, Fellman et al. (2010) and Kothawala et al.
242 (2012) found that this component is a product of
243 autochthonous, microbial processing.

244 Component 4 (C4) (λ_{ex} ~240 and ~320 nm, λ_{em} ~380 nm)
245 is linked to the humic substances, as shown by Stedmon and
246 Markager (2005), Murphy et al. (2008; 2011; 2014), Graeber et
247 al. (2012), Kothawala et al. (2012), Maie et al. (2012), Ishii and
248 Boyer (2012) and Yamashita et al. (2013). These studies
249 demonstrate that C4 indicates recent biological production and
250 is often defined as a microbial humic-like component (Murphy
251 et al., 2011; Maie et al., 2012; Yamashita et al., 2013). Ishii and
252 Boyer (2012) report that C4 has an intermediate molecular
253 weight, between C1 and C2.

254 The mean fluorescence values of component scores and
255 the relative abundance of each component to the total
256 fluorescence intensity are presented in Table 2. C1 and C2 are
257 most abundant at the brook and lake samples, followed by the
258 river and pond samples and are the least abundant at the canal
259 and storm sewer samples. The abundance of C3 and C4 is
260 higher at the canal and storm sewer samples compared to the
261 other samples. A correlation between C1 and C2 was observed
262 ($r_s = 1.00$, $n = 7$, $p < 0.001$), which indicated that all samples
263 contained both high and low molecular weight DOM
264 compounds and with hydrophobic and hydrophilic characters,
265 in almost equal proportions. In addition, a strong correlation
266 between C3 and C4 was calculated ($r_s = 0.93$, $n = 7$, $0.01 > p >$
267 0.005) showing a close relationship between the tryptophan-
268 like compound and the microbial humic-like fraction. Despite
269 the low degrees of freedom for both correlations ($df = 5$), given
270 by the replication in the dataset, the correlations were
271 considered significant since the components tendencies were
272 similar.

273 Based on these results, it was observed that the brook,
274 lake and river samples, which were collected from relatively
275 pristine areas, contained DOM with a strong humic-like
276 character, indicating low anthropogenic contamination. While
277 canal and storm sewer samples showed a high abundance of
278 tryptophan, typically associated with microbial material

279 (Kothawala et al., 2012), indicating the presence of
280 anthropogenic-derived matter (Meng et al., 2013; Carstea et al.,
281 2014). The distinction between urban and rural samples is
282 better reflected by the C3/C1 ratio (Table 2): brook, lake and
283 river samples with a rural character had the lowest values, pond
284 sample had an intermediate urban and rural character due to the
285 sampling location in an urban park, and canal and storm sewer
286 with an urban impact showed the highest C3/C1 values. Canal
287 and storm sewer also presented similar values for DOC and
288 absorbance (Table 3). Furthermore, rural samples showed
289 higher DOC and absorbance values compared to the other
290 samples. The highest conductivity values were detected at the
291 canal and pond samples, while the lowest values were seen at
292 the storm sewer sample. The values for pH were recorded
293 within the range of 6.7 and 8.1.

294 **3.2 Thermal quenching of humic-like components**

295 The fluorescence response to temperature variation,
296 between 20⁰ C and 0⁰ C, for the humic-like components C1, C2
297 and C4 is shown in Figure 3 (a, c and e). All three components
298 exhibit a linear fluorescence increase with temperature
299 decrease. Similar linearity was reported in the studies of Baker
300 et al. (2005), Seredynska-Sobecka et al. (2007) and Watras et
301 al. (2011) on thermal quenching of DOM fluorescence, in the
302 range of 45⁰ C - 5⁰ C. Although, PARAFAC components

303 showed similar linear trends at all samples, the degree of
304 temperature impact was highly variable.

305 Figure 3 (b, d, f) presents the slope of fluorescence
306 intensity decrease per degree Celsius. C1 shows the highest
307 slope at the rural samples, lake and brook, followed by the
308 pond and storm sewer samples, while the lowest values have
309 been seen at the river and canal samples. Similar sample
310 variability of slope was observed at C2. The last humic-like
311 component, C4, presents the highest slope at the urban samples,
312 storm sewer and canal, whilst the lowest have been seen at the
313 rural samples. It must be noted that although the PARAFAC
314 model is consistent across all samples, the degree of thermal
315 quenching is variable between them. This suggests that each
316 humic-like PARAFAC component is comprised of more than
317 one fluorophore.

318 Overall, C1 exhibits a higher slope of fluorescence
319 intensity decrease compared to C2 and C4, indicating that this
320 component might be more environmentally impacted.
321 Seredynska-Sobecka et al. (2007) reported that the humic-like
322 fraction has high sensitivity to thermal quenching, especially at
323 the small size fractions ($< 0.1 \mu\text{m}$). Furthermore, Ohno et al.
324 (2008), Yamashita and Jaffe (2008) and Mounier (2010)
325 proved, by studying the interaction between DOM and metal
326 ions, that this component was more likely to suffer fluorescence
327 quenching, compared to the other humic-like components. This

328 indicated that C1 is more sensitive to environmental changes
329 relative to C2 and C4. Moreover, C2 and C4, which are
330 resistant to further degradation, after photochemical and
331 biological production and degradation (Ishii and Boyer, 2012),
332 are probably less affected by temperature changes. The high
333 slope of C1 could also be associated with the relative
334 abundance of fluorescence intensity, as higher slope has been
335 observed at samples with high abundance. Hence, C1 could be
336 more readily available for thermal quenching compared to C2
337 and C4.

338 **3.3 Tryptophan-like component behaviour to** 339 **temperature changes**

340 Tryptophan-like component, C3, shows the same linearity
341 as the humic-like components (Fig. 4a), in accordance with the
342 results of Baker (2005) and Elliott et al. (2006). Furthermore,
343 variable gradients of fluorescence decrease per degree Celsius
344 (slope) have been observed (Fig. 4b). The highest slope has
345 been seen at the storm sewer and canal samples, followed by
346 the lake, pond and river samples, while the lowest has been
347 observed at the brook sample.

348 In contrast to the humic-like components, C3 slope could
349 be associated to a lesser extent with the relative abundance of
350 fluorescence intensity (Table 2). Although, C3 is more
351 abundant in the canal sample, compared to storm sewer, it
352 shows a lower slope value. According to Baker (2005) the

353 degree of thermal quenching relates to the exposure of the
354 fluorophore to the heat source. These findings suggest that C3,
355 belonging to storm sewer DOM, contains more exposed
356 tryptophan compared to the canal sample. The same
357 assumption could apply to the lake sample C3, which presents a
358 high slope value, despite the low abundance relative to river
359 and pond samples. The results suggest that free tryptophan
360 could be a dominant component in storm sewer and lake
361 samples and is, therefore, more easily quenched with increasing
362 temperature.

363 The various responses of PARAFAC components scores
364 to temperature fluctuations can have a large impact on *in situ*
365 fluorescence measurements, especially when comparing
366 experiments from several locations made in different seasons or
367 times of the day. Consequently, the fluorescence spectra need
368 to be corrected for temperature before comparison studies can
369 be made. The temperature correction tool, developed by Watras
370 et al. (2011), uses a temperature coefficient, which is the ratio
371 between the slope of the fluorescence intensity as a function of
372 temperature change, from 20⁰ C to 5⁰ C and the intercept, at the
373 reference temperature of 20⁰ C. However, their studies have
374 been performed on lake water and could not account for
375 variations between different types of water samples. The slope,
376 calculated in the present study, shows the same linear trend of
377 increase below 5⁰ C, indicating that the temperature correction

378 tool developed by Watras et al. (2011) can be applied even to
379 fluorescence spectra of samples measured below 5⁰ C.

380

381 **4. Conclusions**

382 This study presents the first investigation of DOM
383 fluorescence properties, at low temperatures, with EEM-
384 PARAFAC. The impact of temperature on the individual
385 PARAFAC components in DOM, from several water samples,
386 was evaluated by decreasing the temperature from 20⁰ C to 0⁰
387 C. This analysis extends the fluorescence thermal quenching
388 studies, made by other researchers, in the range of 45⁰ C – 5⁰ C.
389 Results have shown that fluorescence intensity has a linear
390 increase, as temperature decreased from 20⁰ C to 0⁰ C. Thus,
391 the temperature correction tools developed by Watras et al.
392 (2011) can be applied to fluorescence spectra of samples
393 measured at temperatures below 5⁰ C.

394 It has been found that temperature affects the PARAFAC
395 components associated with the tryptophan-like and humic-like
396 fractions differently, depending on DOM character of each
397 sample. The humic-like components, C1 and C2 present the
398 highest thermal quenching at the rural samples and the lowest
399 at the urban samples, while C4 show opposite results. The data
400 indicate that, while the PARAFAC model is consistent across
401 all samples, the degree of thermal quenching varies between
402 them, suggesting that each humic-like PARAFAC component

403 is comprised of more than one fluorophore. Furthermore,
404 thermal quenching has shown that, among the humic-like
405 components, C1 is more environmentally impacted but, at the
406 same time, more readily available to quenching compared to C2
407 and C4. The tryptophan-like component presents the highest
408 slope of fluorescence decrease per degree Celsius in the urban
409 samples and the lowest at the rural samples. Thermal quenching
410 has evidenced that free tryptophan residues, from the
411 tryptophan-like fraction, are dominant at the storm sewer and
412 lake samples, due to the direct exposure of the fluorophore to
413 the heat source.

414 Considering that a growing body of literature stresses the
415 importance of using fluorescence for *in situ* measurements, the
416 analysis of temperature effects on DOM is highly important, as
417 the fluorescence signal of each DOM component is variably
418 quenched depending on temperature. Therefore, we recommend
419 correction of the fluorescence spectra recorded at temperatures
420 below 20⁰ C. However, it is necessary to be aware of the
421 potential multi-fluorophoric nature of the PARAFAC humic-
422 like components, which may lead to variable results between
423 sites.

424

425 **Acknowledgements**

426 The work of E.M. Carstea was partly supported by the
427 European Commission Framework Programme 7, through a

428 Marie Curie Intra-European Fellowship (PIEF-GA-2012-
429 329962).

430

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629 Figure captions

630 Fig. 1 Map with the sampling points from Birmingham and
631 Buxton (Map of UK adapted from © OpenStreetMap
632 contributors, CC BY-SA, Open Database License 2010).

633 Fig. 2. Excitation and emission matrices of the four PARAFAC
634 components.

635 Fig. 3 Linear relationship between PARAFAC scores and
636 temperature, and the slope: (a) and respectively (b) component
637 1, (c) and (d) component 2, (e) and (f) component 4.

638 Fig. 4 Linear relationship between PARAFAC scores and
639 temperature (a) and the slope (b) for component 3.

Table 1. A summary of the PARAFAC models fitted to fluorescence dataset with the following constraints: sample mode – non-negativity, excitation and emission modes – non-negativity and unimodality

Number of components	Convergence (Yes, No)	Sum of squares of errors	Total variance explained (%)	Core-consistency (%)	Split-half analysis validation (Yes, No)
1	Yes	27056	96	100	Yes
2	Yes	23183	96	-87	Yes
3	Yes	5540	99	41	Yes
4	Yes	4860	99	6	Yes
5	Yes	4124	99	0	Yes
6	Yes	4021	99	2	Yes
7	Yes	3669	99	1	Yes

Table 2. DOM fluorescence results of the water samples.

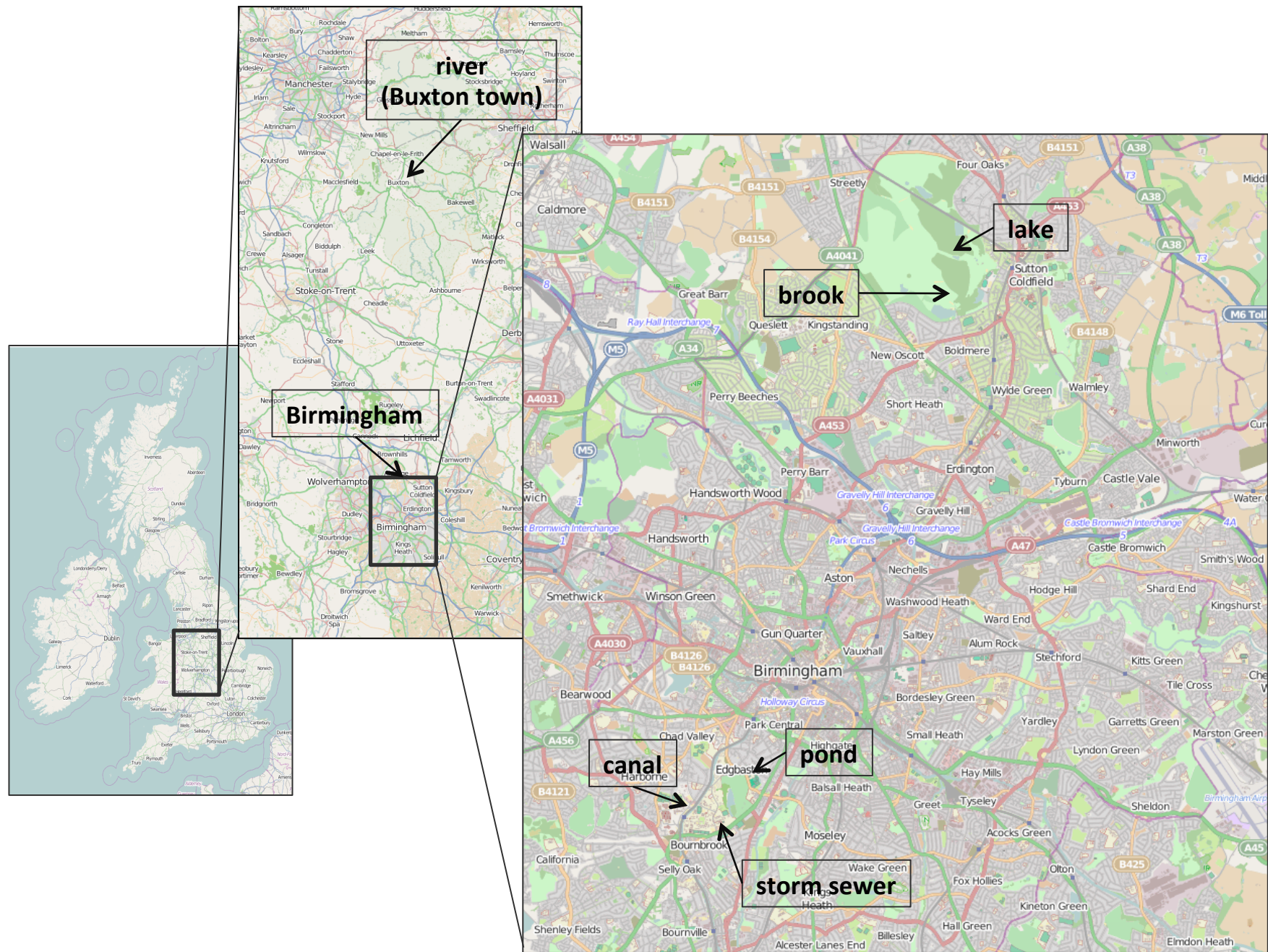
Samples	Mean value of component scores (a.u.) (SD*)						Relative abundance of fluorescence intensity (%)**			
	C1	C2	C3	C4	C3/C1	Total	C1	C2	C3	C4
Brook	30.9 (1.4)	20.0 (1.1)	1.7 (0.2)	0.2 (0.1)	0.1	52.8	59	38	3	0
Lake	23.0 (1.0)	16.5 (1.0)	4.3 (0.4)	0.9 (0.1)	0.2	44.7	51	37	10	2
River	9.5 (0.4)	5.7 (0.3)	3.0 (0.3)	1.2 (0.1)	0.3	19.3	49	29	15	6
Pond	14.9 (0.7)	11.7 (0.7)	6.8 (0.4)	6.6 (0.3)	0.5	39.9	37	29	17	16
Storm Sewer	13.6 (0.5)	11.5 (0.6)	15.0 (0.8)	9.7 (0.4)	1.1	49.8	27	23	30	19
Canal	6.6 (0.3)	4.9 (0.3)	9.8 (0.4)	6.4 (0.3)	1.5	27.6	24	18	35	23
Blank	0.1 (0.0)	0.3 (0.0)	0.1 (0.0)	0.0 (0.0)	-	0.6	22	52	19	7

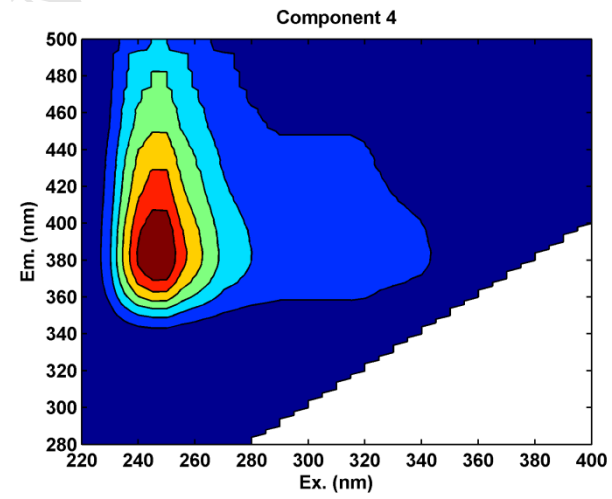
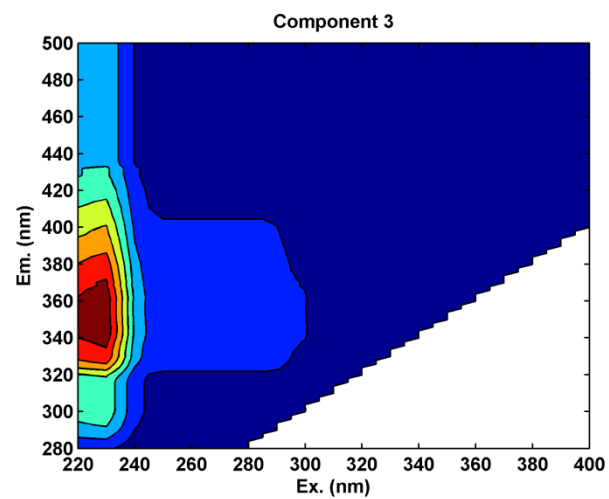
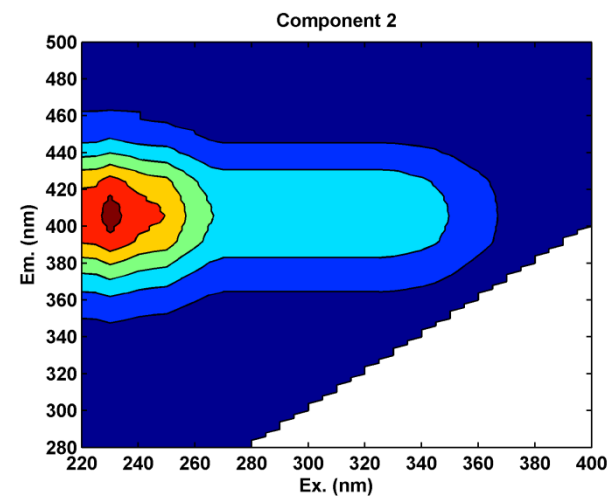
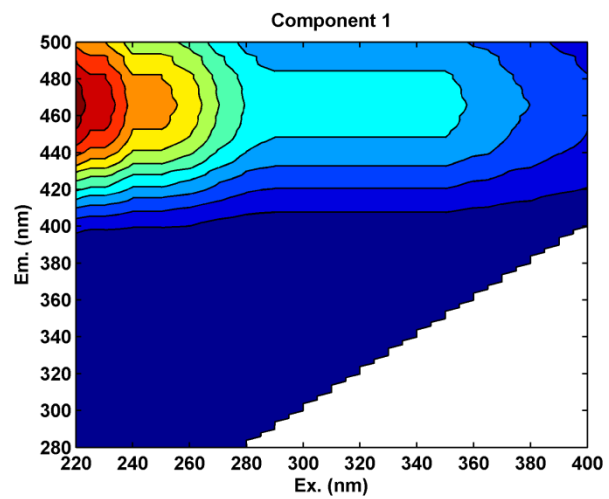
*SD – standard deviation

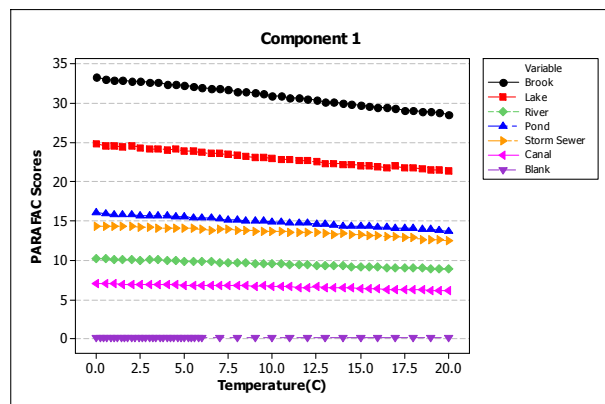
**Calculated according to Yamashita and Jaffe (2008) as percentage of the total fluorescence.

Table 3. Standard data for the analysed water samples.

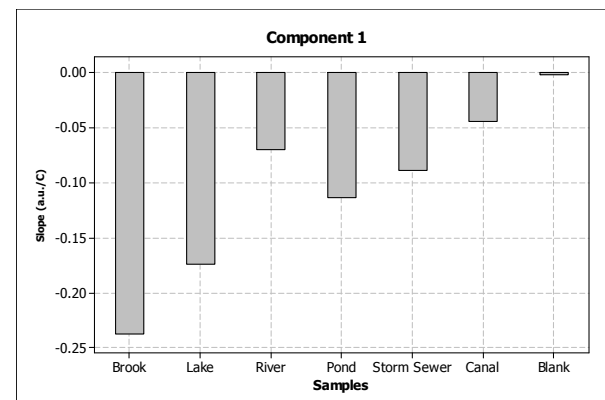
Samples	DOC (mg/L)	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Absorbance at 350 nm (cm^{-1})
Brook	7.75	8.1	413	0.089
Lake	8.71	6.8	288	0.078
River	5.55	6.7	340	0.021
Pond	2.96	7.3	687	0.023
Storm Sewer	4.96	7.0	98	0.035
Canal	4.79	6.8	747	0.039



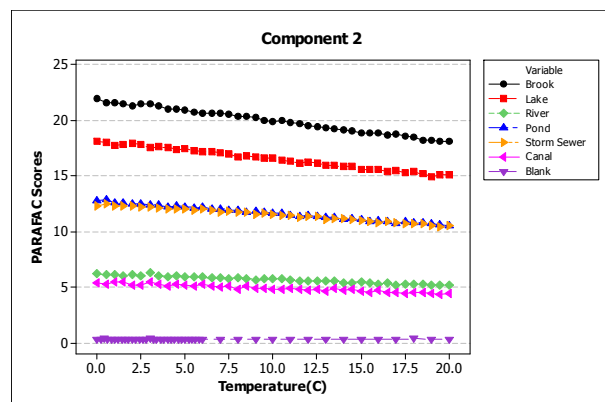




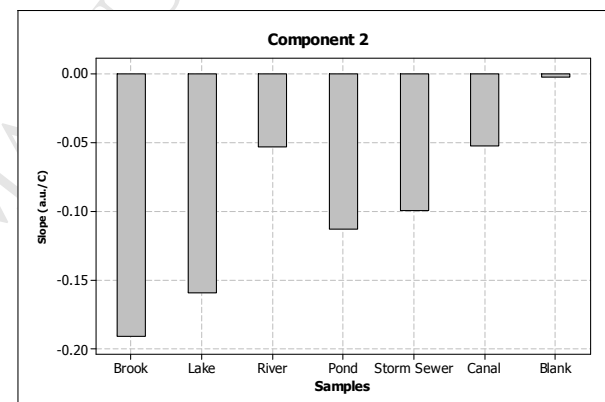
(a)



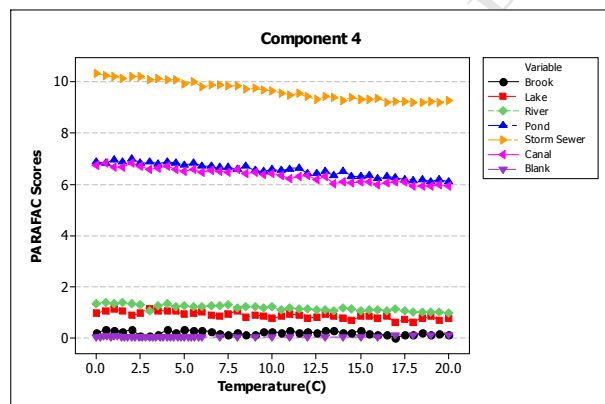
(b)



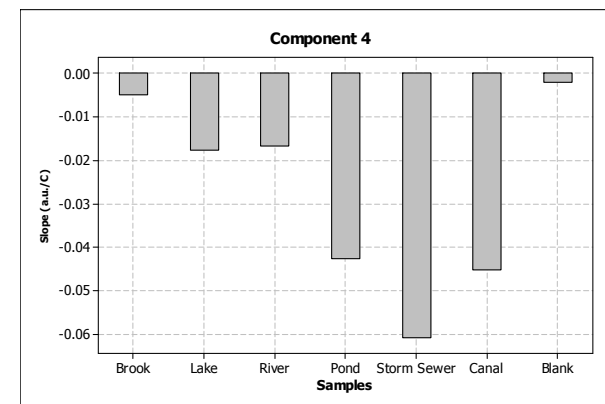
(c)



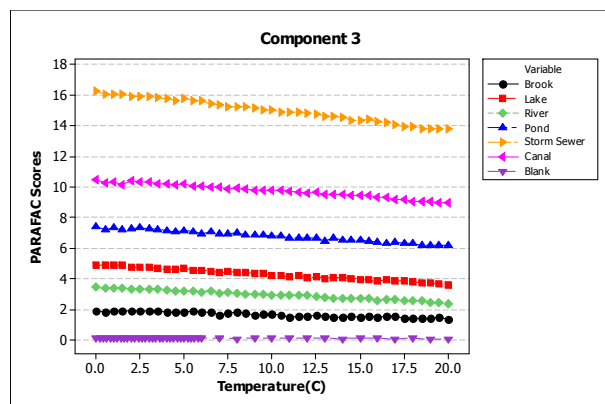
(d)



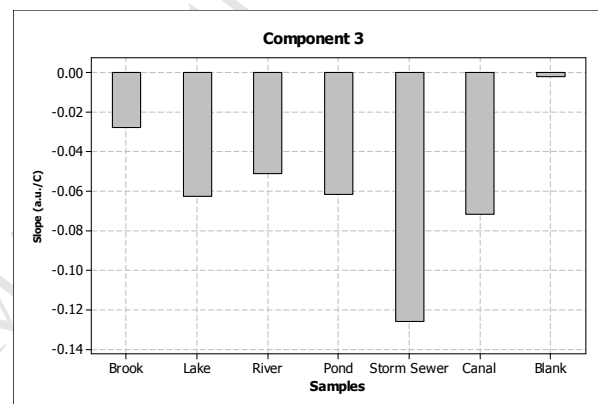
(e)



(f)



(a)



(b)

- We investigated DOM fluorescence properties, at low temperatures, with EEM-PARAFAC
- Fluorescence intensity increases linearly as temperature decreases from 20^o C to 0^o C
- DOM PARAFAC components are variably quenched and this is sample specific
- Each humic-like PARAFAC component might be comprised of more than one fluorophore

ACCEPTED MANUSCRIPT