Response to Referee 2 comment on "Technical note: Effects of iron(II) on fluorescence properties of dissolved organic matter at circumneutral pH"

Please note the reviewer's comments are in *purple italic Verdana font* and our responses are in Times New Roman, with proposed revisions to the text in blue.

Anonymous Referee #2

This study presents results on the influence of Fe(II) on the fluorescence properties of DOM. The concept is novel and addresses a relevant scientific question. However, the results fail to support the conclusions, specifically the PARAFAC modeling results. My recommendation is that the paper should be reconsidered after major revisions. I have three major comments and the rest are addressed in the comments of the attachment.

1. Papers with PARAFAC modeling results typically publish the modeled components and sometimes also the modeled excitation and emission spectra, or at least include them for closer inspection in supplementary material. I'm not sure familiar HESS audience is with PARAFAC, but seeing the modeled components help with understanding.

There was also no mention made of your validation techniques. Validating a model can be very subjective. What steps did you take to resolve this dataset into 13 components? I found it difficult to comment on your PARAFAC results without first knowing how you arrived at your modeled results.

We agree that modeled excitation and emission spectra should be available to support the findings. Indeed, we published these data to a data repository (Zenodo) and referenced the dataset in the "Data availability" section of our paper (http://doi.org/10.5281/zenodo.3737108, please see components_excitation.txt, components_emission.txt, and components_description.txt). HESS policy requests that data should be archived in a FAIR-aligned data repository, not in the supplementary material. In the revised manuscript, we will add a citation to the repository to the references list.

We wish to clarify that we applied a "pre-solved" model, the 13-component model from Cory and McKnight (2005), rather than creating our own model components based on the dataset from Kidd 2. We agree that derivation of a unique PARAFAC model typically requires a large sample set composed of samples from a common organic matter context. The purpose of the manuscript is not to characterize DOM at the site. Rather, it is to understand how ferrous iron affects the fluorescence properties of DOM in groundwater, in which ferrous iron concentrations can be up to 300 mg/L. To ensure that the PARAFAC model is based on a large and diverse sample set and not biased by the presence of ferrous iron, we applied the 13-component model from Cory and McKnight (2005).

We plan to add the following text to the introduction.

Proposed revision:
This study fit EEM spectra to a previously derived 13-component PARAFAC model (Cory & McKnight, 2005). We chose to use this robust and well-developed PARAFAC model, which was developed using DOM from a wide range of aquatic environments and has been subsequently applied to interpret EEMs from a large variety of aquatic systems (Jaffé et al., 2008; Larsen et al., 2010). The use of this 13-component model also facilitates the derivation of the redox index (RI), calculated by summing the reduced quinine-like inputs over total quinone-like inputs from components within the model. Finally, derivation of a unique, site-specific PARAFAC model typically requires a large sample set composed of samples from a common organic matter context (Cory & McKnight, 2005; Ishii & Boyer, 2012). As the aim of this study was to capture how spectral attributes are quenched upon addition of Fe(II), rather than characterization of the underlying organic matter properties, the application of a pre-resolved model ensures that model fitting is not biased by Fe(II) addition.

A residual analysis was conducted to assess whether the Cory and McKnight (2005) model was suitable to reproduce the results in this study and we found that there were no significant nor systematic patterns in the residuals. We acknowledge that it was not clear in the original manuscript that we used "pre-solved" components, as Reviewer 1 also had similar misunderstandings regarding the origin of the PARAFAC components used in this study.

2. I strongly recommend defining optical measurements (absorbance and fluorescence), then describe why they are useful to study DOM, and then define the specific optical properties (FI, HIX, etc.) that were used diagnostically to describe DOM and Fe interactions in this study.

We agree. Based on feedback from both reviewers, we have expanded the introduction to provide more background and definitions related to absorbance and fluorescence spectroscopy and their use in studying DOM. The end of the introduction now mentions the additional operical properties that were measured, but we leave their definitions for later in the manuscript where the results are reported.

Proposed revision:
Moreover, commonly-used fluorescence indices, including fluorescence index (FI) (Cory & McKnight, 2005), humification index (HIX) (Ohno, 2002; Parlanti et al., 2000), the redox index (RI) (Miller et al., 2006), and freshness index (FrI, β/α) (Parlanti et al., 2000; Zsolnay et al., 1999) were also applied to provide further DOM characterization (section 4.1.3).

3. This paper could be vastly improved by a more complete and thorough literature review. Some statements were not attributed appropriately/completely. See comments within doc.

We will update the citations throughout the manuscript and revise the references that were out of date or inappropriately cited. The revised manuscript has ~50% more citations than the original version. A partial list of new references we plan to cite is included in the references list for this document (Aiken, 2014; Bahram et al., 2006; Baker & Spencer, 2004; Bro, 1997; Coble et al., 2014; Hansen et al., 2018; Helms et al., 2008; R. Jaffé et al., 2008; Rudolf Jaffé et al., 2014; Larsen et al., 2010; Murphy, 2011; Murphy et al., 2013; Nieke et al., 1997; Shen et al., 2020; Stedmon et al., 2003; Stedmon & Bro, 2008; Weishaar et al., 2003; Zepp et al., 2004).

Comments within PDF:
Comment 1 (line 10): Please include a statement about why this study was important from a hydrologic perspective.

Agreed.

Original: The effect of soluble reduced iron, Fe(II), on EEM spectra can be significant, but is difficult to quantitatively assign.

Proposed revision: The effect of soluble reduced iron, Fe(II), on EEM spectra can be significant, but is difficult to quantitatively assign, despite the prevalence of groundwater containing high levels of DOM and Fe(II) in deltaic sediments as well as sites contaminated with organics.

Comment 2 (line 20): This seems like a lot of components for samples that contain NOM.

We used a pre-resolved model, the Cory and McKnight (2005) 13-component model to characterize the impacts of dissolved Fe(II) on fluorescence properties of DOM. See proposed revisions in response to your major comment 1 above.

Comment 3 (line 28): Before launching directly into a discussion about fluorescence it is important to first discuss absorbance. I think you would do a great service to your audience to slow down and first define optical measurements (absorbance and fluorescence), then describe why they are useful to study DOM, and finally define the specific optical properties (FI, HIX, etc.) that were used diagnostically to describe DOM:Fe interactions in this study.

Thank you for this suggestion. We have completely rewritten the Introduction section in response to both referees' feedback (e.g., see our response to Referee 1 major comment 3, and to your major comment 1). We now include background information on the optical measurements and the fluorescence index in this section.

We have retained the explicit definitions of the HIX, RI, etc. in section 4.1.3, close to where the data is reported. Since several indices are presented we think it is useful to report their exact definitions close to where the results are discussed.

For example the following text is retained in section 4.1.3:
"FI is defined as the ratio of emission measured at 470 nm and 520 nm at excitation of 370 nm for instrument-corrected spectra (Cory & McKnight, 2005). High values of FI (approximately

1.80) indicate that DOM is derived from extracellular microbial activity, whereas low values of FI (approximately 1.20) suggest that DOM comes from terrestrial plant and soil organic matter (Cory & McKnight, 2005). Measured FI values increased from an initial 1.62 to 1.80 (ΔFI = +0.18 FI units) with increased Fe(II) concentrations (Figure 4b), indicating the susceptibility of FI to the iron-quenching effect."

Comment 4 (line 30): This is a review paper...citation is ok, but this statement should be attributed to some others who have advanced work in this field Cory, McKnight, Coble, Aiken, Baker there are so many others!!!

We agree with the reviewer's literature review assessment. Please see our response to your general comment 3, which details the many of the citations we plan to add in the revised manuscript.

Comment 5 (line 31): Again so many others to cite here, not just Senesi...Helms, Weishaar for example.

We have rewritten this section and added the references you mentioned (Helms et al., 2008; Weishaar et al., 2003), among others.

Comment 6 (line 35): For clarity: In this study we incrementally added Fe(II) to assess the quenching effect on DOM fluorescence in a groundwater sample from a...

Thank you for the suggestion. The manuscript will be revised as follows:

Proposed revision: We incrementally added up to 300 mg/L Fe(II) to DOM-containing groundwater to assess the influence of Fe(II) on the fluorescence properties of DOM. For our analysis, we used groundwater in contact with deltaic sediments in Richmond, British Columbia, Canada, that is representative of other deltaic aquifers, where Fe(II) concentrations can reach >100 mg/L.

Comment 7 (line 45): Choppy. Should be rewritten more within the spirit of how your work builds upon the work in Poulin et al 2014.

Agreed.

Original: Similarly, Fe(II) may also interact with DOM to form organometal complexes that could interfere with fluorescence measurements (Poulin et al., 2014). Limited previous research has addressed the quenching effect of Fe(II) interference. Poulin et al. (2014) first demonstrated that fluorescence intensity decreased due to Fe(II)-DOM interactions. Nevertheless, the iron titration experiments were only designed to characterize the Fe(II) quenching effect for surface water with moderately elevated DOM concentrations (2.3 to 5.0 mg/L) under low Fe (II) concentrations (0-1.5 mg/L).

Proposed revision: However, limited research has focused on the quenching effect of Fe(II) interference in anoxic groundwater, where reducing conditions are present. Poulin et al. (2014) first demonstrated that Fe(II) and DOM can form organometal complexes that decrease fluorescence intensity. Their experiments were only designed to characterize the Fe(II) quenching effect for surface water with moderately elevated DOM concentrations (2.3 to 5.0 mg/L) under low Fe (II) concentrations (0-1.5 mg/L). To our knowledge, the extent of fluorescence quenching in groundwater with higher Fe(II) concentrations is not known.

Comment 8 and 9: Are there more citations you could include here to demonstrate a better need for your particular study? Where else are Fe(II) concentrations this high in groundwater? ...see previous comment. What kind of contamination? Are there other studies you can cite?

Agreed, we have added more citations and context here.

Proposed revision: The fluorescence quenching effect in Fe(II)-rich groundwater is still poorly understood and warrants further investigation, given the prevalence of high DOM and Fe(II) in groundwater in deltaic sediments (Bolton & Beckie, 2011) and sites contaminated with organics, for example, from landfills or fuel spills (van Breukelen & Griffioen, 2004; Christensen et al., 2001; Heron et al., 1994). In most instances, these high Fe(II) groundwaters are found when the oxidation of organic matter is coupled to solid-phase Fe(III) reduction, dissolving Fe(II) into groundwater at circumneutral pH. For example, 1.5-10 mg/L Fe(II) in groundwater is commonly observed in the organic-rich groundwaters of the Bengal Basin (Harvey et al., 2002), and up to 90 mg/L Fe(II) has been observed in landfill leachate in the Netherlands (van Breukelen & Griffioen, 2004).

Comment 10 (line 60): Abstract reports Fluorescence Index, Freshness Index, and HIX. Here you mention Fluorescence Index and Redox Index. Make sure you are consistent.

Thank you for pointing this out, the manuscript has been revised.

Proposed revision: We identified the degree of quenching by Fe(II) based on the excitation−emission matrix (EEMs) regions and peaks, as evaluated using a 13-component parallel factor analysis (PARAFAC) model and commonly-used fluorescence indices, including fluorescence index (FI) (Cory & McKnight, 2005), humification index (HIX) (Ohno, 2002; Parlanti et al., 2000), the redox index (RI) (Miller et al., 2006), and freshness index (β/α) (Parlanti et al., 2000; Zsolnay et al., 1999).

Comment 11 (line :65) Single space after a period throughout.

We will use a single space after a period throughout the revised manuscript.

Comment 12: Just one site and one sample is used as your "stock"? Please explain why this was a representative sample for Kidd 2.

The purpose of the manuscript is not to characterize DOM at the site where we collected this groundwater sample, but rather to understand how ferrous iron affects the fluorescence properties of DOM in groundwater. We selected our single stock solution because it was typical of the deltaic – aquifer groundwaters that can contain high ferrous-iron concentrations. We have revised the manuscript to make this objective clearer. The "stock" sample was collected at the particular groundwater monitoring well where it had lowest ferrous iron concentration (1.3 mg/L) across the site and therefore most suitable for Fe(II) addition experiment.

Comment 13 (line 80): Just one 1L bottle was collected? Surely more was collected to conduct your Fe concentration experiments? If I'm confused, so too will be your audience. Please clarify your methods.

Yes, just one bottle was collected.

Proposed revision: For the measurements in this study, we used a single stock solution of natural DOM-containing groundwater, to which we added (titrated) increasing concentrations of Fe(II).

We also quote our response to Referee 1, major comment 1: "The purpose of the manuscript is not to characterize DOM at the site from which the sample (stock solution) was collected. Rather, it is to understand how ferrous iron affects the fluorescence properties of DOM in groundwater. We selected our single stock solution because it was typical of the deltaic aquifer groundwaters that can contain high ferrous iron concentrations."

Comment 14 (line 88): DOM after filtration will continue to degrade. Are you worried about this? 30 days seems like a lot of time. Many studies of optical properties of DOM show degradation occur in a matter of days, which is why holding time for optical analyses is usually within 2 days of sample collection/filtration. Please provide a justification for this.

Please also see our response to Referee 1, line 86, who had similar questions.

Original

Groundwater was filtered through 0.45 µm cellulose filters, then stored in a 1 L amber glass bottle- with a Teflon-lined cap, without acidification. The bottle was filled with no headspace and duct tape was used to further seal the sample and minimize the oxidation of Fe(II). The collected sample was refrigerated at 4°C until analysis (within 30 days).

Proposed revision

The groundwater was filtered through 0.45 µm cellulose filters, then stored in a 1 L amber glass bottle with a Teflon-lined plastic cap, without acidification. The bottle was filled with no headspace and duct tape was used to further seal the sample and minimize the oxidation of Fe(II). The collected 1 L stock solution was refrigerated at 4°C until fluorescence analysis (within ~14 days). Although some degradation of the DOM may have occurred during the holding period, we expect that this would not significantly affect our conclusions as our intention was to determine how Fe(II) addition affects the fluorescence properties of DOM, rather than to characterize the properties of DOM at the Kidd 2 site.

Comment 15: (line 115) This sentence should be omitted. The analysis and modeling are two separate things….

We agree.

Proposed revision: The procedures of measuring absorbance and fluorescence of DOM was described by (Hansen et al, 2018). The PARAFAC model was applied to decompose the fluorescence EEMs into chemical meaningful components and provide quantification of the DOM fluorescence spectra (Stedmon, C.A., and Bro, R., 2008). In this study, EEMs were analyzed using previously established 13-component PARAFAC models (Cory & McKnight, 2005).

Comment 16 (line 125): water Raman. Please cite Murphy 2011 A Note on Determining the Extent of the Water Raman Peak in Fluorescence Spectroscopy

Thank you, we will cite this reference in the revised manuscript (Murphy, 2011).

Comment 17 (line 125): I don't know what this means. Do you mean the spectra were normalized to the daily water Raman, hence fluorescence intensity units are reported as RU...?

Thanks for pointing this out. The fluorescence EEMs were generated, not intensity. The manuscript will be revised.

Original: Subsequently, the fluorescence intensity (presented in Raman units (RU)) was generated as a function of the excitation and emission wavelengths.

Proposed revision:
Fluorescence intensity within all EEM data is presented in Raman units (RU) due to the way that raw EEM spectra are corrected prior to analysis via PARAFAC modelling or calculation of associated indices. As per standard practice, raw EEMs were instrument corrected via software provided by the instrument manufacturer. Spectra were then corrected for inner filter effects (Ohno, 2002), then normalized to the area under the Raman curve (Nieke et al., 1997; Stedmon et al., 2003); second order Raleigh scatter and Raman bands were excised at a bandpass of 12 nm (Bahram et al., 2006; Zepp et al., 2004) while first order Raleigh scatter was excised at a bandwidth of 50 nm to remove all spectral artefacts (Bro, 1997; Stedmon & Bro, 2008). Specifically, normalization to the area under the Raman curve (which occurs due to the inelastic scatter of light by water) contributes to instrument correction that allows for the comparison of spectra between different instruments and thus different studies.

Comment 18 (line 135): How many spectra were included in your PARAFAC dataset? I would hesitate to try to validate a model containing just a few samples. Typically PARAFAC is best applied to large datasets (n>100). I would like to see the modeled excitation and emission spectra. Additionally, please also provide the modeled components. Modeled components are usually published in a paper that contains this method. Also, seeing the components will be especially helpful to an audience not familiar with the model output. Also not included in your text here is the model validation results. Validating a model can be very subjective. What steps did you take to resolve this dataset into 13 components? Please read Murphy et al 2013 for a detailed walthrough of PARAFAC model validation techniques. Fluorescence spectroscopy and multi-way techniques. PARAFAC. I have to say that 13 components seems like a lot for groundwater.

Please see our detailed response to your general comment 1. We used pre-solved components from Cory and McKnight (2005) and will update the manscript to clarify this. Our dataset published on Zenodo already includes the excitation and emission spectra for all 13 components. As recommended in the author guidelines for HESS, we published the data in a FAIR-compliant data repository rather than as supplementary materials on the journal website.

Comment 19 (line 149): Refer here to the sample name, not the site. One location is not indicative of DOM at the entire site.

Please refer to our response to your comment # 12. The stock water represents deltaic aquifer groundwater at Kidd2 site, where groundwater that can contain high ferrous iron concentrations.

Comment 20 (line 154): Careful the study by Poulin et al 2014 used samples where concentration ranges were not comparable to those used here.

Original: The magnitude of quenching effect was more pronounced in this study than that performed by Poulin et al. (2014), who observed nonlinear fluorescence quenching (7% to 23%) in four different surface water samples, by addition of Fe(II) up to 1.5 mg/L

Proposed revision:
The magnitude of quenching effect was more pronounced in this study than that performed by Poulin et al. (2014), who observed nonlinear fluorescence quenching (7% to 23%) in four different surface water samples, by addition of Fe(II) up to 1.5 mg/L, significantly lower than the Fe(II) concentrations used in this study.

Comment 21 (line 175): Please clarify this sentence.
Original:
Similar to relative OFI, the relative intensity of Peak A decreased by ~60% as Fe(II) increased from 1 to 306 mg/L, and over 65% of the quenching was occurring when Fe(II) reached to 101 mg/L (Figure 4c).

Proposed revision:
The relative intensity of Peak A decreased approximately 60% as Fe(II) increased from 1 to 306 mg/L. Of the total decreased intensity, over 65% of the quenching occurred when Fe(II) increased from 1 to 101 mg/L (Figure 4c).

Comment 22 (line 176): For clarity I would say: shorter (higher energy) emission wavelengths.

Original: In addition, the position of Peak A continuously migrated toward the shorter emission wavelengths with a constant excitation wavelength of 239 nm and increasing Fe(II) concentration.

Proposed revision: In addition, the position of Peak A continuously migrated toward the shorter (i.e., higher energy) emission wavelengths with a constant excitation wavelength of 239 nm and increasing Fe(II) concentration.

Comment 23 (line 178): Again for clarity because the wavelengths didn't change, the location of the fluorescence response changed: ...overall the emission of the fluorescence response shifted from 441 nm to 409 nm as Fe(II) increased...

Original: Although the linear relationship was not observed, overall the emission wavelength gradually changed from 441 to 409 nm as Fe(II) increased from 1 to 306 mg/L.

Proposed revision: Although the linear relationship was not observed, overall the location of fluorescence response gradually changed from 441 to 409 nm as Fe(II) increased from 1 to 306 mg/L.

Comment 24 (line 191): When defining each of these optical properties, it would be useful to state an expected (typical) range of concentrations from indices used in other groundwater DOM studies. There will be lots of studies to cite here and will provide readers with an idea of where results from this study fall within the literature as a whole.

We agree. The typical range for fluorescence index (FI) and redox index (RI) were already reported (line 194 and 243). We will add ranges for HIX and FrI.

Proposed revisions:
A higher FrI indicates more recently created DOM, with values >1 indicating freshly released DOM and lower values (0.6-0.7) correspond to older DOM with a predominant terrestrial source (Parlanti et al., 2000). Overall, the freshness index ranged between 0.72 to 0.85 and increased with Fe(II).

The humidification index (HIX) is defined as the peak area under the emission spectra from 435-480 nm, divided by the peak area from 300-345 nm + 435-480 nm, at an excitation of 254 nm and typically ranges from 0-1 (Ohno, 2002). Higher values of HIX (closer to 1) indicate greater humic content and extent of humidification. HIX values decreased with the addition of Fe(II) (from about 0.93 to 0.84)

Comment 25 (line 210) *As represented by a change in slope.*

Original: Moreover, the decrease in HIX occurred in two stages with increasing Fe(II)

Proposed revision: Moreover, the decrease in HIX occurred in two phases with increasing Fe(II), as represented by a change in slope.

Comment 26 (line 215). *Check this definition. It should be The ratio of emission intensity at 380 nm divided by the maximum emission intensity between 420 and 435 nm at excitation 310 nm.*

We agree that our wording was imprecise, we will revise it.

Original: The freshness index (FrI or β/α) is defined as the intensity at emission at 380 nm (β) at an excitation of 310 nm, divided by maximum intensity between emission 420-435 nm (α) (Parlanti et al., 2000; Wilson & Xenopoulos, 2009).

Proposed revision: The freshness index (FrI or β/α) is defined as the ratio of emission at 380 nm (β) divided by maximum emission between 420 and 435 nm (α), all at an excitation of 310 nm (Parlanti et al., 2000; Wilson & Xenopoulos, 2009).

We thank both referees for their thorough and constructive reviews of the mansucript.

References

Aiken, G. (2014). Fluorescence and Dissolved Organic Matter. In A. Baker, D. M. Reynolds, J. Lead, P. G. Coble, & R. G. M. Spencer (Eds.), *Aquatic Organic Matter Fluorescence* (pp. 35–74). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781139045452.005

Bahram, M., Bro, R., Stedmon, C., & Afkhami, A. (2006). Handling of Rayleigh and Raman scatter for PARAFAC modeling of fluorescence data using interpolation. *Journal of Chemometrics*, *20*(3–4), 99–105. https://doi.org/10.1002/cem.978

Baker, A., & Spencer, R. G. M. (2004). Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Science of The Total Environment*, *333*(1), 217–232. https://doi.org/10.1016/j.scitotenv.2004.04.013

van Breukelen, B. M., & Griffioen, J. (2004). Biogeochemical processes at the fringe of a landfill leachate pollution plume: potential for dissolved organic carbon, Fe(II), Mn(II), NH4, and CH4 oxidation. *Journal of Contaminant Hydrology*, *73*(1–4), 181–205. https://doi.org/10.1016/j.jconhyd.2004.01.001

Bro, R. (1997). PARAFAC. Tutorial and applications. *Chemometrics and Intelligent Laboratory Systems*, *38*(2), 149–171. https://doi.org/10.1016/S0169-7439(97)00032-4

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., et al. (2001). Biogeochemistry of landfill leachate plumes. *Applied Geochemistry*, *16*(7), 659–718. https://doi.org/10.1016/S0883-2927(00)00082-2

Coble, P. G., Lead, J., Baker, A., Reynolds, D. M., & Spencer, R. G. M. (2014). *Aquatic Organic Matter Fluorescence*. Cambridge University Press.

Cory, R. M., & McKnight, D. M. (2005). Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter. *Environmental Science & Technology*, *39*(21), 8142–8149. https://doi.org/10.1021/es0506962

Hansen, A. M., Fleck, J., Kraus, T. E. C., Downing, B. D., von Dessonneck, T., & Bergamaschi, B. (2018). *Procedures for using the Horiba Scientific Aqualog® fluorometer to measure absorbance and fluorescence from dissolved organic matter* (USGS Numbered Series No. 2018–1096). *Procedures for using the Horiba Scientific Aqualog® fluorometer to measure absorbance and fluorescence from dissolved organic matter* (Vol. 2018–1096). Reston, VA: U.S. Geological Survey. https://doi.org/10.3133/ofr20181096

Helms, J. R., Stubbins, A., Ritchie, J. D., Minor, E. C., Kieber, D. J., & Mopper, K. (2008). Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, *53*(3), 955–969. https://doi.org/10.4319/lo.2008.53.3.0955

Heron, G., Crouzet, C., Bourg, A. C. M., & Christensen, T. H. (1994). Speciation Of Fe(Ii) And Fe(Iii) In Contaminated Aquifer Sediments Using Chemical-Extraction Techniques. *Environmental Science & Technology*, *28*(9), 1698–1705.

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. https://doi.org/10.1021/es2043504

Jaffé, R., McKnight, D., Maie, N., Cory, R., McDowell, W. H., & Campbell, J. L. (2008). Spatial and temporal variations in DOM composition in ecosystems: The importance of longterm monitoring of optical properties. *Journal of Geophysical Research: Biogeosciences*, *113*(G4). https://doi.org/10.1029/2008JG000683

Jaffé, Rudolf, Cawley, K. M., & Yamashita, Y. (2014). Applications of Excitation Emission Matrix Fluorescence with Parallel Factor Analysis (EEM-PARAFAC) in Assessing Environmental Dynamics of Natural Dissolved Organic Matter (DOM) in Aquatic Environments: A Review. In F. Rosario-Ortiz (Ed.), *ACS Symposium Series* (Vol. 1160, pp. 27–73). Washington, DC: American Chemical Society. https://doi.org/10.1021/bk-2014-1160.ch003

Larsen, L. G., Aiken, G. R., Harvey, J. W., Noe, G. B., & Crimaldi, J. P. (2010). Using fluorescence spectroscopy to trace seasonal DOM dynamics, disturbance effects, and hydrologic transport in the Florida Everglades. *Journal of Geophysical Research: Biogeosciences*, *115*(G3). https://doi.org/10.1029/2009JG001140

Murphy, K. R. (2011). A Note on Determining the Extent of the Water Raman Peak in Fluorescence Spectroscopy. *Applied Spectroscopy*, *65*(2), 233–236. https://doi.org/10.1366/10-06136

Murphy, K. R., Stedmon, C. A., Graeber, D., & Bro, R. (2013). Fluorescence spectroscopy and multi-way techniques. PARAFAC. *Analytical Methods*, *5*(23), 6557–6566. https://doi.org/10.1039/C3AY41160E

Nieke, B., Reuter, R., Heuermann, R., Wang, H., Babin, M., & Therriault, J. C. (1997). Light absorption and fluorescence properties of chromophoric dissolved organic matter (CDOM), in the St. Lawrence Estuary (Case 2 waters). *Continental Shelf Research*, *17*(3), 235–252. https://doi.org/10.1016/S0278-4343(96)00034-9

Ohno, T. (2002). Fluorescence Inner-Filtering Correction for Determining the Humification Index of Dissolved Organic Matter. *Environmental Science & Technology*, *36*(4), 742–746. https://doi.org/10.1021/es0155276

Shen, J., Liu, B., Wu, J., Chai, Y., Cheng, C., Liu, C., et al. (2020). Characterization of fluorescent dissolved organic matters in metalworking fluid by fluorescence excitation-emission matrix and high-performance liquid chromatography. *Chemosphere, 239*, 124703. https://doi.org/10.1016/j.chemosphere.2019.124703

Stedmon, C. A., & Bro, R. (2008). Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography: Methods*, *6*, 572–579. https://doi.org/10.4319/lom.2008.6.572

Stedmon, C. A., Markager, S., & Bro, R. (2003). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, *82*(3), 239–254. https://doi.org/10.1016/S0304-4203(03)00072-0

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., & Mopper, K. (2003). Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology*, *37*(20), 4702–4708. https://doi.org/10.1021/es030360x

Zepp, R. G., Sheldon, W. M., & Moran, M. A. (2004). Dissolved organic fluorophores in southeastern US coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation–emission matrices. *Marine Chemistry*, *89*(1–4), 15–36. https://doi.org/10.1016/j.marchem.2004.02.006