https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

Hydrology and Earth System Sciences
Discussions

Technical note: Effects of iron(II) on fluorescence properties of dissolved organic matter at circumneutral pH

Kun Jia[1,*], Cara C. Manning[1], Ashlee Jollymore[2,#], and Roger D. Beckie[1]

[1]University of British Columbia, Department of Earth, Ocean and Atmospheric Sciences, Vancouver, BC, Canada
[2]University of British Columbia, Institute for Resources, Environment and Sustainability, Vancouver, BC, Canada
*Currently at: AECOM Canada
[#]Currently at: Province of British Columbia, Ministry of Forest, Lands, Natural Resource Operations and Rural Development

Correspondence to: Kun Jia (kun418@gmail.com) and Roger Beckie (rbeckie@eoas.ubc.ca)

Abstract. Modern fluorescence spectroscopy methods, including excitation-emission matrix (EEMs) spectra parsed using parallel factor analysis (PARAFAC) statistical approaches, are widely used to characterize dissolved organic matter (DOM) pools. The effect of soluble reduced iron, Fe(II), on EEM spectra can be significant, but is difficult to quantitatively assign. In this study, we examine the effects of Fe(II) on the EEM spectra of groundwater samples from an anaerobic deltaic aquifer containing up to 300 mg/L Fe(II), located a few kilometers from the ocean, adjacent to the Fraser River in Richmond, British Columbia, Canada. We added varying quantities of Fe(II) into groundwater samples to evaluate Fe(II)-DOM interactions. Both the overall fluorescence intensity and the intensity of the primary peak, a humic-like substance at excitation/emission wavelengths 239/441-450 nm (Peak A), decreased by approximately 60% as Fe(II) concentration increased from 1 to 306 mg/L. Furthermore, the quenching effect was non-linear and proportionally stronger at Fe(II) concentrations below 100 mg/L. This non-linear relationship suggests a static quenching mechanism. In addition, DOM fluorescence indices are substantially influenced by the Fe(II) concentration. With increasing Fe(II), the fluorescence index (FI) tends to shift to a more microbial-derived origin, and both the humidification index (HIX) and freshness index (FrI) indicate more freshly produced DOM. Nevertheless, the 13-component PARAFAC model showed that the component distribution was relatively insensitive to Fe(II) concentration, and thus, PARAFAC may be a reliable method for obtaining information about the DOM composition and its redox status in Fe(II)-rich waters. By characterizing the impacts of up to 300 mg/L Fe(II) on EEMs using groundwater from an aquifer which contains similar Fe(II) concentrations, we advance previous works which characterized impacts of lower Fe(II) concentrations (less than 2 mg/L) on EEMs.

1 Introduction

Fluorescence spectroscopy has been widely used to characterize the properties of organic matter as it is highly sensitive to the structures and functional chemistry of aquatic organic matter (Fellman et al., 2010) . Nevertheless, the fluorescence properties of organic matter appear to also depend on a number of parameters, including the origin, molecular weight, concentration, pH, and interactions with metal ions and organic chemicals (Senesi, 1990).

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

It is well accepted that dissolved organic matter (DOM) fluorescence is quenched or enhanced by interactions with metal ions, including Fe(III) (Ohno et al., 2007; Poulin et al., 2014; Pullin et al., 2007; Senesi, 1990), Fe(II) (Poulin et al., 2014), Al(III) (Ohno et al., 2007), Cu(II) (Senesi, 1990) and Hg(II) (Senesi, 1990). In this study, an Fe(II) addition experiment was performed to assess the quenching effect of Fe(II) in groundwater samples from a deltaic aquifer in Richmond, British Columbia, Canada, where natural Fe(II) concentrations reach over 300 mg/L (5.4 mM).

Fe(III) is recognized as an important source of interference for fluorescence measurements (Ohno et al., 2007; Pullin et al., 2007). Previous studies have reported a significant quenching effect caused by the binding of Fe(III) to organic ligands. A possible mechanism that may account for quenching is the formation of organometal complexes at the fluorescent sites (Rue & Bruland, 1995; Senesi, 1990). Such complexes can efficiently decrease the fluorescence intensity of the fluorophore. Furthermore, the degree of quenching varies among different organic-matter compounds, which increases the complexity and uncertainty in characterizing and predicting the iron-binding effect across a range of DOM types (Ohno et al., 2007). 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).

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 contaminated sites (Christensen et al., 2001). Fe(II) is often present in groundwater in organic-rich settings where the oxidation of organic matter is coupled to solid-phase Fe(III) reduction, dissolving Fe(II) into groundwater. 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).

In this study, up to 300 mg/L Fe(II) was added to DOM-containing groundwater to assess the influence of Fe(II) on the fluorescence properties of DOM. 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 two commonly-used fluorescence indices - the fluorescence index (FI) and the redox index (RI) (Miller et al., 2006).

2 Study area

The field study area, known as the Kidd 2 site, is located adjacent to the Fraser River a few kilometers upstream from its outlet to the ocean, near Vancouver, Canada (49°11’53.34”N, 123° 6’53.25”W), where a near-surface sandy aquifer is found between 5 m and 22 m below ground surface (Bolton & Beckie, 2011; Jia, 2015). In the anaerobic deltaic aquifer, Fe(II) is

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

released to groundwater by oxidation of dissolved organic matter (DOM) in a process that is affected by the circulation of saline ocean water. At the site, a wedge of denser, saline ocean water enters the aquifer in the hyporheic zone at the river bottom, flows inland along the base of the aquifer to a maximum distance of approximately 500 m inland where it overturns flows back towards the river under a regional gradient from freshwater recharged inland (Neilson-Welch & Smith, 2001). Along the flow path, the saline water mixes with fresh groundwater. The saline-freshwater mixture eventually discharges to the river at the top of the saline wedge. Two mixing zones can be identified along the saline wedge: at the bottom of the wedge, freshwater from the lower confining silt flows up into the overlying sandy aquifer as the saline water flows inland (the "lower mixing zone") and at the top of the wedge, terrestrial recharge from inland flows on top of the saline water as it flows back to the river (the "upper mixing zone"). High concentrations of Fe(II) are observed along the circulation flow path, especially in the upper mixing zone, where pore water Fe(II) concentrations peak above 300 mg/L (5.4 mM) (Figure 2).

3 Methodology

3.1 Sample collection

Groundwater sampled from W3-14 at a depth of 20.03 m was selected as the DOM stock solution as previous measurements showed that it had the lowest Fe(II) concentration (1.3 mg/L) at the Kidd 2 site (Jia, 2015). The multilevel sampling port consisted of a 0.635 cm inner diameter low-density polyethylene tube with a 5 cm fiberglass-mesh screen (Neilson-Welch & Smith, 2001). Three tubing volumes of groundwater were purged with a peristaltic pump while pH was monitored using an OAKTON™ pH/mV/°C meter in a sealed flow-through cell to prevent degassing. The pH and temperature stabilized at 7.44 and 11°C, respectively. 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). The stock solution DOM concentration of 10.7 mg/L was measured using high temperature combustion with a HACH™ IL 550 TOC-TN analyzer, detection limit 1 mg/L, at the Environmental Engineering Laboratory in UBC's Department of Civil Engineering.

3.2 Fe(II) addition experiment

Experimental solutions were prepared from the stock DOM solution in an anaerobic glove box (Coy Labs, MI, USA), filled with an N_2/H_2 mixture (95% of N_2 and 5% of H_2) with a palladium catalyst inside the chamber, which maintains O_2 levels of less than 5 ppm. The stock DOM solution was deaerated in the glove box by purging with pure N_2 for 30 minutes. The sample addition experiments used glass cuvettes that were acid-washed with 10% HNO_3, and rinsed with deionized distilled water.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

As the highest observed Fe(II) concentration in the groundwater at the Kidd 2 site was approximately 300 mg/L (Jia, 2015), the Fe(II) addition experiment was designed for a range of Fe(II) concentrations (from 1 to 300 mg/L). A Fe spiking solution of 1000 mg/L Fe(II) was prepared with $FeSO_4(H_2O)_7$ using the DOM stock solution so that spiking with Fe(II) would not change the overall concentration of DOM. Experiments were performed by sequentially adding Fe(II) spiking solution to an initial volume of 250 ml of DOM stock solution to reach 10 different concentrations between 1.3 to 306 mg/L. If necessary, the pH of the experimental solution was adjusted using 0.1 M NaOH or 0.1 M HCl (to 7.4 ± 0.3) to match the target pH (7.44) of the original DOM stock solution. After each Fe(II) addition, 10 ml of the Fe(II) solution was pipetted into each of two glass cuvettes. The cuvette of Fe(II) solution used for fluorescence analysis was capped tightly, and transferred out of the glove box for immediate analysis. The other cuvette was acidified with concentrated HCl to a pH of approximately 2, and used to determine the total dissolved Fe(II).

3.3 Sample analysis

Fe(II) concentrations were determined inside the glove box using a HACH™ DR/2010 spectrophotometer via the colorimetric method (Hach ferrozine method) (Stookey, 1970).

The fluorescence analysis and the 13-component PARAFAC modeling are described by Ishii & Boyer (2012). All fluorescence spectra were obtained by using a Horiba Aqualog® (Horiba Scientific, Edison, NJ, USA) spectrofluorometer, equipped with subtractive double excitation monochromators. A 150 W ozone-free vertically mounted xenon arc lamp was used as the excitation source. Both excitation and emission were collected at a bandpass at 5 nm. Fluorescence intensities, as a function of the excitation and emission wavelengths, were measured across excitation wavelengths ranging from 240 to 800 nm in 3 nm increments; emission wavelengths, ranging from 250 to 830 nm, were measured over an integration time of 0.1 s. Water samples were analyzed in 1 cm quartz cuvettes. Between the samples, the quartz cuvette was rinsed 3 times with Milli-Q water, followed by 3 times with the sample, to reduce possible cross-contamination. If necessary, water samples were quantitatively diluted with Milli-Q water until the UV absorbance was lower than 0.2 units (at 254 nm) to minimize inner filter effects between the Milli-Q water and the water samples. The EEM spectra for each sample was obtained by subtracting the Milli-Q (blank) spectra to eliminate the Rayleigh scatter and water Ramen peak. Subsequently, the fluorescence intensity (presented in Raman units (RU)) was generated as a function of the excitation and emission wavelengths. The overall fluorescence intensity (OFI) was determined for each sample by adding the fluorescence intensities across all EEMs (Poulin et al., 2014). The relative fluorescence (OFI/OFI_0) can be used to quantitatively determine the quenching effect of Fe(II) (Poulin et al., 2014), where OFI and OFI_0 represent the Fe(II) addition samples and the original groundwater sample, respectively. Similarly, the intensity of the primary peak (Peak A) at excitation wavelength = 239 nm, and at broad emission wavelengths ranging from 380 to 460 nm, was determined for each sample, and the parameter $A/A_{o\ was}$ used to quantify the Fe(II) quenching effect on this diagnostic peak.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

The PARAFAC model resolved the EEM dataset into 13 components, including three oxidized quinones (Q1, Q2, and Q3), four reduced quinones (SQ1, SQ2, SQ3, and HQ), two amino acid-like components (C8, tryptophan, and C13, tyrosine) fluorophores, and four remaining unknown fluorophores (C1, C3, C6, and C10) (Cory & McKnight, 2005). Each component gave rise to a unique excitation and emission spectrum, and can be considered as a single fluorophore or a group of similar fluorophores (Cory & McKnight, 2005). No obvious residuals were found after adapting the EEMs to the PARAFAC model, suggesting that the 13-component PARAFAC model was able to represent the samples, and that Fe(II) additions did not significantly change the structure of fluorophores in groundwater samples from the Kidd 2 site. After the components were identified, they were quantified by their relative contributions (%) to the total fluorescence.

4 Results

4.1 Quenching effect on the EEM fluorescence

4.1.1 Relative fluorescence intensity (OFI/OFI_0)

Figure 3 shows that the fluorescence intensities of sample EEMs decrease with increasing Fe(II), indicating that the DOM fluorescence of groundwater at the Kidd 2 site was quenched by the addition of Fe(II). Figure 4a presents the decrease in the relative fluorescence intensity (OFI/OFI_0) as Fe(II) increases from 1 to 306 mg/L. Approximately 60% of the fluorescence intensity found in the 1 mg/L Fe(II) sample was quenched in the 306 mg/L Fe(II) sample. The fluorescence intensity decreased more rapidly at lower Fe(II) concentrations: as Fe(II) increased from 1 to 101 mg/L the OFI decreased by ~40% and as Fe(II) increased from 101 to 306 mg/L, the OFI decreased by an additional ~20%. 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.

Fe(III) also interacts with DOM and quenches fluorescence intensities (Ohno et al., 2007; Poulin et al., 2014). In this experiment, it was expected that some portion of Fe(II) would have oxidized to Fe(III) since the samples were directly exposed to the atmosphere when they were transferred to the sample cuvette for analysis. Nevertheless, the oxidation from Fe(II) to Fe(III) was limited due to the minimal exposure, and no visible Fe(III) colloids were observed prior to the analysis. The analysis took about 5-10 minutes for each sample; the full analysis was completed within an hour. Therefore, the quenching effect was unlikely to be caused by dynamic colloid formation. Furthermore, Poulin et al. (2014) found that almost no quenching was observed by Fe(III) from the oxidation of Fe(II) to Fe(III) at pH 6.7. Hence, the quenching effect in this experiment was believed to be primarily due to Fe(II)-DOM interactions.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

4.1.2 Relative Peak A fluorescence intensity (A/A_0)

Coble (1996; 1990) identified five primary peaks from a visual inspection of EEMs, including humic-like Peaks A, C, and M; and protein-like Peaks B and T. The peaks are believed to be linked to the organic matter properties, and have been used for fluorescence comparisons in numerous studies. We observed only one distinct humic-like fluorescence peak (Peak A) in the EEMs from W3-14. Peak A was in the UV region at an excitation wavelength of 239 nm, and at broad emission wavelengths ranging from 380 to 460 nm.

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). 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. Figure 4e presents the emission positions of Peak A along with Fe(II) concentrations at excitation 239 nm. 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. This result is consistent with quenching experiments conducted with Everglades F1 water samples, where they observed a distinct shift in the quenching locations with increasing ratio of Fe(II) to DOM (Poulin et al., 2014).

4.1.3 Fluorescence intensities

Iron quenching also affects several indices that are used to quantify DOM fluorescence properties. The most common indices are the 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). By defining the ratios of fluorescence intensity in different regions of the EEMs, indices can provide insight into the source of DOM, the degree of humification, and the relative age of the recently produced DOM. Similar to OFI and Peak A intensity, all of the indices exhibited non-linear changes with the addition of Fe(II) (Figure 4).

The fluorescence index (FI) is the most widely used index that provides information about the source of organic matter. 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 ($\Delta FI = +0.18$ FI units) with increased Fe(II) concentrations (Figure 4b), indicating the susceptibility of FI to the iron-quenching effect. As FI is a ratio of emission intensities, non-uniform changes in component emissions are responsible for the increase in FI values with Fe(II). Nevertheless, the effect of iron-

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

quenching on DOM fluorescence and FI were only observed in the 1 to 101 mg/L Fe(II) concentration range. As the Fe(II) increased from 101 to 306 mg/L, the measured FI remained stable at ~1.80. Poulin et al. (2014) also observed that FI values increased more rapidly at low Fe(II) concentrations, and began leveling off approaching the maximum Fe(II) concentrations that he studied, 1.5 mg/L. A stable value of FI was not reached in Poulin et al. (2014) Fe(II) addition experiment, probably because 1.5 mg/L of added Fe(II) did not saturate all available DOM ligands.

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 (Ohno, 2002). Higher values of HIX indicate greater humic content and extent of humidification. HIX values decreased with the addition of Fe(II), indicating that the emission spectra of fluorescence tends to shift toward shorter wavelengths (Figure 4d). Moreover, the decrease in HIX occurred in two stages with increasing Fe(II). The steeper decrease of approximately 4.5% in HIX was observed as the Fe(II) concentration changed from 1 to 72 mg/L. Above 72 mg/L Fe(II), the decrease in HIX decreased only 2.9%. The results indicated that changes in emission spectra were therefore more sensitive to relatively low Fe(II) concentrations.

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). It is a measure of the proportion of recently produced DOM, where β represents freshly produced DOM and α represents more decomposed DOM (Parlanti et al., 2000; Wilson & Xenopoulos, 2009). A higher FrI indicates more recently created DOM. Overall, the freshness index increased with Fe(II), except for the slight decrease seen at the Fe(II) concentration of 101 mg/L (Figure 4f). Similar to HIX, the more pronounced change of 10.4% occurred as the Fe(II) concentration ranged from 1 to 72 mg/L and changed by 6.3% when the Fe(II) concentration ranged between 72 and 306 mg/L.

4.2 Quenching effect on PARAFAC modeling and component distribution

Of the 13 components, seven components were identified as quinone-like organic components (including three oxidized quinones, Q1, Q2, and Q3 and four reduced quinones, SQ1, SQ2, SQ3, and HQ), based on the similarity of the positions and relative intensities of the component excitation peaks compared to the absorbance and excitation peaks of model quinones (Cory & McKnight, 2005). Two components were defined as resembling amino acid fluorophores (C8, tryptophan, and C13, tyrosine). The remaining four components (C1, C3, C6, and C10) have not yet been associated with any class of molecule. The three most abundant components were Q1, Q2, and Q3; together they contributed 49-52% to the total fluorescence (Figure 5).

The fluorescence intensity of each component peaked between 1 to 44 mg/L Fe(II) and then steadily decreased as Fe(II) increased to 306 mg/L (Figure 5a). For all components except tryptophan (C8), the fluorescence at 306 mg/L Fe(II) was less than the fluorescence at 1 mg/L Fe. As Fe(II) increased from 1 to 306 mg/L, the total fluorescence intensity of the 13

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

components decreased by approximately 50%, from 207 to 101 RU. However, the relative proportions of the components were relatively stable. The maximum deviation was seen in C6 (unknown classification) which decreased in relative proportion from 9 to 5% as Fe(II) increased from 1 to 306 mg/L. For the other components, the changes in proportions were restricted to be within ±3%. We conclude that the proportions of the 13 components are relatively insensitive to the Fe(II) concentration for the DOM at the Kidd 2 site.

The trends in component distribution can be further evaluated using the redox index (RI), which is calculated as the sum of reduced quinone-like inputs over the total quinone-like input. RI measures the oxidation state of the DOM and redox reactivities (Miller et al., 2006; Mladenov et al., 2008). The RI can be used to determine whether the quinone-like components within the DOM are more reduced (RI closer to 1) or more oxidized (RI closer to 0). A shift in the RI usually indicates changes in the redox status. The total oxidized (Q1, Q2, and Q3) and reduced (SQ1, SQ2, SQ3, and HQ) quinones changed their contributions to total fluorescence from 52 to 50% and from 21 to 20%, respectively. The small changes for both oxidized and reduced quinones are responsible for the relatively stable values of RI, which slightly decreased from 0.30 to ~0.285 (Figure 5b).

5 Discussion

Fe(II) is responsible for the observed quenching of DOM fluorescence. Both the total fluorescence intensity of EEMs and Peak A intensity decreased with increasing Fe(II). The likely mechanism accounting for the quenching is formation of organic-metal complexations, which occupy the fluorescent sites and efficiently decrease the fluorescence intensity of fluorophores (Senesi, 1990; Waite & Morel, 1984). The non-linear quenching of the fluorescence intensity with Fe(II) concentrations indicates a static quenching mechanism, where quenching primarily depends on the fraction of DOM ligands that are complexed to Fe(II), rather than on the Fe(II) concentration itself (Senesi, 1990). Poulin et al. (2014) found that Fe(II)-quenching of fluorescence ranged from 7% to 23% in four different hydrophobic acid (HPoA) fractions and surface water samples, with DOC concentrations ranging from 2.3 to 5.0 mg/L, and the degree of quenching was not related to DOM concentrations. In our study, both total fluorescence intensity and Peak A intensity decreased by approximately 60% as Fe(II) increased from 1 to 306 mg/L. The differences in Fe(II) quenching at the Kidd 2 site compared to Poulin's study could be related to the high Fe(II) background in the organic-rich environment and the mixing of saline water and groundwater. It should be noted that the DOC concentration in this study was 10.7 mg/L, significantly greater than that used in previous study (Poulin et al. 2014).

Poulin et al. (2014) mainly examined the effect of Fe(II) addition to terrestrial-derived fresh surface water with undetectable Fe(II) levels. In contrast, the DOM at the Kidd 2 site is hypothesized to be derived from microbial sources and may respond to high Fe(II) concentrations differently than freshwater terrestrial-derived DOM. The quenching mechanism for this DOM is not well understood.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

Similar metal quenching of humic-like peaks has been observed by other researchers. Ohno et al. (2007) conducted experiments on the impact of Fe(III) and Al(III) addition to the deciduous water-soluble organic matter (WSOM) fluorescence spectra. This result showed that the fluorescence intensity was quenched by about 30% in the presence of 25 μM (1.4 mg/L) Fe for Peak A (Ohno et al., 2007)

The fluorescence indices FI, HIX, β/α, provided evidence of the susceptibility to Fe(II) quenching, while RI was relatively insensitive to Fe(II) concentration. FI values increased by approximately 0.18 units from the addition of 300 mg/L of Fe(II), and shifted towards greater microbial-derived origin. This relatively small change may not affect the utility of FI towards inferring DOM origin. Moreover, FI values are more sensitive at low Fe(II) concentrations and they gradually reach a plateau once Fe(II) is above 101 mg/L, as all available DOM ligand sites are likely fully occupied by Fe(II)-DOM interactions. This result also supports a static quenching mechanism, since quenching does not depend on the Fe(II) concentration (Senesi, 1990). Similar to FI, both HIX and β/α show more pronounced changes at low Fe(II) concentrations (Fe(II) < 72 mg/L). The decrease in HIX and increase of the β/α are consistent, as they both indicate that the DOM shifted to more freshly produced, with a higher H:C ratio and less polycondensation (Ohno, 2002). Nevertheless, the HIX and the β/α are not likely to reach a constant value with Fe(II) concentration. Therefore, conclusions about organic matter origin based on humidification and freshness indices must consider the DOM sensitivity to Fe(II) concentration when reporting values.

In the 13 component PARAFAC model (Cory & McKnight, 2005), all components except C8 were quenched in terms of their fluorescence intensities. Nevertheless, changes in their "relative" component distribution were relatively small (within ±4% contribution to total fluorescence). This result was consistent with Poulin et al.'s (2014) observation, where changes in the component distributions were within ±3% of the total fluorescence in their 13-component PARAFAC model. The small or negligible change in component distribution results in the relatively constant RI. Although few fluctuations were observed in the Fe(II) addition experiment, the overall trend of the RI is stable; thus, it can be used as a reliable index to infer the redox condition of the aquatic environment. Still, the component distribution may vary in different PARAFAC models. There were more pronounced distribution changes in a 7-component model than in a 13-component model (Poulin et al., 2014). This suggests that the degree of quenching should always be associated with fluorophore classification. Besides fluorophore classification, DOM composition is another important factor that controls the quenching characterization. Ohno (2009) found that quenching of fluorescence intensity varies depending on the DOM composition. In their 3-component PARAFAC model, Fe(III) quenching was observed in all three components for the deciduous WSOM sample. For the coniferous WSOM sample however, only component 1 and 2 were quenched, and component 3 increased slightly with the initial addition of Fe(III), and decreased with further Fe(III) additions.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

6 Conclusions

This study demonstrates the quenching effect of Fe(II) in organic-rich anoxic groundwater from the Kidd 2 site, where Fe(II) concentrations range from 1 to 300 mg/L and DOM concentrations are 10 mg/L. In our experiments, total fluorescence intensity decreased by approximately 60% as Fe(II) increased from 1 to 300 mg/L. The proportionally stronger quenching effect at low Fe(II) concentration (below 100 mg/L) suggests that the degree of quenching is difficult to predict. Indeed, because quenching intensity does not change uniformly with excitation and emission wavelengths, it would be difficult to quantify the quenching effect on fluorescence indices in other water samples without performing a similar Fe(II) addition experiment. In this study, FI values tended to shift to be more autochthonous in origin with increasing Fe(II), but the small changes are unlikely to invalidate conclusions about the source of DOM. Changes in the humidification index and freshness index both indicated that the addition of Fe(II) would shift these two indices towards more recently produced DOM. Therefore, the sensitivity of these indices should be evaluated when water samples contain Fe(II). The non-linear relationship between the indices and the Fe(II) can be seen in all of the indices, especially the FI.

Although the intensities of all 13 components varied as a function of Fe(II), the relatively stable component distribution suggests that the Fe(II) quenching effect has a negligible effect on the 13-component PARAFAC model. As a result, the PARAFAC model can be a reliable method for obtaining information about DOM composition in their relative distributions and redox status via the RI.

Data availability

The data presented in this manuscript has been published on Zenodo: http://doi.org/10.5281/zenodo.3737108

Author contribution

KJ, AJ, and RB designed experiments and KJ conducted experiments. KJ and AJ performed data analysis. KJ and CM prepared figures. CM compiled and archived the data. KJ and CM prepared the manuscript with contriubtions from AJ and RB.

Acknowledegments

We thank Mark Bolton for assistance with sample analysis.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

330 **References**

Bolton, M., & Beckie, R. (2011). Aqueous and mineralogical analysis of arsenic in the reduced, circumneutral groundwater and sediments of the lower Fraser River delta, British Columbia, Canada. *Applied Geochemistry*, *26*(4), 458–469. https://doi.org/10.1016/j.apgeochem.2011.01.005

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*(7–8), 659–718.

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, *51*(4), 325–346. https://doi.org/10.1016/0304-4203(95)00062-3

Coble, P. G., Green, S. A., Blough, N. V., & Gagosian, R. B. (1990). Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature*, *348*(6300), 432–435. https://doi.org/10.1038/348432a0

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

Fellman, J. B., Hood, E., & Spencer, R. G. M. (2010). Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography*, *55*(6), 2452–2462. https://doi.org/10.4319/lo.2010.55.6.2452

Harvey, C. F., Swartz, C. H., Badruzzaman, A. B. M., Keon-Blute, N., Yu, W., Ali, M. A., et al. (2002). Arsenic mobility and groundwater extraction in Bangladesh. *Science*, *298*(5598), 1602–1606.

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.

Jia, K. (2015). *Iron and manganese reduction driven by organic matter and mixing of fresh and saline groundwater in the Fraser River delta aquifer, Vancouver, Canada*. University of British Columbia. https://doi.org/10.14288/1.0167190

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

Miller, M. P., McKnight, D. M., Cory, R. M., Williams, M. W., & Runkel, R. L. (2006). Hyporheic Exchange and Fulvic Acid Redox Reactions in an Alpine Stream/Wetland Ecosystem, Colorado Front Range. *Environmental Science & Technology*, *40*(19), 5943–5949. https://doi.org/10.1021/es060635j

Mladenov, N., Huntsman-Mapila, P., Wolski, P., Masamba, W. R. L., & McKnight, D. M. (2008). Dissolved organic matter accumulation, reactivity, and redox state in ground water of a recharge wetland. *Wetlands*, *28*(3), 747–759. https://doi.org/10.1672/07-140.1

Neilson-Welch, L., & Smith, L. (2001). Saline water intrusion adjacent to the Fraser River, Richmond, British Columbia. *Canadian Geotechnical Journal*, *38*(1), 67–82.

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

Ohno, T., Amirbahman, A., & Bro, R. (2007). Parallel Factor Analysis of Excitation–Emission Matrix Fluorescence Spectra of Water Soluble Soil Organic Matter as Basis for the Determination of Conditional Metal Binding Parameters. *Environmental Science & Technology*, *42*(1), 186–192. https://doi.org/10.1021/es071855f

Parlanti, E., Wörz, K., Geoffroy, L., & Lamotte, M. (2000). Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry*, *31*(12), 1765–1781. https://doi.org/10.1016/S0146-6380(00)00124-8

Poulin, B. A., Ryan, J. N., & Aiken, G. R. (2014). Effects of Iron on Optical Properties of Dissolved Organic Matter. *Environmental Science & Technology*, *48*(17), 10098–10106. https://doi.org/10.1021/es502670r

Pullin, M. J., Anthony, C., & Maurice, P. A. (2007). Effects of Iron on the Molecular Weight Distribution, Light Absorption, and Fluorescence Properties of Natural Organic Matter. *Environmental Engineering Science*, *24*(8), 987–997. https://doi.org/10.1089/ees.2006.0040

Rue, E. L., & Bruland, K. W. (1995). Complexation of iron(III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Marine Chemistry*, *50*(1–4), 117–138. https://doi.org/10.1016/0304-4203(95)00031-L

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020


Hydrology and Earth System Sciences
Discussions

Senesi, N. (1990). Molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals : Part II. The fluorescence spectroscopy approach. *Analytica Chimica Acta*, *232*, 77–106. https://doi.org/10.1016/S0003-2670(00)81226-X

Stookey, L. L. (1970). Ferrozine---a new spectrophotometric reagent for iron. *Analytical Chemistry*, *42*(7), 779–781. https://doi.org/10.1021/ac60289a016

Waite, T. D., & Morel, F. M. M. (1984). Ligand exchange and fluorescence quenching studies of the fulvic acid-iron interaction: Effects of ph and light. *Analytica Chimica Acta*, *162*, 263–274. https://doi.org/10.1016/S0003-2670(00)84247-6

Wilson, H. F., & Xenopoulos, M. A. (2009). Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geoscience*, *2*(1), 37–41. https://doi.org/10.1038/ngeo391

Zsolnay, A., Baigar, E., Jimenez, M., Steinweg, B., & Saccomandi, F. (1999). Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. *Chemosphere*, *38*(1), 45–50. https://doi.org/10.1016/S0045-6535(98)00166-0

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

Hydrology and Earth System Sciences
Discussions

Figures

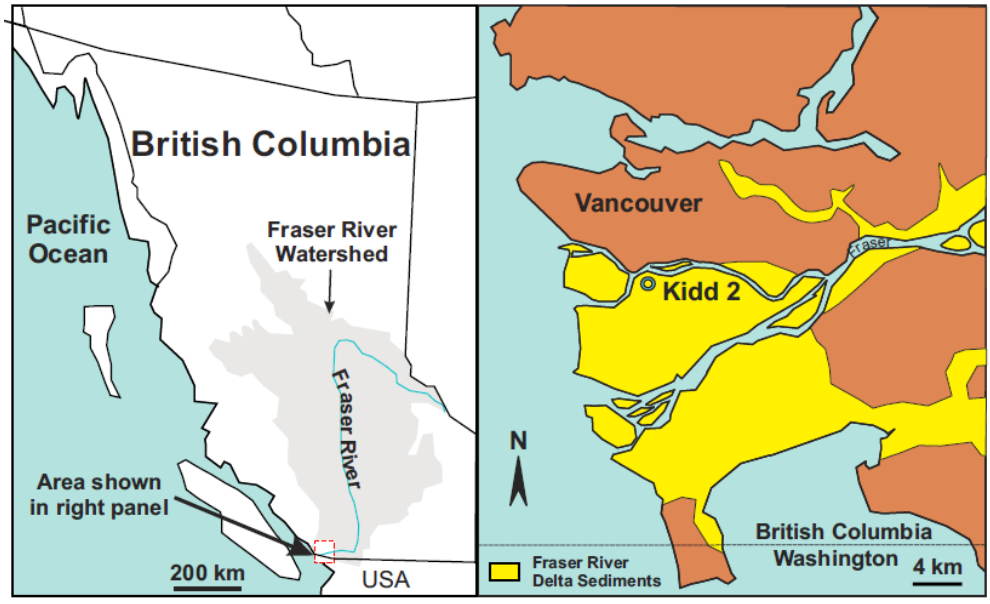


Figure 1: The Kidd 2 site is located in the Fraser River delta in southwest British Columbia.

395

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

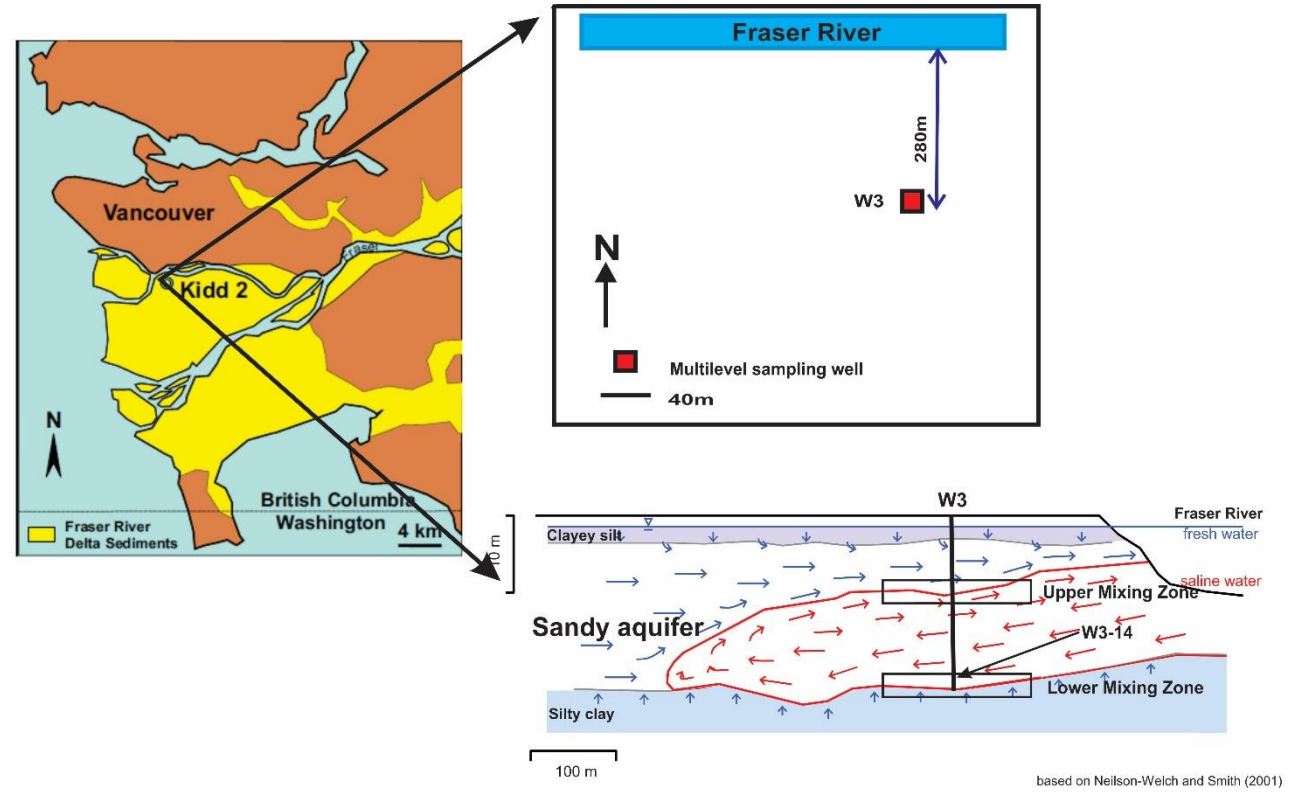


Figure 2: Plan view of the W3 well location and cross-section of the saline wedge, after Neilson-Welch and Smith (2001).

400

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

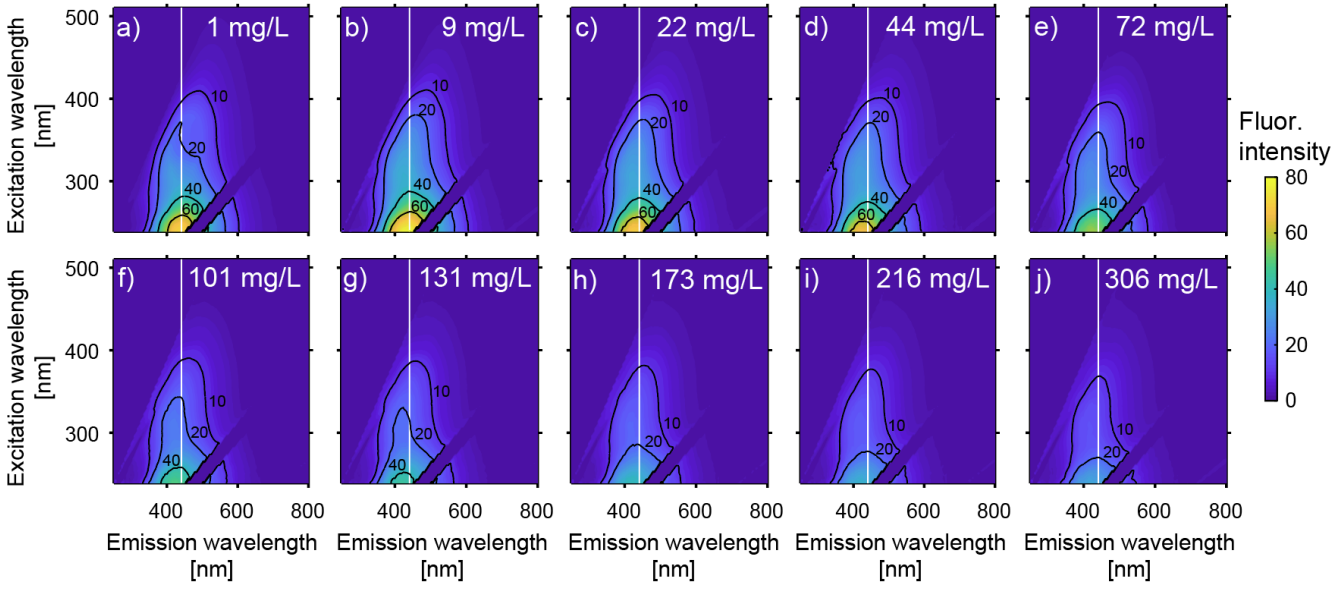


Figure 3: Excitation-emission matrices (EEMs) of groundwater from Fraser River aquifer over a range of Fe(II) concentrations. The Fe(II) concentration for each sample is indicated in mg/L in the top right of each plot, from a) 1 mg/L to j) to 306 mg/L. To distinguish changes in the center of the primary peak (peak A), a vertical dashed line at an emission wavelength of 441 nm is shown (the peak emission wavelength at excitation 239 nm for the 1 mg/L solution, see Figure 4). The fluorescence intensities are reported in Raman units (RU).

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020

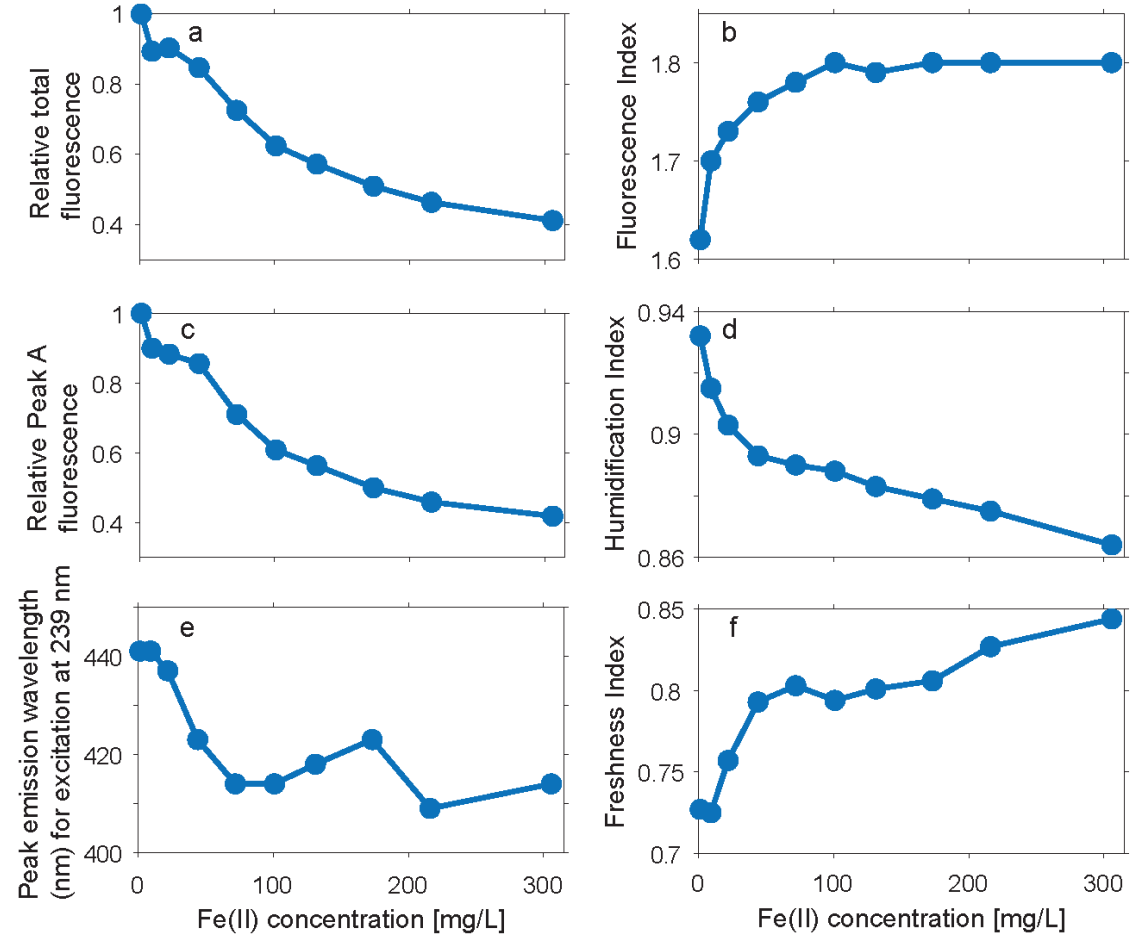
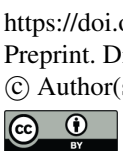
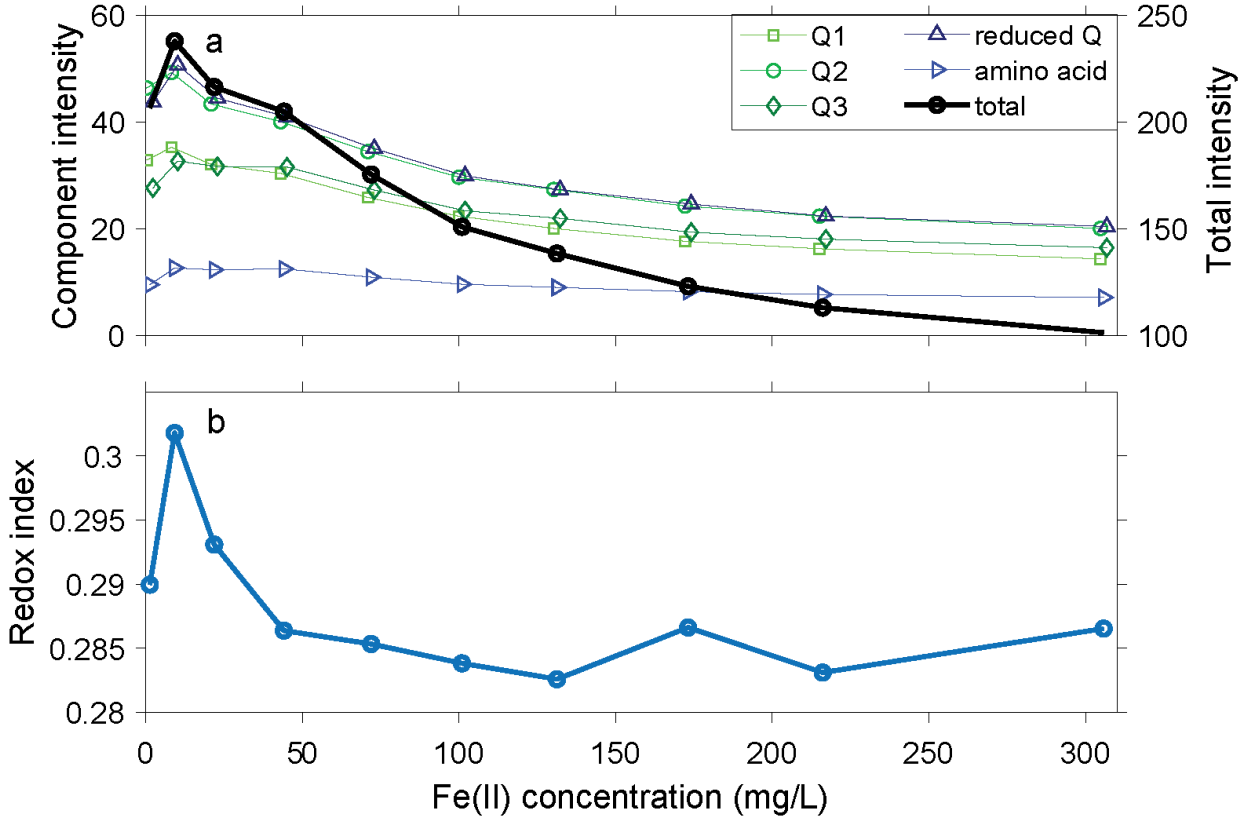


Figure 4: Effect of varying Fe(II) concentrations at pH 7.4 on: a) relative total fluorescence intensity (OFI/OFI$_0$); c) relative fluorescence intensity of Peak A (A/A$_0$) and e) peak fluorescence emission wavelength (nm) at excitation at 239 nm (peak A). Effect of varying Fe(II) concentration at pH 7.4 on various indices: b) fluorescence index (FI), d) humidification index (HIX), and f) freshness index (FrI). The results show that the fluorescence intensities, indices and peak emission wavelength change as Fe(II) is increased from 1 to 306 mg/L.

https://doi.org/10.5194/hess-2020-150
Preprint. Discussion started: 11 May 2020



Hydrology and Earth System Sciences
Discussions
Open Access
EGU



415

Figure 5: a) Intensity of components at varying Fe including quinones Q1, Q2, and Q3 (green), reduced quinones (reduced Q, calculated as the sum of SQ1, SQ2, SQ3, and HQ), amino acid (tryptophan and tyrosine) and total intensity, and b) redox index (RI) in the presence of varying Fe(II). In a) the Fe concentrations have been slightly offset to better show overlapping symbols.