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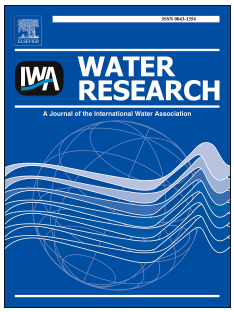

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Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching

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Abstract

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

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

Keywords: fluorescence spectroscopy; thermal quenching; dissolved organic matter; parallel factor analysis; temperature correction

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1. Introduction

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

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

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

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

2. Materials and Methods

2.1 Sample preparation and analysis

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

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

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

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

The temperature was decreased gradually from 20^0 C to 0^0 C, by the use of a Peltier temperature controller, recording EEMs at every 0.5^0 C. Each set of measurements lasted for 90 min, to ensure gentle cooling of the sample. Below 6^0 C,

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

2.2 PARAFAC analysis

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

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

3. Results and Discussion

3.1 Fluorescence properties of DOM

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

Component 2 (C2), found at λ_{ex} ~225 nm and ~330 nm, λ_{em} ~410 nm, belongs to the group of humic fluorophores,

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

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

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

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

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

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

3.2 Thermal quenching of humic-like components

The fluorescence response to temperature variation, between 20^0 C and 0^0 C, for the humic-like components C1, C2 and C4 is shown in Figure 3 (a, c and e). All three components exhibit a linear fluorescence increase with temperature decrease. Similar linearity was reported in the studies of Baker et al. (2005), Seredynska-Sobecka et al. (2007) and Watras et al. (2011) on thermal quenching of DOM fluorescence, in the range of 45^0 C - 5^0 C. Although, PARAFAC components

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

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

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

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

3.3 Tryptophan-like component behaviour to temperature changes

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

In contrast to the humic-like components, C3 slope could be associated to a lesser extent with the relative abundance of fluorescence intensity (Table 2). Although, C3 is more abundant in the canal sample, compared to storm sewer, it 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 20^0 C to 5^0 C and the intercept, at the
373 reference temperature of 20^0 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 5^0 C, indicating that the temperature correction

tool developed by Watras et al. (2011) can be applied even to fluorescence spectra of samples measured below 5^0 C.

4. Conclusions

This study presents the first investigation of DOM fluorescence properties, at low temperatures, with EEM-PARAFAC. The impact of temperature on the individual PARAFAC components in DOM, from several water samples, was evaluated by decreasing the temperature from 20^0 C to 0^0 C. This analysis extends the fluorescence thermal quenching studies, made by other researchers, in the range of 45^0 C – 5^0 C. Results have shown that fluorescence intensity has a linear increase, as temperature decreased from 20^0 C to 0^0 C. Thus, the temperature correction tools developed by Watras et al. (2011) can be applied to fluorescence spectra of samples measured at temperatures below 5^0 C.

It has been found that temperature affects the PARAFAC components associated with the tryptophan-like and humic-like fractions differently, depending on DOM character of each sample. The humic-like components, C1 and C2 present the highest thermal quenching at the rural samples and the lowest at the urban samples, while C4 show opposite results. The data indicate that, while the PARAFAC model is consistent across all samples, the degree of thermal quenching varies between 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 20^0 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

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References

Baghoth, S.A., Sharma, S.K., Amy, G.L., 2011. Tracking natural organic matter (NOM) in a drinking water treatment plant using fluorescence excitation−emission matrices and PARAFAC. Water Research 45 (2), 797−809.

Baker, A., 2005. Thermal fluorescence quenching properties of dissolved organic matter. Water Research 39 (18), 4405-4412.

Bieroza, M., Baker, A., Bridgeman, J., 2009. Relating freshwater organic matter fluorescence to organic carbon removal efficiency in drinking water treatment. Science of the Total Environment 407 (5), 1765–1774.

Bieroza, M., Baker, A., Bridgeman, J., 2011. Classification and calibration of organic matter fluorescence data with multiway analysis methods and artificial neural networks: an operational tool for improved drinking water treatment. Environmetrics 22 (3), 256-270.

Birdwell, J.E., Valsaraj, K.T., 2010. Characterization of dissolved organic matter in fogwater by excitation-emission matrix fluorescence spectroscopy. Atmospheric Environment 44 (27), 3246-3253.

Birmingham City Council, http://www.birmingham.gov.uk/suttonpark [accessed in January 2014].

Bridgeman, J., Baker, A., Carliell-Marquet, C.M., Carstea, E., 2013. Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. Environmental Technology 34 (23), 3069-3077.

Bro, R., Kiers, H.A.L., 2003. A new efficient method for determining the number of components in PARAFAC models. Journal of Chemometrics 17 (5), 274-286.

458 Carstea, E.M., Baker, A., Bieroza, M., Reynolds D.M., 2010.
459 Continuous fluorescence excitation emission matrix monitoring of river
460 organic matter. Water Research 44 (18), 5356-5366.
461 Carstea, E.M., Baker, A., Savastru, R., 2014. Comparison of river
462 and canal water dissolved organic matter fluorescence within an urbanised
463 catchment. Water and Environment Journal 28 (1), 11-22.
464 Coble, P.G., 1996. Characterisation of marine and terrestrial
465 dissolved organic matter in seawater using excitationemission matrix
466 spectroscopy. Marine Chemistry 51, 325-346.
467 Cumberland, S., Bridgeman, J., Baker, A., Sterling, M., Ward, D.,
468 2012. Fluorescence spectroscopy as a tool for determining microbial quality
469 in potable water applications. Environmental Technology 33 (6), 687-693.
470 Drozdowska, V., 2007. Seasonal and spatial variability of surface
471 seawater fluorescence properties in the Baltic and Nordic Seas: results of
472 lidar experiments. Oceanologia 49 (1), 59–69.
473 Elliott, S., Lead, J.R., Baker, A., 2006. Thermal quenching of
474 fluorescence of freshwater, planktonic bacteria. Analytica Chimica Acta 564
475 (2), 219-225.
476 Fellman, J.B., Spencer, R.G.M., Hernes, P.J., Edwards, R.T.,
477 D'Amore, D.V., Hood, E., 2010. The impact of glacier runoff on the
478 biodegradability and biochemical composition of terrigenous dissolved
479 organic matter in near-shore marine ecosystems. Marine Chemistry 121 (1–
480 4), 112–122.
481 Ferretto, N., Tedetti, M., Guigue, C., Mounier S., Redon. R., Goutx,
482 M., In press. Identification and quantification of known polycyclic aromatic
483 hydrocarbons and pesticides in complex mixtures using fluorescence
484 excitation–emission matrices and parallel factor analysis, Chemosphere.
485 Ghervase, L., Cordier, A., Ibalot F., Parlanti, E., 2012. Storage effect
486 on fluorescence signal of dissolved organic matter components. Romanian
487 Reports in Physics 64 (3), 754-760.

Graeber, D., Gelbrecht, J., Pusch, M.T., Anlanger, C., von Schiller, D., 2012. Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams, Science of the Total Environment 438, 435–446.

Guéguen, C., Granskog, M.A., McCullough, G., Barber, D.G., 2011. Characterisation of colored dissolved organic matter in Hudson Bay and Hudson Strait using parallel factor analysis. Journal of Marine Systems 88 (3), 423–433.

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

Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell-Marquet, C., Browning, S., 2008. Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? An example from South West England. Science of the Total Environment 391 (1), 149–158.

Ishii, S.K.L., Boyer, T.H., 2012. Behavior of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review. Environmental Science & Technology 46 (4), 2006−2017.

http://www.peakdistrict.gov.uk/microsites/sopr/landscape/river-quality [accessed in April 2014]

Hur, J., Kong, D.S., 2008. Using synchronous fluorescence spectra to estimate biochemical oxygen demand (BOD) of urban rivers affected by treated sewage. Environmental Technology, 29 (4), 435-444.

Hur, J., Cho J.W., 2012. Prediction of total nitrogen, BOD, and COD concentrations in a typical urban river using fluorescence excitation-emission matrix with PARAFAC and UV absorption indices. Sensors 12, 972-986.

517 Kelton, N., Molot, L.A., Dillon, P.J., 2007. Spectrofluorometric
518 properties of dissolved organic matter from Central and Southern Ontario
519 streams and the influence of iron and irradiation. Water Research, 41 (3),
520 638 – 646.

521 Korak, J.A., Dotson, A.D., Summers, R.S., Rosario-Ortiz, F.L., 2014.
522 Critical analysis of commonly used fluorescence metrics to characterize
523 dissolved organic matter. Water Research 49, 327-338.

524 Kothawala, D.N., von Wachenfeldt, E., Koehler, B., Tranvik, L.J.,
525 2012. Selective loss and preservation of lake water dissolved organic matter
526 fluorescence during long-term dark incubations. Science of the Total
527 Environment 433, 238-246.

528 Kowalczuk, P., Durako, M.J., Young, H., Kahn, A.E., Cooper, W.J.,
529 Gonsior, M., 2009. Characterization of dissolved organic matter
530 fluorescence in the South Atlantic Bight with use of PARAFAC model:
531 Interannual variability, Marine Chemistry 113 (3–4), 182–196.

532 Lakowicz, J.R., 2006. Principles of Fluorescence Spectroscopy.
533 Third edition, Publisher Springer New York, Lakowicz, J.R., 2006.
534 Principles of Fluorescence Spectroscopy. Third edition, Publisher Springer
535 New York., ISBN 978-0-387-46312-4.

536 Lawaetz, A., Stedmon, C., 2009. Fluorescence intensity calibration
537 using the Raman scatter peak of water. Applied Spectroscopy 63 (8), 936-
538 940.

539 Maie, N., Yamashita, Y., Cory, R.M., Boyer, J.N., Jaffé, R., 2012.
540 Application of excitation emission matrix fluorescence monitoring in the
541 assessment of spatial and seasonal drivers of dissolved organic matter
542 composition: Sources and physical disturbance controls. Applied
543 Geochemistry 27 (4), 917–929.

544 Meng, F., Huang, G., Yang, X., Li, Z., Li, J., Cao, J., Wanga, Z.,
545 Sun, L., 2013. Identifying the sources and fate of anthropogenically

546 impacted dissolved organic matter (DOM) in urbanized rivers. Water
547 Research 47 (14), 5027-5039.

548 Mounier, S., Zhao, H., Garnier, C., Redon, R., 2010. Copper
549 complexing properties of dissolved organic matter: PARAFAC treatment of
550 fluorescence quenching. Biogeochemistry 106 (1), 107−116.

551 Murphy, K., Stedmon, C.A., Waite, D., Ruiz, G., 2008.
552 Distinguishing between terrestrial and autochthonous organic matter sources
553 in marine environments using fluorescence spectroscopy. Marine Chemistry
554 108 (1-2), 40-58.

555 Murphy, K.R., Hambly, A., Singh, S., Henderson, R.K., Baker, A.,
556 Stuetz, R., Khan, S.J., 2011. Organic matter fluorescence in municipal water
557 recycling schemes: toward a unified PARAFAC model. Environmental
558 Science & Technology, 45 (7), 2909-2916.

559 Murphy, K.R., Stedmon, C.A., Wenig, P., Bro R., 2014. OpenFluor–
560 an online spectral library of auto-fluorescence by organic compounds in the
561 environment. Analytical Methods 6 (3), 658-661.

562 Ohno, T., Amirbahman, A., Bro, R., 2008. Parallel factor analysis of
563 excitation-emission matrix fluorescence spectra of water soluble soil organic
564 matter as basis for the determination of conditional metal binding
565 parameters. Environmental Science and Technology 42 (1), 186−192.

566 OpenStreetMap contributors, CC BY-SA, Open Database License.
567 (2010) Map of the United Kingdom Ordnance Survey data © Crown
568 copyright and database right 2010–12. http://www.openstreetmap.org/
569 [accessed January 2014].

570 Patsayeva, S., Reuter, R., Thomas, D.N., 2004. Fluorescence of
571 dissolved organic matter in seawater at low temperatures and during ice
572 formation, EARSel eProceedings 3, 227-238.

573 Pollard, P., 2012. Fluorescence instrument for ***in situ*** monitoring of
574 viral abundance in water, wastewater and recycled water. Journal of
575 Virological Methods 181 (1), 97-102.

Reynolds, D.M., Ahmad, S.R., 1997. Rapid and direct determination of wastewater BOD values using a fluorescence technique. Water Research 31(8), 2012–2018.

Sanchez, N.P., Skeriotis, A.T., Miller, C.M., 2014. A PARAFAC-based long-term assessment of DOM in a multi-coagulant drinking water treatment scheme, Environmental Science and Technology 48 (3), 1582–1591.

Seredynska-Sobecka, B., Baker, A., Lead, J.R., 2007. Characterisation of colloidal and particulate organic carbon in freshwaters by thermal fluorescence quenching. Water Research 41 (14), 3069 – 3076.

Shutova, Y., Baker, A., Bridgeman, J., Henderson, R.K., 2014. Spectroscopic characterisation of dissolved organic matter changes in drinking water treatment: From PARAFAC analysis to online monitoring wavelengths. Water Research 54, 159–169.

Spencer, R.G.M., Pellerin, B.A., Bergamaschi, B.A., Downing, B.D., Kraus, T.E.C., Smart,D.R., Dahlgren, R.A., Hernes, P.J., 2007. Diurnal variability in riverine dissolved organic matter composition determined by *in situ* optical measurement in the San Joaquin River (California, USA). Hydrological Processes 21 (23), 3181-3189.

Stedmon, C.A.; Markager, S., 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. Limnology and Oceanography 50 (2), 686−697.

Stedmon, C.A., Bro, R., 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnology and Oceanography-Methods 6, 572-579.

Vodacek, A., Hoge, F.E., Swift, R.N., Yungel, J.K., Peltzer, E.T., Blough, N.V., 1995. The use of in situ airborne fluorescence measurements to determine UV absorption coefficients and DOC concentrations in surface waters. Limnology and Oceanography, 40 (2), 411-415.

606 Watras, C.J., Hanson, P.C., Stacy, T.L., Morrison, K., Mather, J., Hu,
607 Y.H., Milewski, P., 2011. A temperature compensation method for CDOM
608 fluorescence sensors in freshwater. Limnology and Oceanography-Methods
609 9, 296–301.
610 Williams, C.J., Yamashita, Y., Wilson, H.F., Jaffe, R., Xenopoulo,s
611 M.A., 2010. Unraveling the role of land use and microbial activity in
612 shaping dissolved organic matter characteristics in stream ecosystems.
613 Limnology and Oceanography 55 (3), 1159–1171.
614 Yamashita, Y., Jaffé, R., 2008. Characterizing the interactions
615 between trace metals and dissolved organic matter using excitation-emission
616 matrix and Parallel Factor Analysis. Environmental Science & Technology
617 42 (19), 7374-7379.
618 Yamashita, Y., Kloeppel, B.D., Knoepp, J., Zausen, G.L., Jaffé, R.,
619 2011. Effects of watershed history on dissolved organic matter
620 characteristics in headwater streams. Ecosystems 14 (7), 1110-1122.
621 Yamashita, Y., Boyer, J.N., Jaffé, R., 2013. Evaluating the
622 distribution of terrestrial dissolved organic matter in a complex coastal
623 ecosystem using fluorescence spectroscopy. Continental Shelf Research 66,
624 136–144.
625 Yang, L., Shin, H.S., Hur, J., 2014. Estimating the concentration and
626 biodegradability of organic matter in 22 wastewater treatment plants using
627 fluorescence excitation emission matrices and Parallel Factor Analysis.
628 Sensors 14, 1771-1786.

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.

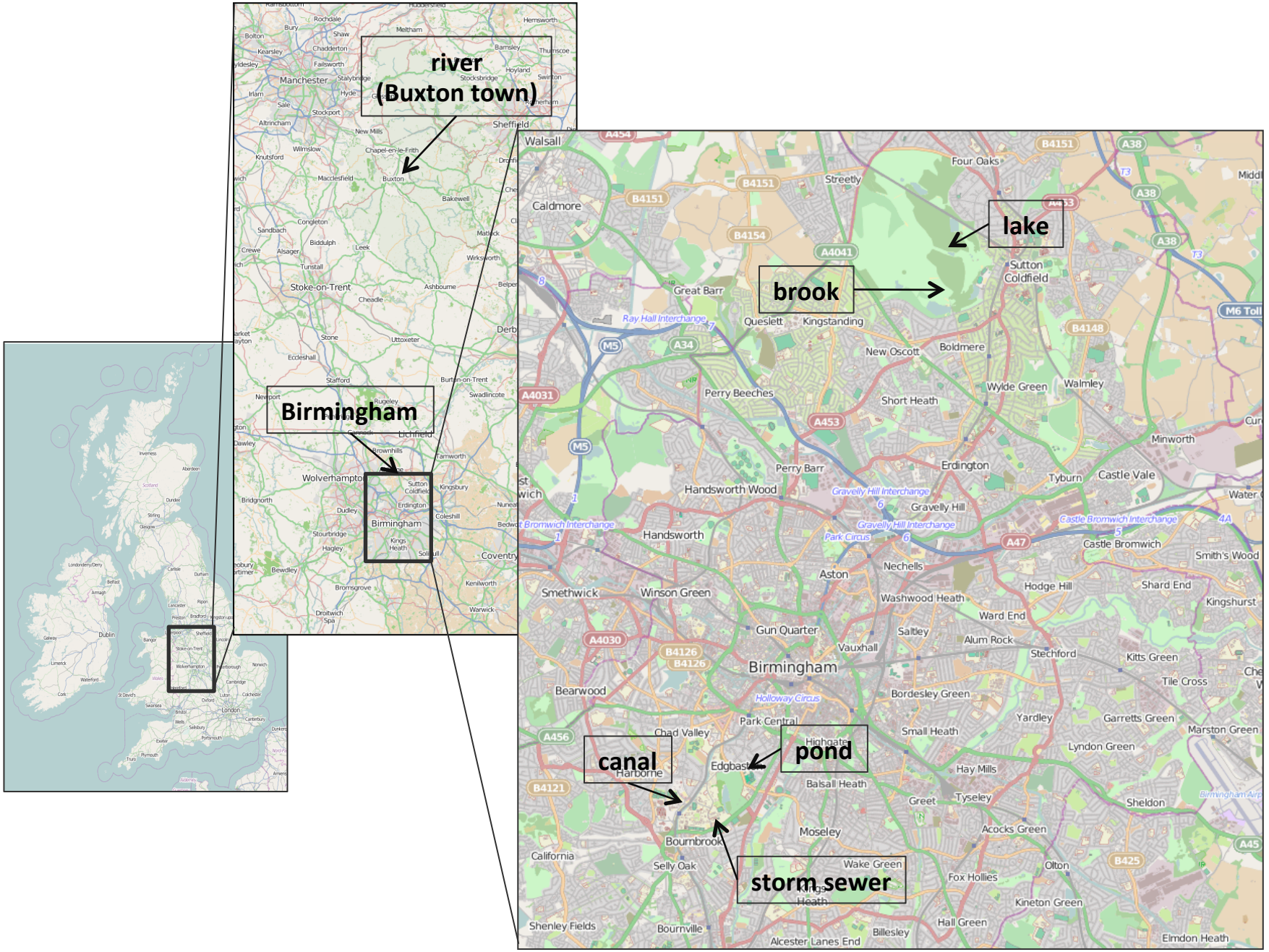
Samples	Mean value of component scores (a.u.) (SD*)						Relative abundance of fluorescence intensity (%)**			
	C1	C2	C3	C4	C3/C1	Total	C1	C2	C3	C4
Brook	30.9 (1.4)	20.0 (1.1)	1.7 (0.2)	0.2 (0.1)	0.1	52.8	59	38	3	0
Lake	23.0 (1.0)	16.5 (1.0)	4.3 (0.4)	0.9 (0.1)	0.2	44.7	51	37	10	2
River	9.5 (0.4)	5.7 (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

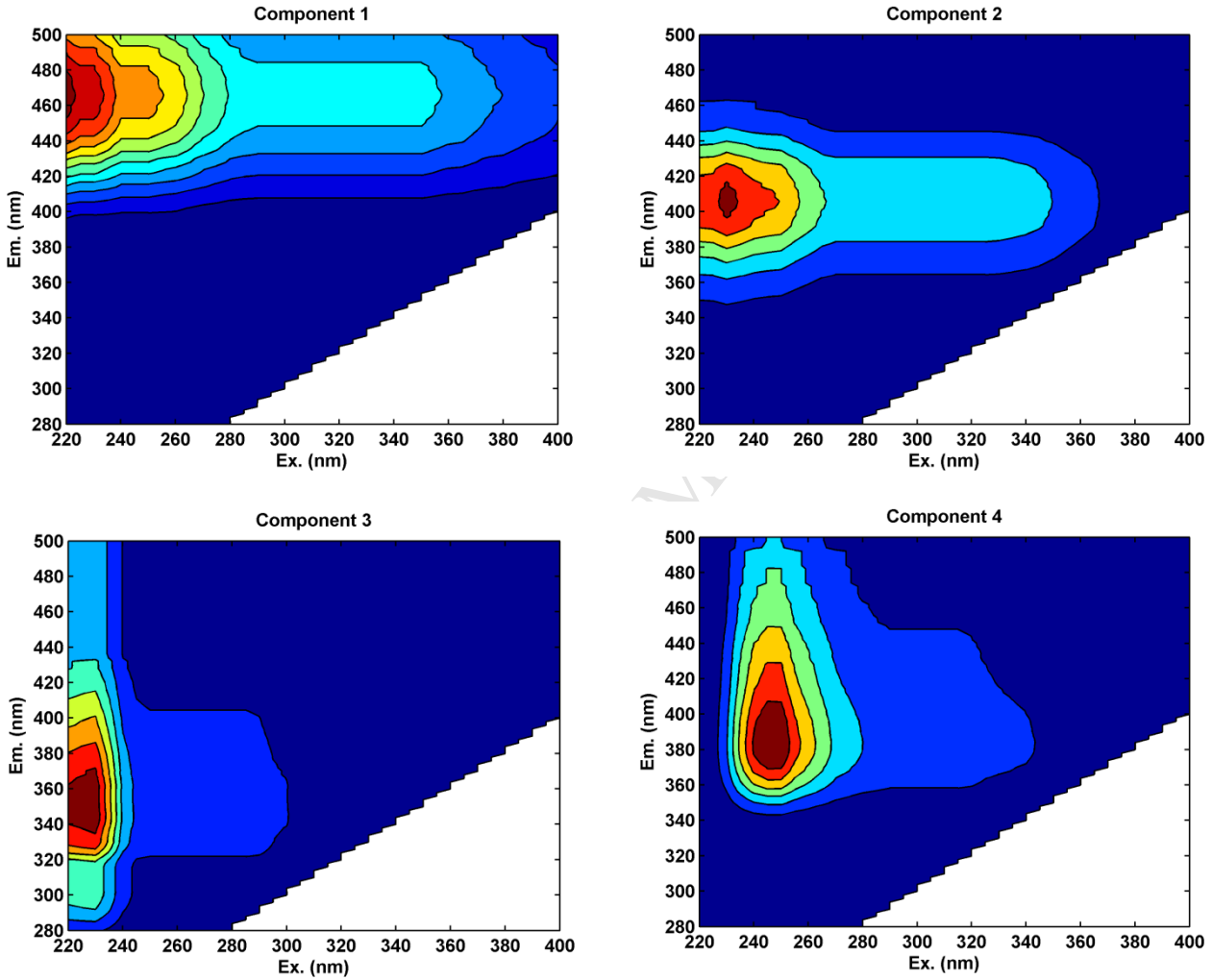
*SD – standard deviation

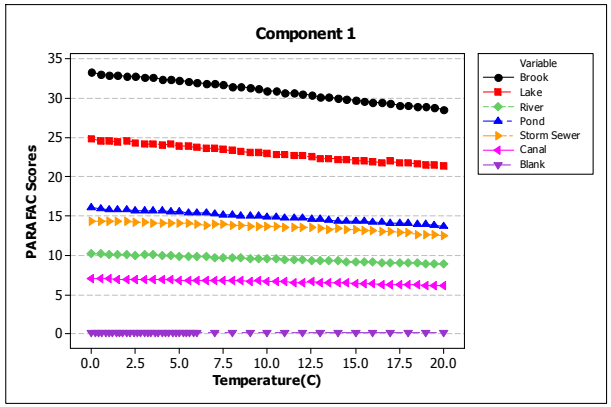
**Calculated according to Yamashita and Jaffe (2008) as percentage of the total fluorescence.

Table 3. Standard data for the analysed water samples.

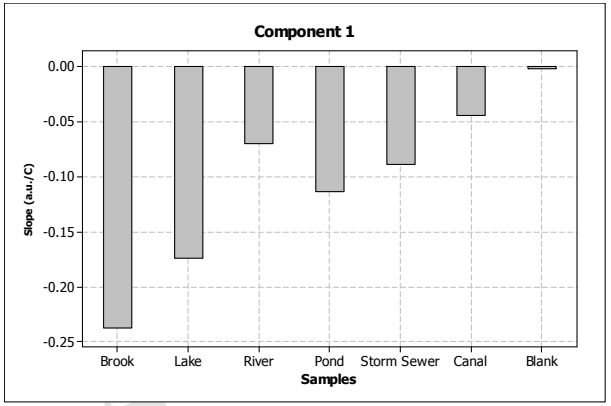
Samples	**DOC (mg/L)**	**pH**	**Conductivity (µS/cm)**	**Absorbance at 350 nm (cm^{-1})**
Brook	7.75	8.1	413	0.089
Lake	8.71	6.8	288	0.078
River	5.55	6.7	340	0.021
Pond	2.96	7.3	687	0.023
Storm Sewer	4.96	7.0	98	0.035
Canal	4.79	6.8	747	0.039


river
(Buxton town)
Birmingham
lake
brook
canal
pond
storm sewer





(a)



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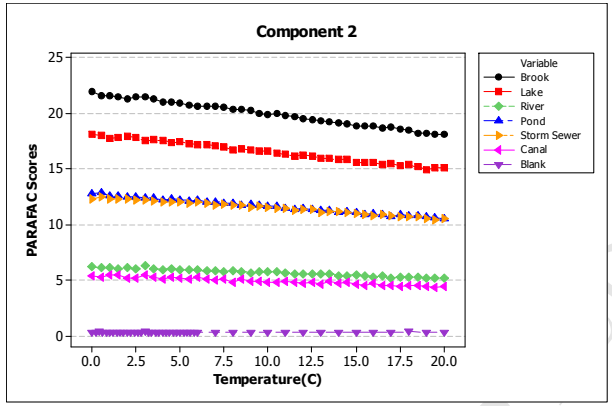

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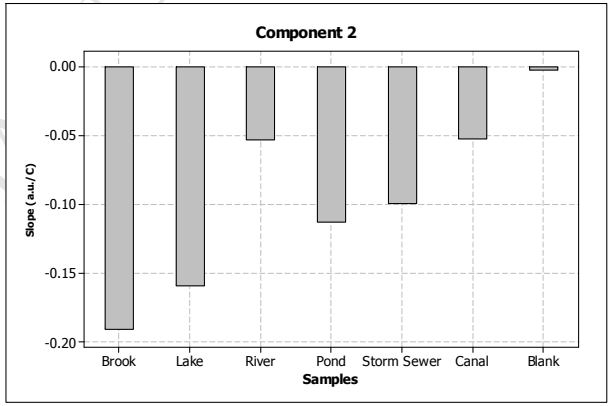

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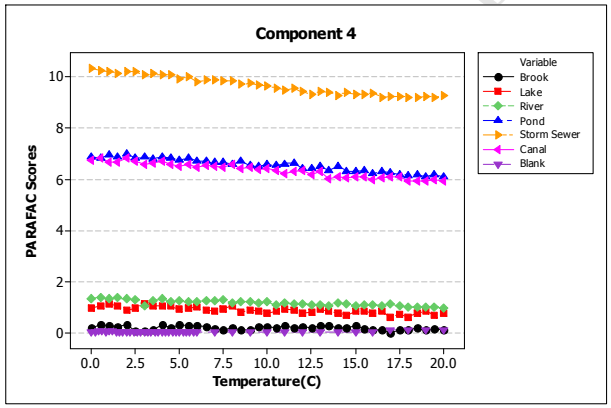

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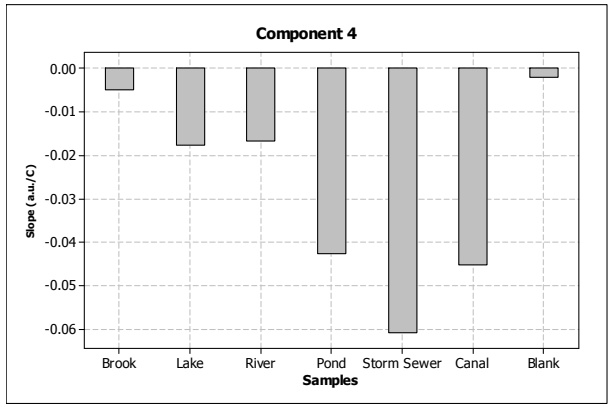

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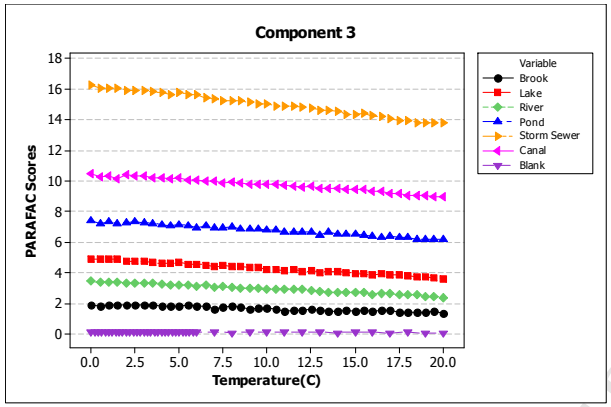

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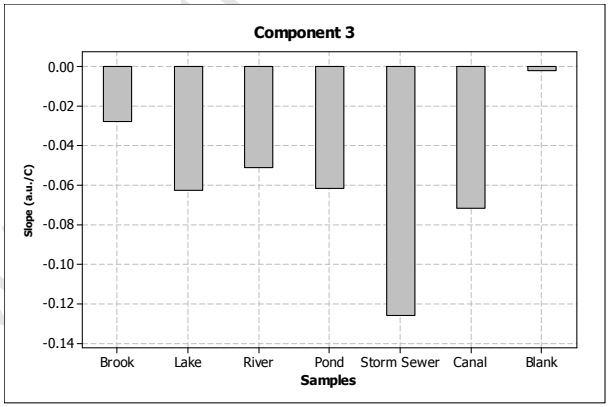

(b)

- We investigated DOM fluorescence properties, at low temperatures, with EEM-PARAFAC
- Fluorescence intensity increases linearly as temperature decreases from 20^0 C to 0^0 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