INTRODUCTION

Virtually nothing is known about the role of chromophoric dissolved organic matter (CDOM) in the redox chemistry of the troposphere. Earlier studies have suggested that CDOM accounts for as much as 50% of the organic fraction in rainwater (Willey et al. 2000). The main component of CDOM is thought to be humic-like substances, which arise from wind blown soil particles or are formed in the atmosphere from oxidation of soot (Decesari et al. 2002). CDOM is the dominant chromophore and fluorophore in rainwater (Kieber et al. 2005) and has profound ramifications with respect to a wide variety of fundamental questions in atmospheric chemistry particularly regarding the attenuation of sunlight and the generation or quenching of free radicals.

One of the most important, yet least studied aspects of CDOM chemistry in rainwater is its impact on the speciation of trace metals. Iron is the dominant focus in this study because of its abundance in atmospheric waters and its affinity for forming chelates with organic ligands. Earlier studies have suggested that a significant fraction of the Fe(II) and Fe(III) in rainwater may also be organically complexed (Kieber et al. 2005, Willey et al. 2000). Complexation of Fe by organics in rainwater has important implications for controlling both the speciation of the metal and the photochemistry of the ligand. Earlier studies have suggested for example that Fe(II) is photochemically produced from an Fe(III) organic complex via a ligand to metal charge transfer generating an oxidized form of the ligand (Kieber et al. 2003). The oxidation of the photoproduced Fe(II) is also directly impacted by the presence of organic ligands with significantly slower oxidation rates relative to synthetic rainwater with no ligands (Willey et al. 2005).

Despite the significance of organic complexation on the speciation of Fe in rainwater the nature of the complexing ligand is unknown. One of the primary objective Sof this thesis is to determine if CDOM can act as an effective ligand of both Fe(II) and Fe(III) in rainwater. Previous studies indicate that CDOM in rainwater is extremely photoreactive where there is a significant loss of CDOM absorbance and fluorescence even when exposed to short term irradiation with simulated sunlight (Reid 2003). A secondary objective of this thesis is to determine if the photodegredation of rainwater CDOM is slowed or accelerated by the formation of chelates with Fe(II) and Fe(III).

A final goal of this thesis is to evaluate the role of CDOM complexation of iron on the speciation of atmospherically deposited Fe in seawater. There have been many total Fe determinations in rainwater from marine areas. Quantifying total Fe concentration does not, however, predict the potential significance of atmospherically deposited iron to oceanic primary productivity because the bioavailability of iron is critically dependent on the speciation of the soluble forms of the metal. Rich (1990), for example, has shown colloidal Fe and Fe oxides are not immediately available for use by phytoplankton. In addition, Fe(II) is much more soluble relative to Fe(III) and therefore more likely to be present in larger amounts for aquatic organisms (Anderson and Morel 1982; Martin and Fitzwater 1988; Martin and Gordon 1988; Banse 1990; Martin et al. 1990; Rich and Morel 1990; Martin et al 1991). Although Fe(II) is more soluble it oxidizes extremely rapidly in the presence of O_2 and H_2O_2 at seawater pH of 8, but has been found to be relatively stable in various oceanic waters (Rijkenberg 2005) and atmospheric rainwater (Kieber et al. 2003) due to an undetermined organic ligand.

METHODS

Sample Collection

Rainwater was collected on the UNCW campus on an event basis over the duration of this project. The UNCW rainwater collection site is a large open site that is approximately one-hectare, located within the turkey oak, long leaf pine, and wire grass community, this is typical of inland coastal areas in southern North Carolina. This site is approximately 8.5 km from the Atlantic Ocean.

Event rain samples were collected using four Aerochem-Metrics Automatic Wet-Dry Precipitation Collectors. One of these contained a 4 L muffled Pyrex glass beaker, which samples for dissolved organic carbon, hydrogen peroxide, pH, inorganic ions, and organic compounds were collected. The remaining 3 collectors for trace metal samples contained a Teflon funnel leading to a 2.2 L Teflon bottle extensively cleaned using trace metal clean procedures and protocols (Bruland et al., 1979; Bruland, 1980; Tramontane et al., 1987).

Reagents

All reagents and standards used for analysis were Fisher Scientific reagent grade (Fairlawn, NJ) unless otherwise noted. SigmaUltra (>99.9% pure) Ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic aceid)-1,2,4-triazine) was received from Sigma Chemical Company (St. Louis, MO). Ferric Chloride, 6-Hydrate, Lump ACS reagent grade was recieved from J.T. Baker (Phillipsburg, NJ). Deionized water was obtained from a Milli-Q Plus Ultra-pure water system (Millipore, Bedford, MA), which provided water (>18MΩ) for all analysis, dilutions, reagents, and standard preparation.

Sample Preparation

All samples were warmed to room temperature and filtered through 0.2 μm, acid-washed Gelman Supor® polysulfonone filters enclosed in a 1 L muffled glass filtration apparatus. All glassware was muffled prior to use at 560ºC for at least 6 hours. Teflon containers were cleaned by immersing into 10% HCl with Milli-Q water for at least 24 hours between uses. Teflon bottles were initially cleaned through a 3 week procedure where they were first rinsed with methanol and then placed in 2% Citrinox (phosphate free acid cleaner and detergent) for one week, then in 3 M HCl for at least 2 weeks, and finally transferred to pH 2 HCl which was prepared by adding 1 mL of trace metal grade 12 M HCl to 1 L Milli-Q water. Bottles were rinsed with DIW between each stage and finally with Milli-Q and dried where they were then stored in two zip-lock bags.

Fe and Ferrozine preparation

A primary stock solution of Fe(II) was prepared by dissolving 0.4970 g $FeCl_2 \cdot 4H_2O$ with 2 mL optima grade HCl (O-HCl) to 250 g with Milli-Q (10mM). A secondary solution was made daily by adding 30 μL of primary Fe^{2+} to 30 mL of pH 4.5 Milli-Q water, which is prepared by diluting 4 mL of 5 mM H_2SO_4 to 1 L with Milli-Q, pH 4.5 Milli-Q is called synthetic rainwater (SRW).

Iron(III) was prepared by dissolving 0.676 g $FeCl_3 \cdot 6H_2O$ with 2 mL O-HCl to 250 g with Milli-Q (10mM). A secondary solution was prepared in the same manner as the Fe^{2+}.

Ferrozine solution for excitation emission analysis (EEM) was prepared from the primary stock solution that was used in the Fe analysis with a concentration of 1.04 M. A secondary solutions was prepared by adding 30 μL of primary Ferrozine to 100 mL SRW

(0.312 mM) and a tertiary solution was prepared for optical analysis from the secondary solution by adding 320 μL to 100 mL SRW (1.00 μM).

Photochemical Experiments

Photolysis experiments were done in accordance with Kieber et al. (2003). Samples were apportioned into 30 mL quartz cells with aliquots removed for initial measurements and dark controls. Light exposed samples were placed in a constant temperature water bath and irradiated for 6.5 hours, unless otherwise noted, which corresponds to one day mid summer at 34º north sun light by using a solar simulator (Spectral Energy solar simulator LH lamp housing with a 1000 watt Xe arc lamp) equipped with a sun lens diffuser and an AM1 filter to remove wavelengths not found in the solar spectrum.

Hydrophobic CDOM Extraction

Chromophoric dissolved organic matter (CDOM) was extracted using C_{18} cartridges (Waters Chromatography, Milford, MA) by the method of Amador et al. (1990). C_{18} was chosen because earlier studies have shown that they were between 22 and 84% more effective at removing CDOM from seawater relative to XAD-2 (Amador et al. 1990). The authors also found that C_{18} cartridges are better able to retain the UV-visible and fluorescence characteristics of the isolated CDOM material relative to XAD-2. Another advantage of C_{18} cartridges over XAD-2 is that acetonitrile is the eluant rather than 0.1M NaOH. Therefore, any degradation due to high pH is avoided.

Before the C_{18} cartridge was used to extract CDOM from rain water they were prepped by washing with 2 X 5 mL acetonitrile followed by 2 X 5 mL Milli-Q water. Samples for CDOM extraction were filtered (0.2 μM) and then loaded onto the C_{18}

cartridges, the maximum volume per cartridge was 500 mL. The cartridges were finally washed with 2 X 5 mL of Milli-Q water to remove residual salts. Samples and wash water was pulled through the C_{18} cartridge by the house vacuum with a 5-port manifold, accompanied with a 1 L side-arm flask.

The bound constituents were eluted off the cartridge by 2 X 3 mL of acetonitrile into a muffled 50 mL round-bottom flask and concentrated to dryness under reduced pressure (Buchi Rotavapor, Model RE 111, Switzerland). Traces of water and acetonitrile were removed under vacuum (Sargent-Welch Model 1400, Skokie, IL). All samples were reconstituted back to solution with SRW and sonicated for 30 seconds.

Iron Analysis

Reagents for iron analysis were all prepared gravimetrically and stored in a class 100 clean hood for up to one month. Ferrozine was prepared by adding 0.514 g of ferrozine and Milli-Q to make a 100 g solution. Hydroxylamine hydrochloride was received from LabChem Inc Pittsburgh, PA, and prepared by adding 25 g of hydroxylamine hydrochloride and Milli-Q to 250 g total solution (10%). Ammonium acetate buffer was prepared by adding 13.2 mL of concentrated NH_4OH, 2.4 mL glacial acetic acid, and Milli-Q to 100 g total solution.

Iron(II) and Fe(III) in rainwater samples were determined by the ferrozine method modified from Stookey (1970). Absorbance measurements were made using a 1 m Ocean Optics (World Precision Instruments) liquid waveguide capillary cell (LWCC) attached to an Ocean Optics Inc. SD2000 spectrophotometer and an Analytical Instrument Systems Inc. Model DT 1000 CE UV/Vis Light Source (Kieber et al. 2003). Iron(II) forms a complex with ferrozine that absorbs visible light most strongly at 562

nm. To correct for baseline shifts and background absorbance, the absorbance readings at 685 nm were subtracted from the absorbance at 562 nm to give the correct absorbance for the Fe(II)-ferrozine complex.

In order to determine Fe(II) 700 μL of ferrozine was added to 30 mL of sample and incubated for 5 min at room temperature after which 1.8 mL of buffer was added. To determine Fe(T) (Fe(II) + Fe(III)), 700 μL of 10% hydroxylamine hydrochloride solution was added to 30 mL of sample to reduce Fe(III) in solution to Fe(II). Iron(III) concentrations were obtained by subtracting the Fe(II) concentration from the Fe(T). Two standard curves were prepared, one for Fe(II) and one for Fe(T). Iron (II) and Fe(T) standards for the 1 m cell were prepared at concentrations of 0, 31, 93, and 155 nM. These solutions were prepared from the secondary Fe(II) solution that is made daily as described in the Fe preparation section.

Iron Stability and Redox Chemistry

Extracted rain was reconcentrated to 150 mL and complexed with Fe(II) for one hour, along with a SRW reference. Samples were then spiked with H_2O_2 to a concentration of 10-30 μM. Ten milliliter aliquots from each sample were taken at 0, 30, 60, 120, and 240 minutes and analyzed in triplicate for Fe(II). Peroxide was analyzed at 0 and 240 minutes and fluorescence was analyzed at the beginning of each experiment.

In the redox experiment, rain extract was reconcentrated to 200 mL, and initial Fe concentrations were determined. If the reconcentrated extract had an Fe(III) concentration less than 20nM a spike of Fe(III) was added and allowed to complex for an hour. Samples were then put under irradiation for 2 hours and stored in the dark for the remaining 22 hours. Iron(II) and Fe(T) were analyzed at initially after 2 hours of

irradiation then stored in the dark where measurements were taken every 10 minutes for the first hour under dark storage, then at 4 and 24 hours from the start of the experiment.

Optical Analysis

In determining the impact of metal species on CDOM EEM spectroscopy will be examined on various types of rain events. EEM can be employed to discriminate between CDOM types and to gain insight into the structural nature of the chromophores of the rainwater (Blough et al. 1995).

Excitation-emission matrix (EEM) fluorescence properties were determined on a Jobin Yvon SPEX Fluoromax-3 scanning fluorometer equipped with a 150 W Xe arc lamp and a R928P detector. The EEMs were constructed by using excitation wavelengths from 250 to 500 nm (5 nm intervals) and scanning emission from 280 to 550 nm (5 nm intervals). The instrument was configured to collect the signal in ratio mode with dark offset using 5 nm bandpasses on both the excitation and emission monochromators. The EEMs were created by integrating emission spectra measured every 5 nm from 250 to 500 nm at 51 separate excitation wavelengths (Del Castillo et al. 1999). Scans were corrected for instrument configuration using factory supplied correction factors, which were determined as described in Method 1 of Coble et al. (1993). Post processing of scans is performed using FLToolbox 1.91 developed by Wade Sheldon (University of Georgia) for MATLAB® (Release 11). The software eliminates Rayleigh and Raman scattering peaks by excising portions of each scan centered on the respective scatter peak. The excised data is replaced using three-dimensional interpolation of the remaining data according to the Delaunay triangulation method and constraining the interpolation such that all nonexcised data is retained. Following

removal of scatter peaks, data were normalized to a daily-determine water Raman intensity (270 ex/300 em) and converted to Raman normalized quinine sulfate equivalents (QSE) in ppb (Coble, Del Castillio et al. 1998). In order to better compare samples peak areas were integrated to a total integration value noted as total fluorescence intensity. Replicate scans are generally within 5% agreement in terms of intensity and within bandpass resolution in terms of peak location. Major fluorescence CDOM component peaks have been designated by letters to describe the unknown chemical composition (Coble 1996). Four peaks will be used to describe rainwater CDOM: A and C indicates terrestrial humic-like substances, M is marine humic-like material, and T indicates the presence of protein-like substances (Figure 1).

RESULTS AND DISCUSSION

Iron (II) Complexation with CDOM

Twenty four different ambient rainwater samples were analyzed for fluorescence with and without addition of Fe(II) in order to see if Fe(II) could complex ambient CDOM as seen by reaction (c) in Figure 2. All samples were spiked with 250 nM Fe(II) which is significantly higher than the 40 nM usually seen in a typical rain event (Kieber et al. 2001).

An addition of Fe(II) enhances fluorescence in 77% of the samples (Figure 3), while in 23% of samples there was little or no change in fluorescence upon addition of Fe(II). The increase in CDOM fluorescence observed in the majority of these samples indicates that inorganic Fe(II) is capable of forming a complex with ambient CDOM.

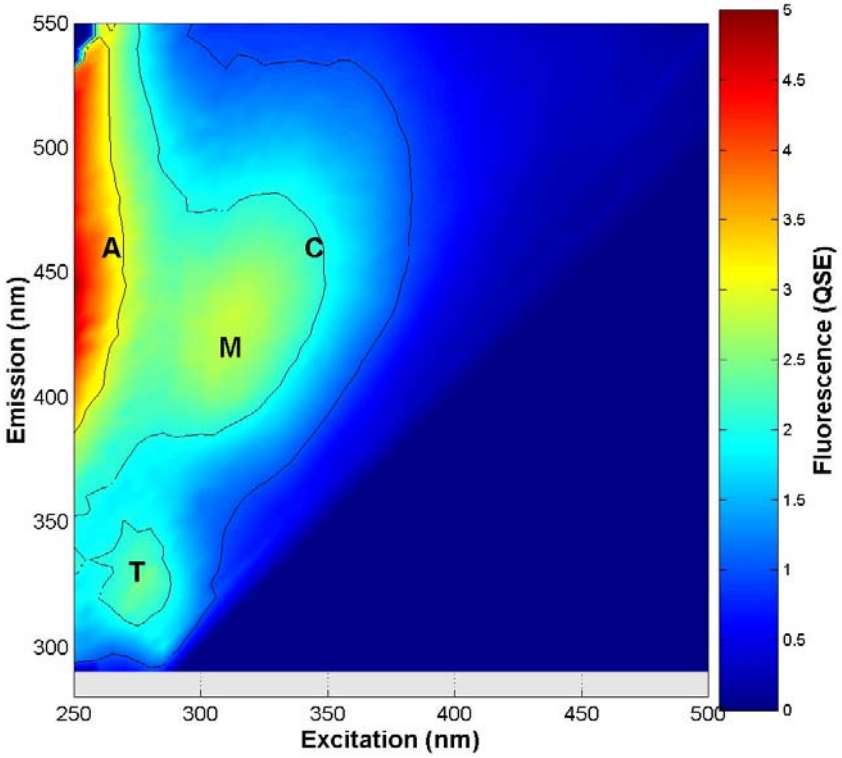


Figure 1. Typical EEM spectra of rainwater. The A, M, C,

and T labels are based upon the work of Coble (1996): where A and C indicate terrestrial humic-like substances, M is marine humic-like material and T indicates the presence of protein-like substances.

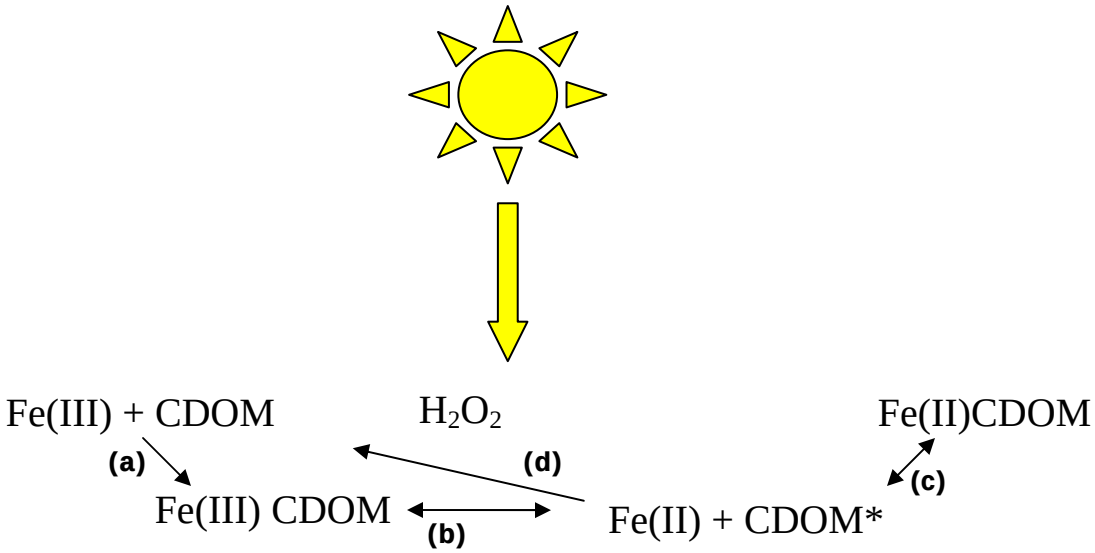


Figure 2. Photochemical redox reaction of Fe(II) and Fe(III) with CDOM.

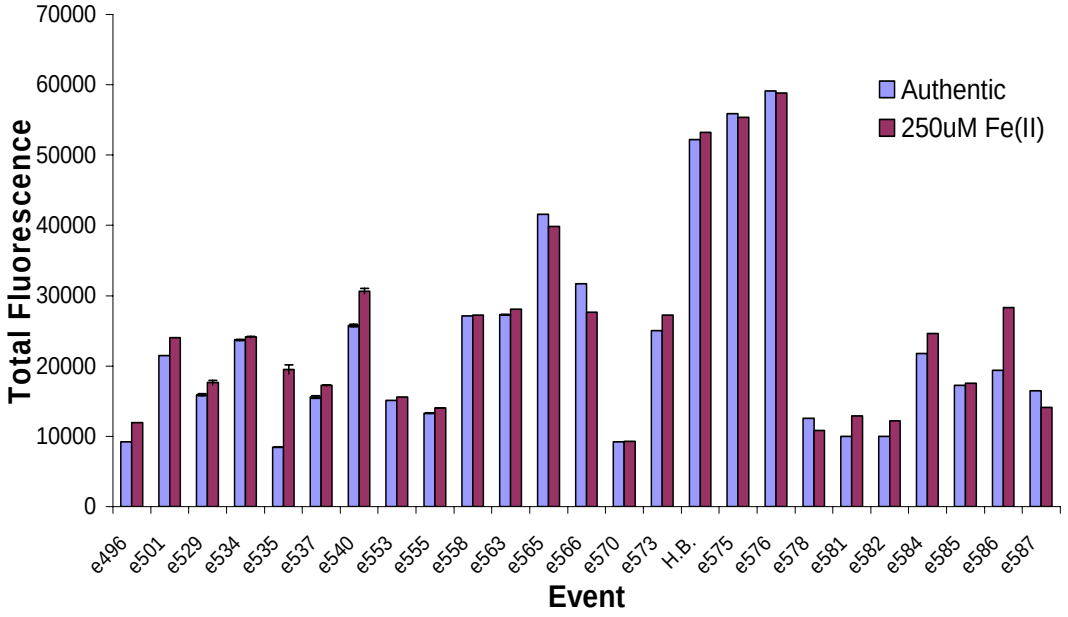


Figure 3. Total fluorescence of EEM spectra of rain events with and without the addition of 250nM Fe(II). Error bars represent ± 1 standard deviation (n =3). Samples were collected between May 3, 2004 and July 14, 2005. H.B. = Horseshoe Bend of the Cape Fear River 1:50 dilution. Summer 2004 are events 501, 529, 534, 535, and 537. Winter 2004 are events 553, 555, and 558. Summer 2005 are events 578, 581, 582, 584, 585, 586, and 587.

In order to better understand the interaction of rainwater CDOM with Fe(II) the fluorescent property of the known ferrous ligand ferrozine (Fz) was studied before and after Fe(II) addition, ferrozine forms a complex with Fe(II) in the form of Fz_2Fe_3 (Stookey et al. 1970). The ferrozine solution was made up in synthetic rain.

The ferrozine and added Fe(II) mimicked the behavior seen in the authentic rain events (Figure 4) where fluorescence increased upon Fe(II) addition. Added Fe(III) also showed a slight increase in fluorescence, however this may result from the photoreduction of Fe(III) to Fe(II) due to the long exposure of the sample to light in the instrument while measuring EEMs or direct reduction of Fe(III) by ferrozine. This suggests that the increase may actually be due to Fe(II). This also suggests that ferrozine can act as a model ligand for the Fe(II) ligand found in rainwater.

Seasonality

Rain events were separated into winter and summer seasons in order to determine if there was any seasonality in the extent of complexation of Fe(II) with CDOM (Table 1) which might explain some of the variability in fluorescence changes observed upon Fe(II) addition presented in Figure 3. Winter was defined as December 1 to February 28, while summer season was defined as June 1 to September 30 similar to previous research done at this location (Reid 2003). Volume weighted integrated values were higher in winter compared to summer, which is consistent with Reid (2003) indicating there is a strong seasonal signal to the ambient CDOM fluorescence in rainwater at this location. When Fe(II) is added there is a larger increase in fluorescence seen in both summer seasons compared to the winter season. Most of the events in Figure 2 that showed little or no change upon addition of Fe(II) were winter or marginal seasoned events while the

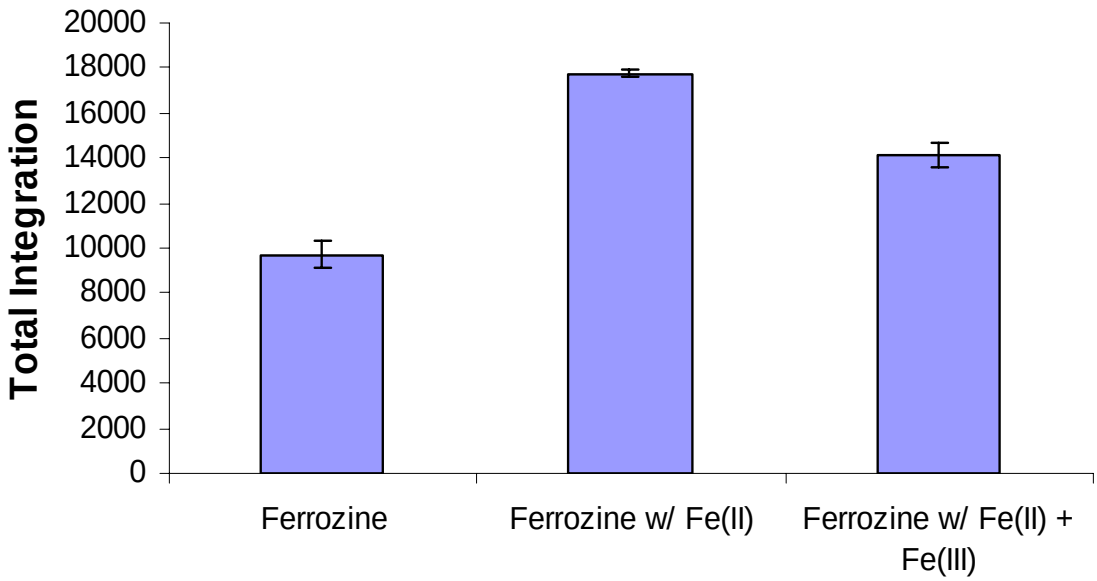


Figure 4. Total integrated fluorescence of synthetic rain in 1 μM Ferrozine with the addition of 250 nM Fe(II) and Fe(III)/Fe(II) mix where the error bars indicate the standard deviation of 3 replicates.

Table 1. Seasonal volume weighted averages from surface volume. Total integration for initial rain events and the difference of integration after addition of Fe(II) is indicated. Samples were collected at Wilmington, NC between May, 3 2004 and July 14, 2005. Integration values are all relative to the polygon integration program designed by Coble (1996), n = number of samples.

	n	Amount (mm)	Initial Fluorescence	Δ Fluorescence by addition of Fe(II)
Summer 2004	5	178	12971	6305
Winter 2004-2005	4	54	17667	453
Summer 2005	7	143	13274	1376

majority of those where there was an increasing fluorescence upon addition of Fe(II) were summer storms. Summer events may be more likely to increase in fluorescence upon Fe(II) addition because of the photochemical history of the samples. The CDOM in summer events has been subjected to much more photobleaching and has had a significant portion of its ambient complexed Fe removed than the CDOM in winter events (Kieber et al. 2003). The photochemical removal of the complexed iron may have allowed more of the added Fe(II) to complex with the CDOM in an ambient rain event similar to the ferrozine with Fe(II) added (Figure 4) causing an increase in fluorescence.

Impact of Strom Origin

Rain events from Figure 3 were also divided by back trajectory classification (Figure 5) to determine if the back trajectory of the rain event could explain some of the variability in fluorescence changes observed upon Fe(II) addition. Back trajectories were compiled by NOAA hysplit and characterized into 5 trajectories by Long 2003. Initial volume weighted fluorescence values were highest in trajectory 2 storms which are almost exclusively continentally influenced. The coastal storms from trajectories 4 and 5, had significantly lower initial volume weighted fluorescence. The trajectory 3 events were a mixture of both coastal and continental influences and had a total initial fluorescence in between trajectory 2 and 5 storm types. When rain amount was plotted against initial fluorescence there was no significant correlation ($p > 0.1$) indicating it's not a function of rain amount. The results presented in Table 2 are consistent with Kieber et al (2003) who found there was a strong storm origin impact on the amount of CDOM fluorescence in rainwater at this location.

In addition to initial fluorescence the effect of Fe(II) addition was dramatically

Figure 5. Plots of air mass back-trajectories calculated from the HYSPLIT model grouped by trajectory classification.

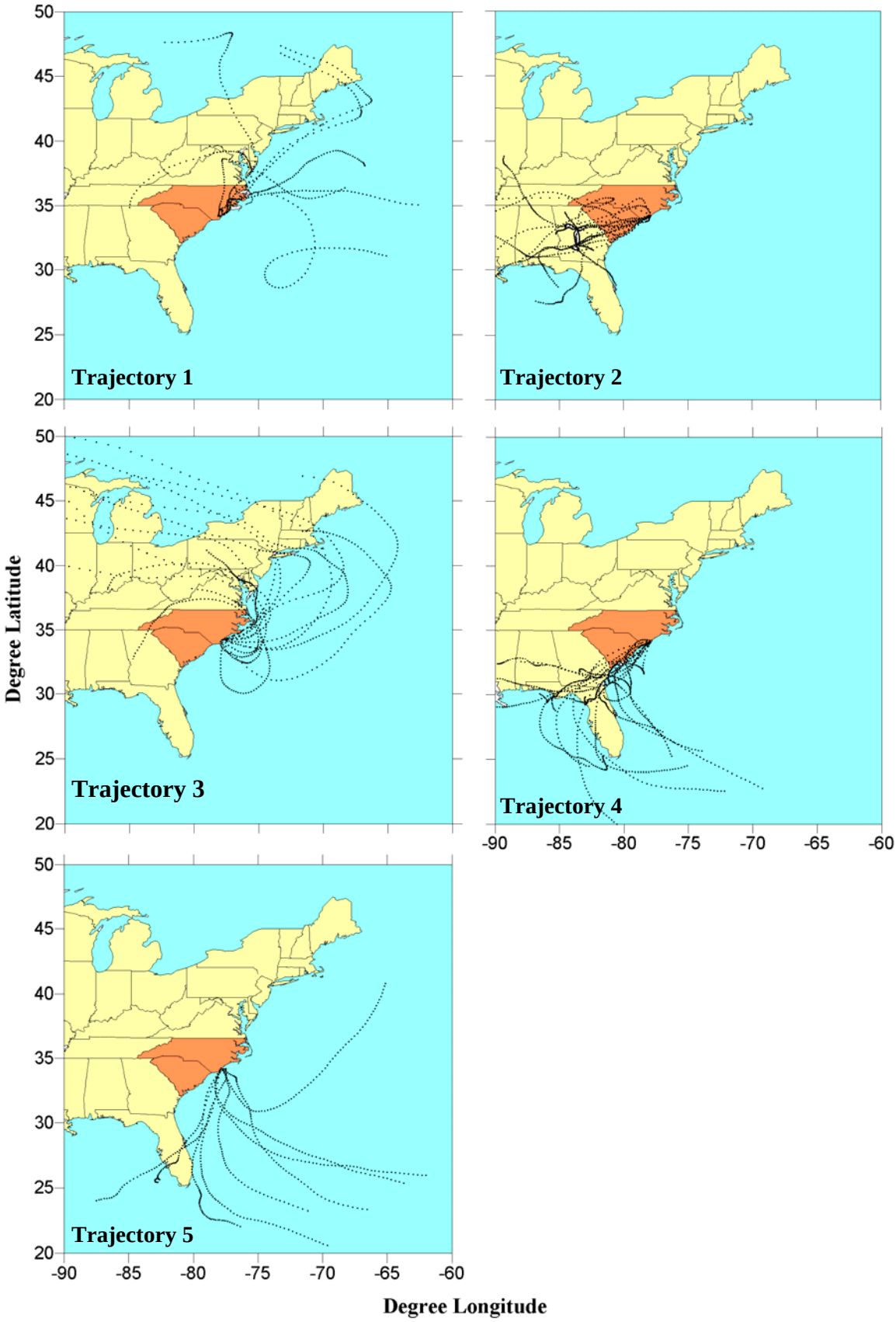

Trajectory 1
Trajectory 2
Trajectory 3
Trajectory 4
Trajectory 5
Degree Latitude
Degree Longitude
50
45
40
35
30
25
20
-90
-85
-80
-75
-70
-65
-60

Table 2. Trajectory volume weighted averages from surface volume. Trajectory category is divided up as according to Figure 5. Total integration for initial rain events and the difference of integration by addition of Fe(II) is indicated. Samples were collected at Wilmington, NC between May, 3 2004 and July 14, 2005. Integration values are all relative to the polygon integration program designed by Coble (1996).

Trajectory	n	Amount (mm)	Initial Fluorescence	Δ Fluorescence by addition of Fe(II)
2	4	59	40831	92
3	7	231	21878	-96
4	6	178	12828	6889
5	6	173	12544	1001

different depending on the back trajectory history of the rain event used. When Fe(II) is added to both trajectory 4 and 5, coastal events there was a much larger increase in fluorescence upon Fe(II) addition compared with trajectory 2 and 3, continental events. The reason why coastal events increase more in fluorescence upon addition of Fe(II) may be because coastal storms typically have less Fe(II) in them initially (Kieber et al. 2001) which could allow more available sites for Fe(II) to complex in the CDOM. It could also be that the coastal event are more photobleached allowing for more Fe(II) to complex. This may also partly explain why lower Fe coastal rain events have lower initial fluorescence values. The continental rains have a higher initial fluorescence suggesting that most of the CDOM may be already complexed with Fe(II) resulting in little or no increase of fluorescence upon addition of Fe(II).

Fe(III) Complexation with CDOM

Seven ambient rain samples and samples from the organically rich Cape Fear River Estuary (CFRE) were analyzed for fluorescence before and after addition of 250 nM Fe(III) in order to see if Fe(III) is capable of complexing with CDOM. In 6 out of 8 samples including the CFRE sample addition of Fe(III) quenched fluorescence while in 2 samples there was an increase or negligible change in fluorescence upon addition of Fe(III) (Figure 6). The decrease in CDOM fluorescence observed in the majority of the samples indicates that inorganic Fe(III) is capable of forming a complex with ambient CDOM following reaction (a) of Figure 2. A study in ocean water by Heihachiro et al. (2003) suggested that CDOM controls the distribution of Fe(III) solubility. When there is a higher quantity of organic mater the solubility of Fe(III) is increased because the organic complexed Fe(III) does not precipitates out as easily as iron oxyhydroxides. Rain

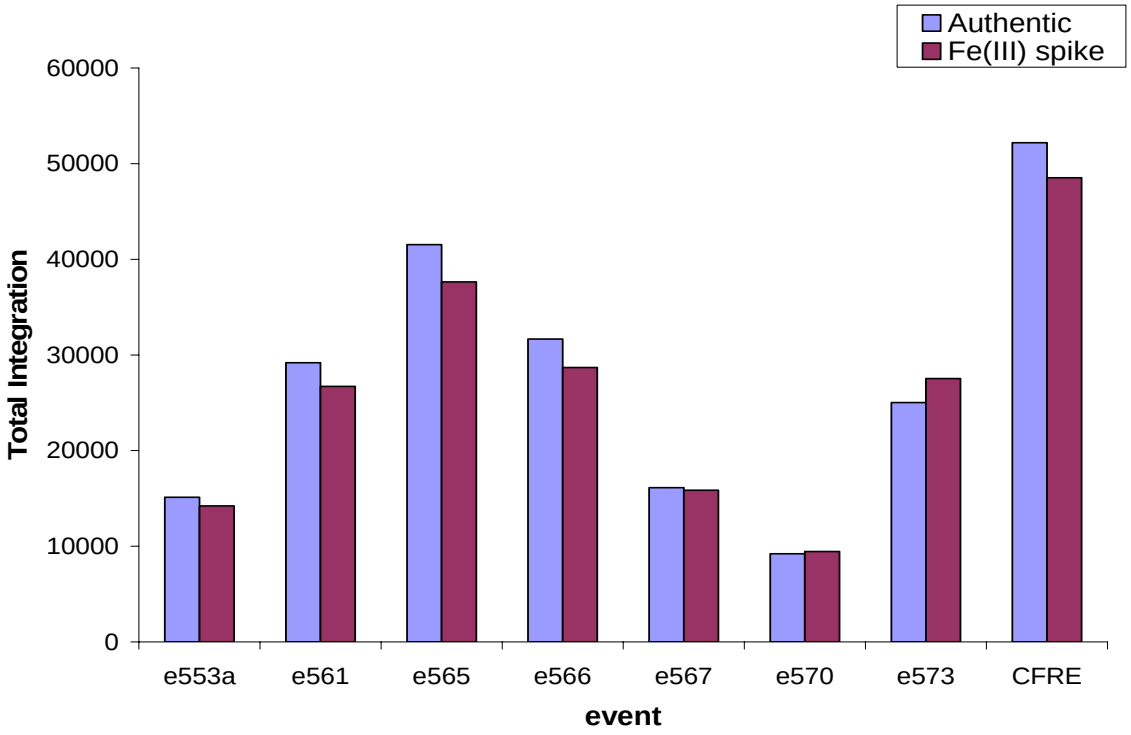


Figure 6. Total integration fluorescence of EEM spectra of rain events with and without the addition of 250nM Fe(III). Samples were collected between January 14, 2005 and July 14, 2005. H.B. = Horseshoe Bend of the Cape Fear River 1:50 dilution; e = event of rain.

samples with higher initial CDOM concentrations (e561, e565, e566, and CFRE) had greater amount of quenching in the presence of added Fe(III) presumable because there was excess Fe(III) ligand to bind with the added iron. The quantity of CDOM available for Fe(III) complexation may explain why in event 570 there was a negligible increase upon addition of Fe(III). This rain sample had the lowest initial fluorescence indicating that there was little CDOM available for complexation upon the added Fe(III). However event 573 had a relatively large initial fluorescence but also had an increase in fluorescence upon addition of Fe(III). When looking at the seasonality of the collection period event 573 was collected at the end of May nearing the summer months while all other samples were collected during the early to mid spring months with the exception of e553 which was the only winter sample. Nearing the end of spring there is more exposure to the solar radiation suggesting that this slight increase might be due to photobleaching of the CDOM thus making it unavailable for Fe(III) complexation. Due to the lack of seasonal differences in the samples collected volume weighted seasonal averages were not compiled. However there is a significant negative linear correlation ($r = 0.9861$, $p < 0.001$) produced when the change in fluorescence (Fe(III) fluorescence – initial fluorescence) is plotted against ambient rainwater fluorescence (Figure 7). There exists one outlier at (25013.8, 2526.8) which is the summer event 573. When fluorescence is normalized (Δ fluorescence/initial fluorescence) and plotted against date of collection (Figure 8), it appears that the winter samples have little change while the samples that near the summer months show a increase in normalization, suggesting that as the days become longer with more exposure to the sun's radiation photobleaching is effecting the ability of Fe(III) to complex with CDOM. Back trajectories for the seven

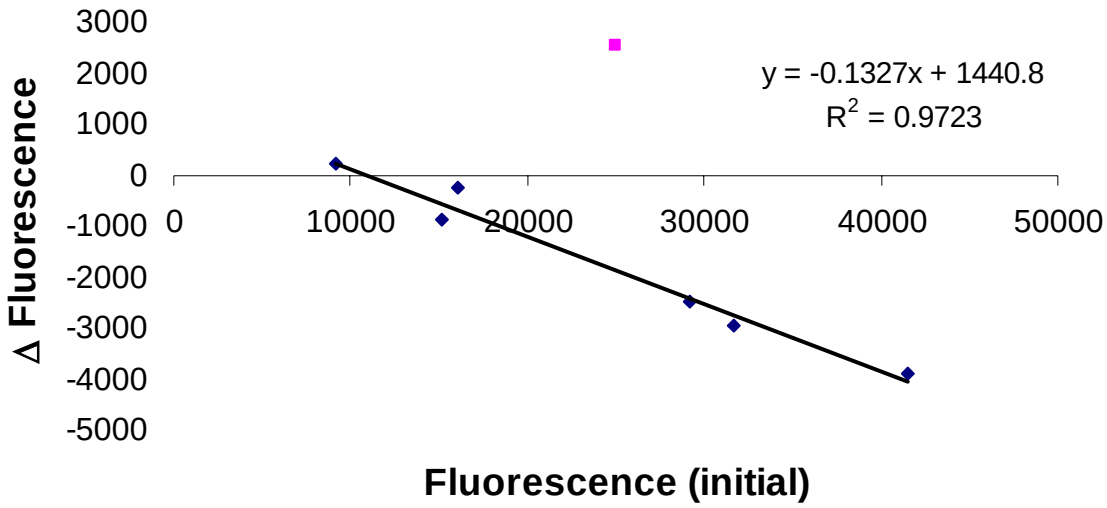


Figure 7. Linear relationship between ambient rainwater fluorescence and Δ fluorescence (Fe(III) fluorescence – initial fluorescence) n = 6, r^2 = 0.97, and $p < 0.001$. The samples at (27540, -0.10) represents the summer event 573.

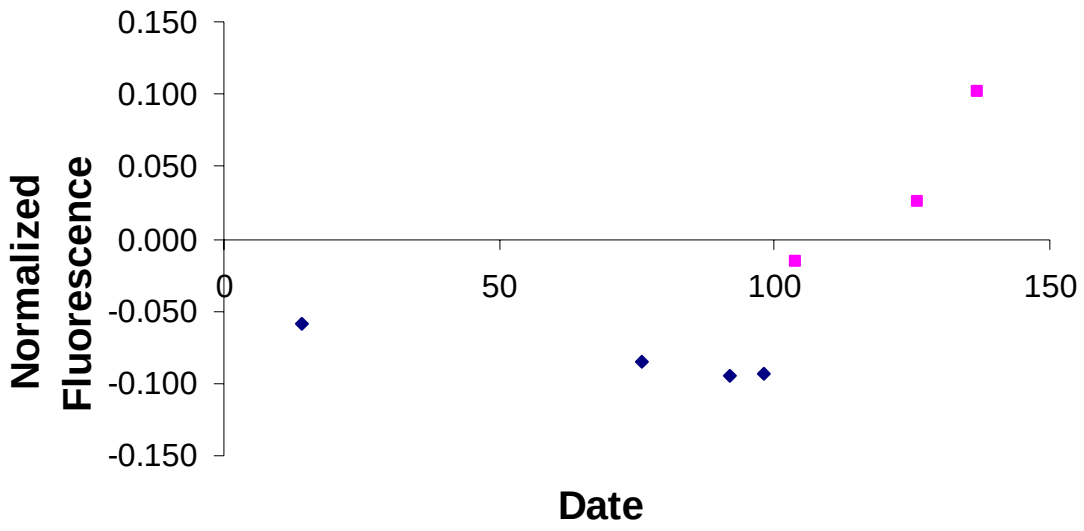


Figure 8. Normalized fluorescence (Δ fluorescence/initial fluorescence) vs. date of sample collection, where the diamonds represents samples collected between January 14, 2005 to April 8, 2005 and the squares represent samples collected from April 14, 2005 to May 17, 2005.

Table 3. Trajectory volume weighted averages from surface volume values where trajectory categories are divided up as according to Figure 5. Total integration for initial rain events and the difference of integration by addition of Fe(III) is indicated. Samples were collected at Wilmington, NC between January 14, 2005 and May 17, 2005.

Trajectory	n	Amount (mm)	Initial Fluorescence	Δ Fluorescence by addition of Fe(II)
3	4	176	23380	-1650
5	3	62	17739	124

rain samples indicated that all samples were either trajectory 3 or 5 as seen in Table 3 where trajectory 3 has a higher initial volume weighted average fluorescence with large changes upon the addition of Fe(III) while trajectory 5 has lower initial volume weighted average fluorescence with less of a change in fluorescence upon the addition of Fe(III).

Photochemistry of Fe

Photochemical experiments were conducted to determine the mechanism by which Fe(II) is photoproduced in rainwater. Iron concentrations and fluorescence intensities were measured both initially and after 6 hours of irradiation of authentic rain events while a separate aliquot was kept in the dark throughout the irradiation period as a control. The fluorescence and Fe(II) changes upon irradiation were positively correlated ($p < 0.05$) (Figure 9). In four out of the six rain samples Fe(II) concentrations increased upon irradiation with a concomitant increase in fluorescence. This suggests that iron in these rainwater samples is predominantly Fe(III) prior to photolysis. When exposed to simulated sunlight Fe(III) is photochemically reduced to Fe(II).

When Fe(III) is photoreduced to Fe(II) in these samples an increase in fluorescence is observed. This is because Fe(III) quenches fluorescence (Figure 6) and as it is removed from the CDOM complexes via reduction to Fe(II) the fluorescence increases. This is similar to what was observed by Kieber et al. (2003) who hypothesized that Fe(II) was photochemically produced from Fe(III) in a variety of authentic rainwater samples. The authors suggested that the amount of Fe(II) photoproduced in rainwater is dependent on the amount of Fe(II), Fe(III), and hydrogen ion initially present in the sample. Rijkenberg (2005) suggests that in seawater an organic ligand that consists of chromophoric characteristics that is bonded to Fe(III) or Fe(II) absorbs light. When the

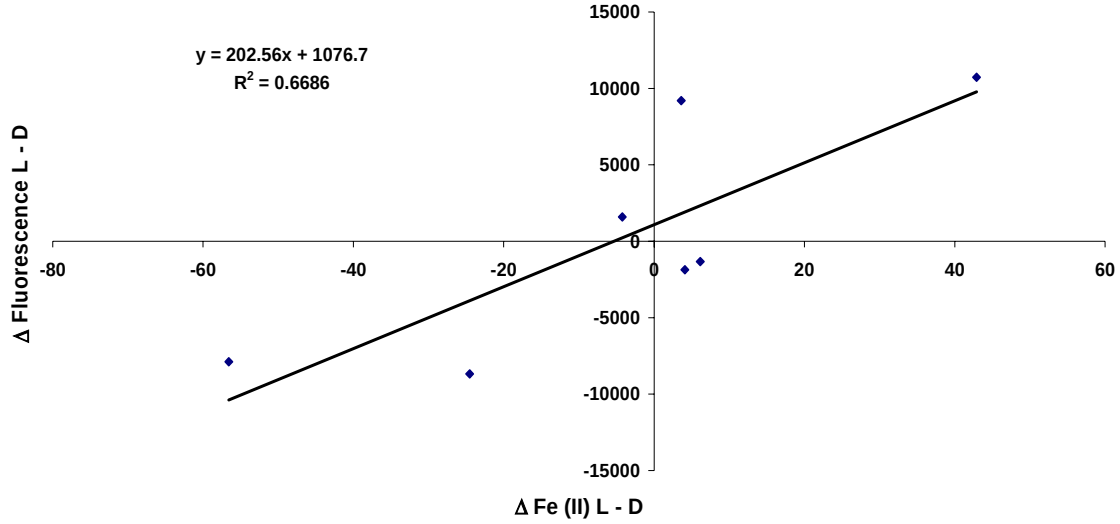


Figure 9. Authentic rain events (e496, e501, e521, e533, e555, e559) that were irradiated for 6 hours. Δ Fe(II) L – D and Δ Total Fluorescence L – D represent the difference between the initial and final Fe(II) concentration and total fluorescence in light exposed and dark controls.

organic is bound to Fe(III) redox chemistry occurs by ligand to metal charge transfer (LMCT) which leads to the photoreduced Fe(II)-organic compound. This increase observed in Figure 9 supports the theory of LMCT by absorption of light via a chromophoric organic ligand causing photoreduced Fe(II) species (Rijkenberg 2005). The predicted reaction that is occurring is seen in Figure 2 reaction (b) where the Fe(III)-CDOM complex undergoes photolysis to produce Fe(II) and CDOM.

In two of the six authentic rain samples Fe(II) and fluorescence concentrations go down upon irradiation. This suggests that sometimes Fe(II) is being oxidized to Fe(III) resulting in the reverse direction of reaction (b) Figure 2 . This trend was also noted by Kieber et al. (2003) and Faust (1994) where there was decrease in Fe(II) after irradiation. The point that had the largest Fe(II) and fluorescence decrease (-24, -8677) had no Fe(III) initially. When Fe(II) concentrations that were photochemically produced were compared with an equation that predicts the photoproduction of Fe(II) in rainwater from initial Fe speciation and H^+ values (Kieber et al. 2003):

$$\Delta [Fe(II)]_{nM} = 0.798 + 0.570[Fe(III)]_{nM} - 0.287[Fe(II)]_{nM} + 0.0565[H^+]_{\mu M} \quad \text{(Equation 1)}$$

there showed a significant correlation where $n = 6$, $p < 0.02$, and $r^2 = 0.7537$ (Figure 10).

In a separate experiment increasing amounts of Fe(II) was added to aliquots of an authentic rain event [0 to 500 nM Fe(II)] and irradiated for 6 hours. In this experiment all aliquots that had added Fe(II) showed an increase in Fe(II) concentration after irradiation with the exception of the authentic aliquot with no added Fe(II) which showed a small loss of Fe(II) due to irradiation (Figure 11). The fluorescence intensity decreased with this increase in Fe(II) production suggesting the decomposition of the Fe(II)-CDOM

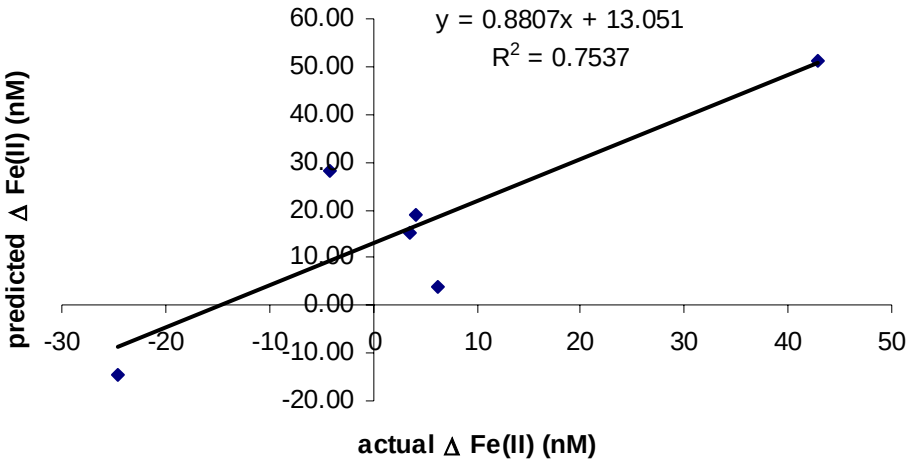


Figure 10. Comparison of actual Fe(II) photoproduction in rainwater vs. predicted Fe(II) (nM) using equation 1 where Δ Fe(II) is the initial - final: n = 6, r^2 = 0.7537, and p < 0.02.

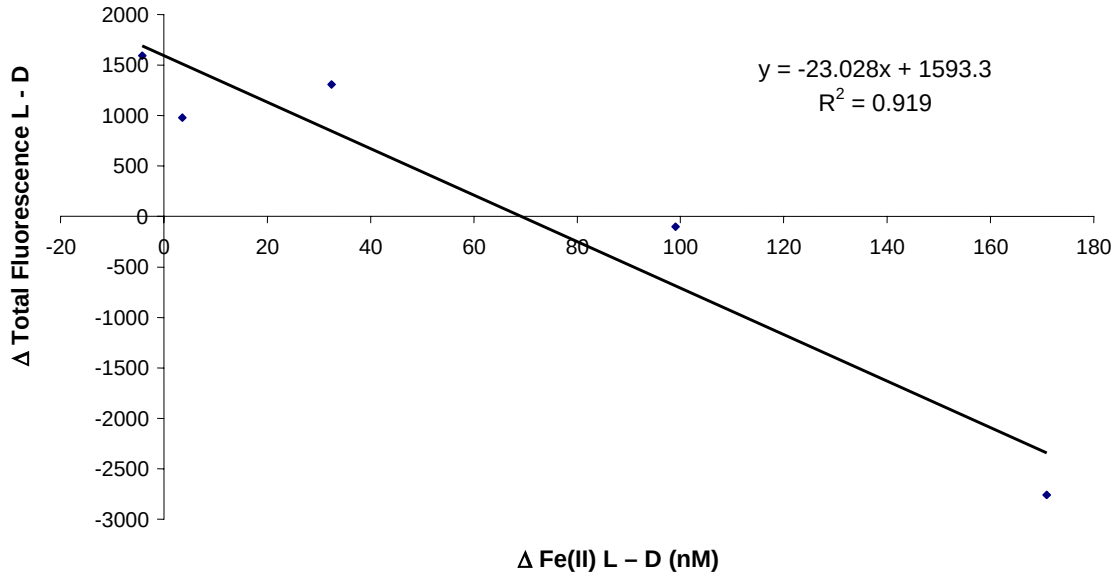


Figure 11. Rain event 521 was spiked with increasing amounts of Fe(II) (0-500 nM) prior to exposure to solar simulation. Iron(II) and total fluorescence concentration were measured initially then exposed to solar simulation for 6 hours. Δ Fe(II) L – D and Δ Total Fluorescence L – D represent the difference between the initial and final Fe(II) concentration and total fluorescence in light exposed and dark controls, n = 5, r^2 = 0.919, p , 0.01.

complex when Fe(II) is in excess. Equation 1 was used to see what the predicted the photoproduction of Fe(II). When plotted against the actual amount of Fe(II) produced (Figure 12) there was a strong correlation, $p < 0.01$ and $r^2 = 0.8753$. This decreasing fluorescence with increase in Fe(II) is the opposite to what was seen when authentic rain was irradiated and produced an increase in fluorescence. Rijkenberg (2005) suggest that the absorption of light by the organically bound Fe(II)-organic does not always undergo redox chemistry. In some cases the organic ligand will become excited and the Fe(II)-organic bond will break resulting in the individual compounds, Fe(II) and CDOM. This is demonstrated in the back reaction of Figure 2 reaction (c).

Rainwater

When authentic rainwater is irradiated there is a dynamic interconversion between Fe(II) and Fe(III) (Figure 13) where Fe(II) is produced upon irradiation after which it oxidizes back to or near its original concentration. Earlier studies have suggested that the behavior of Fe(II) in rainwater presented in Figure 13 is the result of an organic complex (Kieber et al. 2005). CDOM was extracted from 500 mL of two rainwater samples by a C_{18} cartridge and reconstituted in 150 mL SRW in order to determine if the Fe-CDOM complex could reproduce the behavior observed in authentic rain presented in Figure 13. The extracts from events 571 and 575 were spiked with 20 and 100 nM Fe(III) respectively after reconstitution. Samples were then put under solar simulation for 2 hours and stored in the dark for 22 hours at room temperature.

Though the starting concentration of Fe(II) were very different, both experiments showed a large increase in Fe(II) after irradiation (Figure 14) suggesting that Fe(III) photochemically reduces to Fe(II). When equation 1 is used to predict the amount of

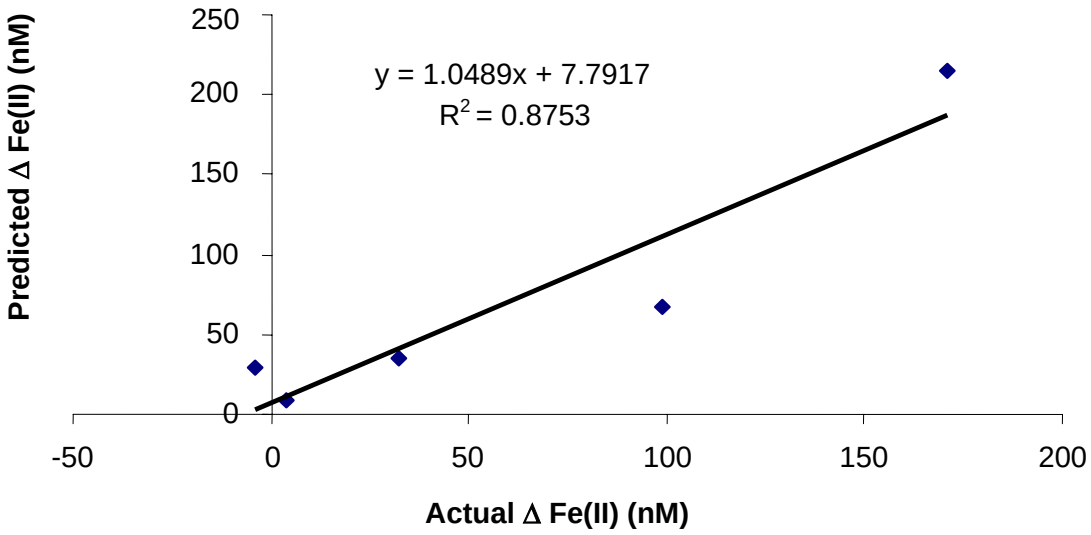


Figure 12. Comparison of actual Fe(II) photoproduced in rain event 521 versus the predicted Fe(II) (nM) when aliquots were spiked with Fe(II) 0 - 500 nM; the only point showing a loss of Fe(II) was the authentic event with no added Fe(II) all other aliquots showed photoproduction of Fe(II) where Δ Fe(II) is L-D: $n = 5$, $r^2 = 0.8753$, and $p < 0.01$.

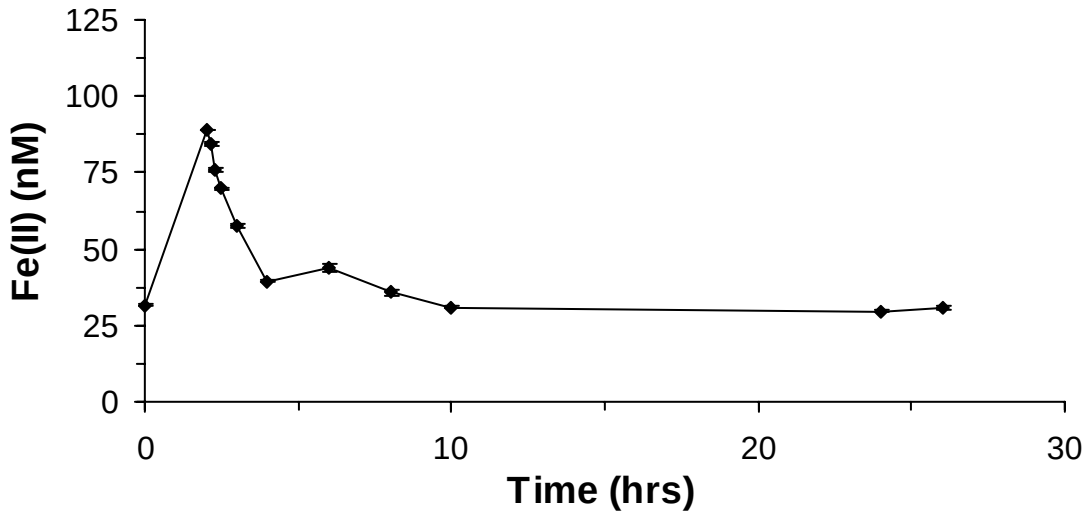
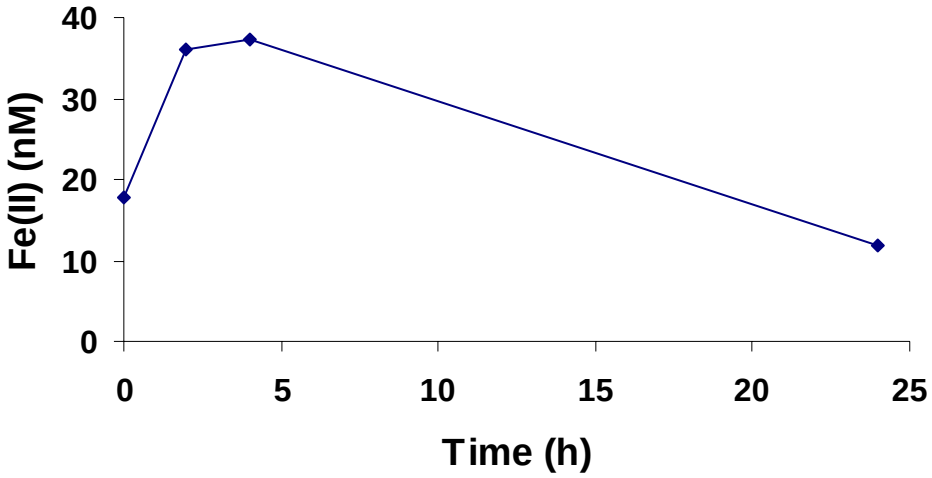


Figure 13. Concentration of Fe(II) (nM) as a function of time (hrs)in a rain sample (event 432) irradiated for 2 h (from time = 0 to time = 2 h) under simulated sunlight and then stored dark for the remainder of the experiment (from time = 2 to time = 26 h). Error bars represent ±1 standard deviation (n = 3) and are usually smaller then the symbol (Kieber et al. 2005).

(a)



(b)

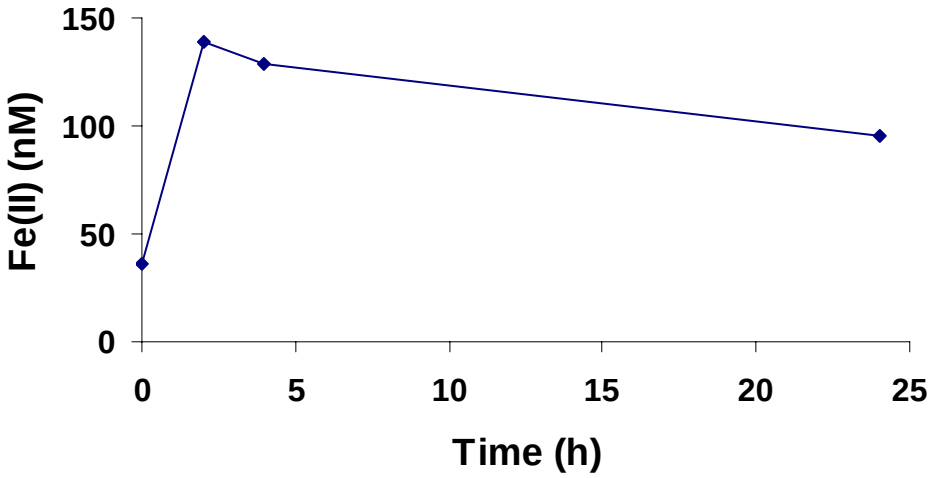


Figure 14. Concentration of Fe(II) (nM) as a function of time (h) in SRW with added CDOM from (a) event 571 and (b) event 575, irradiated for 2 h (from time = 0 to time = 2 h) under solar simulation and then stored in the dark for the remainder of the experiment (from time = 2 to time = 24 h) where each point represents one analysis.

Fe(II) that would be photochemically produced in rainwater it is remarkably close to the actual amount of Fe(II) produced under 2 hours of irradiation (Figure 15) of the reconstituted rainwater.

This suggests that the Fe(III) CDOM complex is capable of accurately mimicking the photochemical production of Fe(II) in rainwater. Once the irradiation ended and samples were stored in the dark Fe(II) slowly oxidized back to Fe(III) (Figure 14) similar to what was observed in the authentic rainwater (Figure 13). The photochemically produced Fe(II) in event 571 oxidized back to the original Fe(II) concentration after 24 hours. The oxidation rate of Fe(II) was 1.3 nM h^{-1}. The photoproduced Fe(II) in event 575 also oxidized but its final concentration was three times higher than initially. The oxidation rate in this sample was 1.7 nM h^{-1}. In both events the rates of Fe(II) oxidation were well with in the range of oxidation rate of photochemically produced Fe(II) in six authentic rain samples of 0.9 – 7 nM h^{-1} (Willey et al. 2005) suggesting that the rate of Fe(II) oxidation in the CDOM extract behaves similar to authentic rainwater.

The initial DOC concentrations in these two samples were remarkably different (e571 was 36 μM and e575 was 315 μM) suggesting that the amount of organic material available influences the stability of photochemically produced Fe(II). Kieber et al. (2005) hypothesized that there are two forms of Fe(II) occurring in rainwater, a labile Fe(II) produced photochemically that oxidized within hours and a much more stable form of complexed Fe(II) that is stable for days. The authors found that in authentic rainwater without excess Fe(II) ligand concentrations photoproduced Fe(II) oxidized back to Fe(III) rapidly with in the first 2 hours of dark storage with a final Fe(II) concentration that was

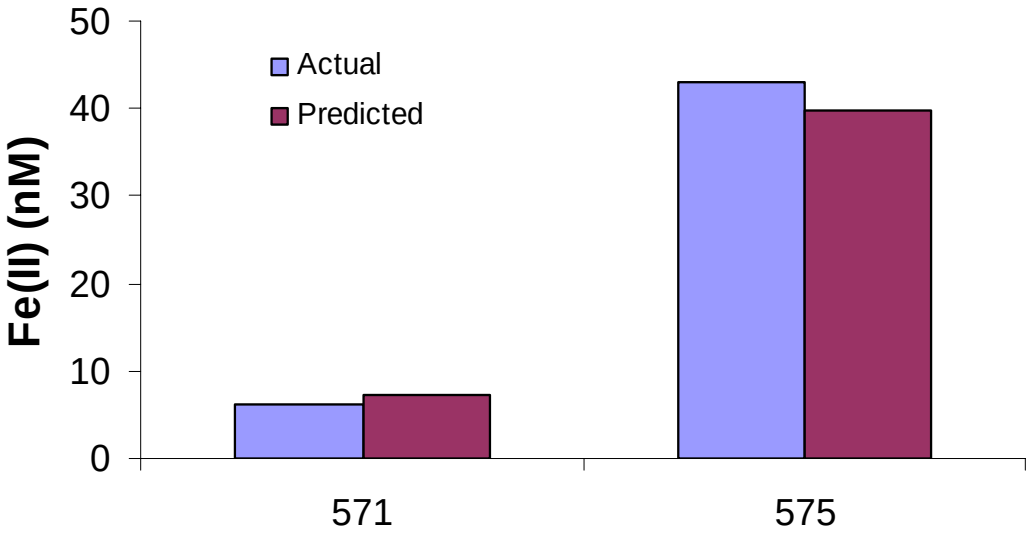


Figure 15. Photoproduced Fe(II) by irradiation for 2 hours of SRW with added CDOM showing both actual Δ Fe(II) and predicted Δ Fe(II) concentrations by equation 1 based on initial Fe(II), Fe(III) concentration and pH.

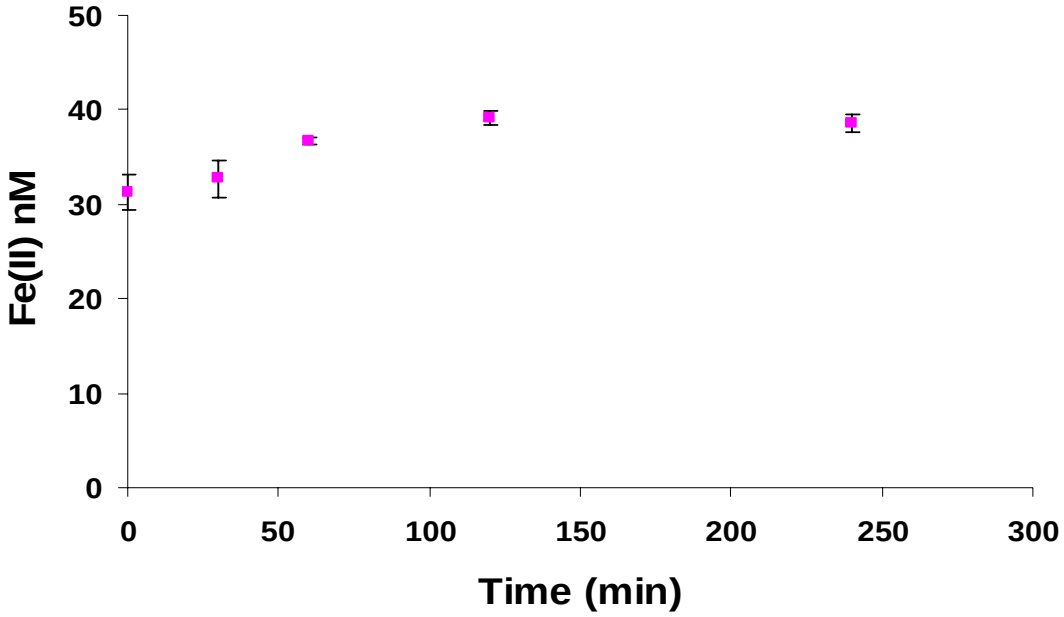
at or near the original concentration of Fe(II) (Kieber et al. 2005). The results of Figure14 suggest that the higher DOC extracted rainwater had excess Fe(II) CDOM ligands resulting in higher Fe(II) concentrations after storage similar to what was reported by Kieber et al. (2005).

The oxidation of Fe(II) in the presence of CDOM in SRW was also explored by adding spikes of ferrous iron and H_2O_2 (10 μM), a known Fe(II) oxidizing agent, to SRW containing CDOM from 2 rain events (Figure 16). In this case however the Fe(II) was added as an inorganic spike rather than generated photochemically as in Figure 14. In event 570 that had high initial extraction fluorescence (33,683) the concentration of Fe(II) increased slightly over the first 2 hours and became stable for the final two hours (Figure 16a). In contrast the Fe(II) in event 581 with a relatively lower initial extraction fluorescence (10,011) decreased over the first 2 hours but once again stabilized for the final 2 hours (Figure 16b) with an oxidation rate of 16.1 nM h^{-1}, suggesting the amount of CDOM available influences the stability of Fe(II). When Fe(II) was added to SRW containing no extracted CDOM the Fe(II) concentration decreased within the first 2 hours (Figure 17) much more rapidly at an oxidation rate of 20.7 nM h^{-1}. Kieber et al. (2005) found that Fe(II) in authentic rain oxidized much slower than inorganic Fe(II) in synthetic rain. The results of Figures 14-17 suggest that CDOM is capable of slowing or stopping the rate of Fe(II) oxidation in SRW in a manner analogous to authentic rainwater.

Rainwater and Seawater Mixing

The significance of atmospherically deposited iron to natural waters depends, to some degree, on the lifetime of the various oxidation states once they are deposited (Kieber et al. 2001). Kieber et al. (2001) analyzed ambient rainwater mixed with

(a)



(b)

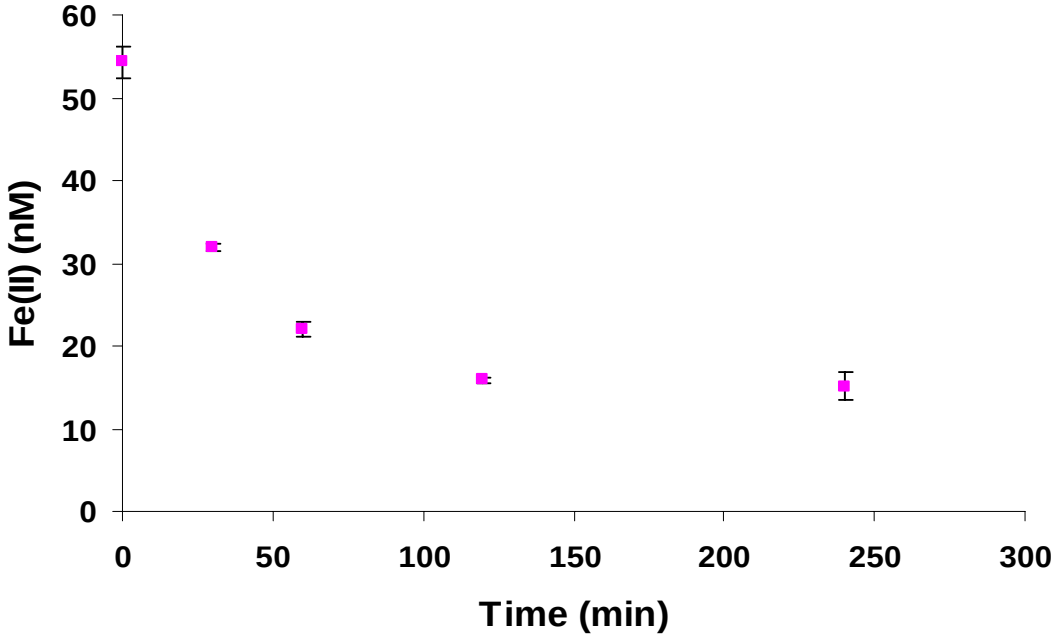


Figure 16. Concentration of Fe(II) (nM) as a function of time (min) in 150 mL SRW in the presence of CDOM extracted from 500 mL and 10μM H_2O_2 (a) event 570 (b) event 581. Error bars represent ±1 standard deviation based on n = 3.

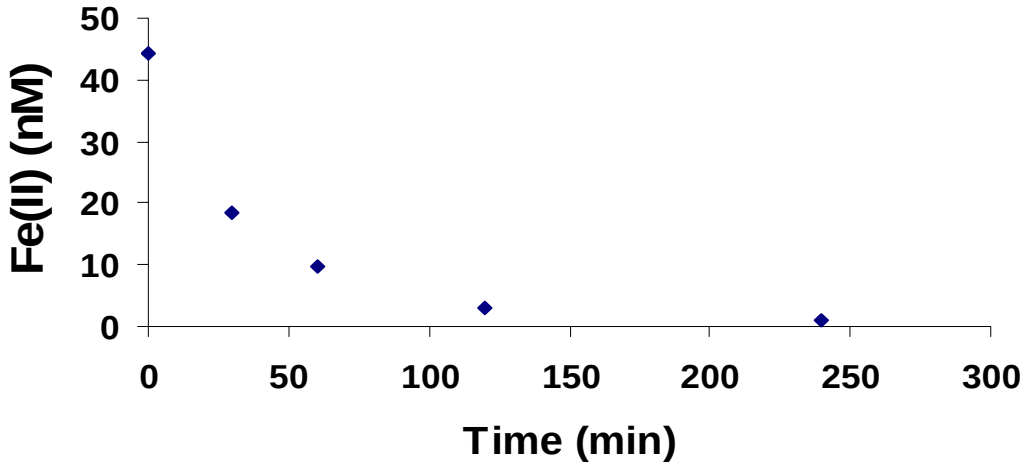
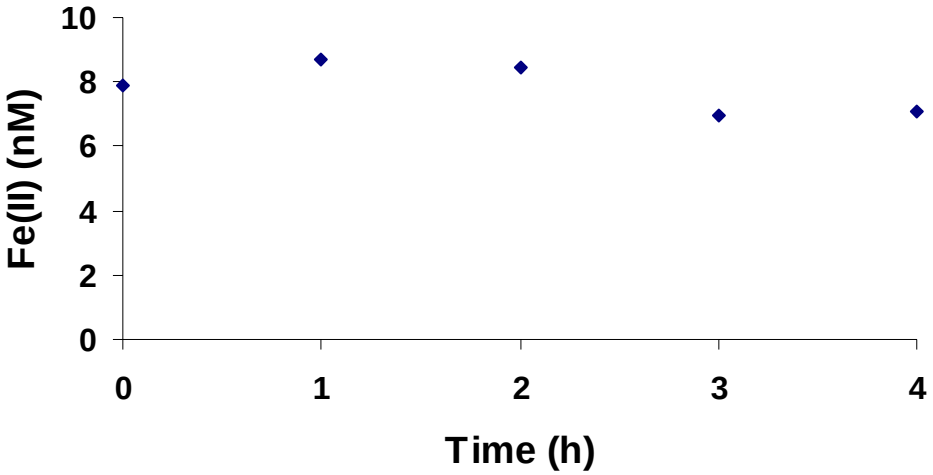


Figure 17. Concentration of Fe(II) (nM) as a function of time (min) in SRW with no added CDOM and 10 μM H_2O_2. Error bars represent ±1 standard deviation based on n = 3 and are usually smaller than the symbols.

seawater and monitored Fe speciation for a period of 4 hours to observe the stability of Fe(II) that is atmospherically deposited. Four hours was chosen because mixing would most likely occur in this duration of time. The authors hypothesized that the Fe(II) was stabilized in seawater because of an undefined organic complex. In order to determine if CDOM could be the stabilizing ligand of Fe(II) a similar set of experiments were done in which CDOM extracted from rain was brought back to volume by mixing equal volumes of SRW and Gulf Stream seawater (Figure 18) and Fe speciation was measured over 4 hours, where initial physical parameters of H_2O_2, pH, salinity, and Fe(II) concentrations are found in Table 4. Gulf Stream sea water was chosen because of its purity and high salinity. Samples were stored in the dark at room temperature and analyzed for Fe(II) as a function of time. Both samples were similar in their reconstituted pH and H_2O_2 concentration of 8.2 and 10 μM respectively while the added starting amount of Fe(II) in the two samples were slightly different, event 570 initial Fe(II) was 7.91 nM and event 589 initial Fe(II) was 19.1 nM.

Over the course of the 4 hours there was a slight loss of Fe(II) in both samples. The concentration of Fe(II) in event 570 showed a slight oxidation with a final Fe(II) concentration relatively similar to the starting amount, while event 589 Fe(II) concentration oxidized more over the 4 hours but became stable for the final 2 hours with a final Fe(II) concentration just over half the starting amount. This trend is similar to Figure 16 where the extracted rain was brought back to concentration with just SRW and stabilized for the final 2 hours of the 4 hour duration. Once again the stability of Fe(II) in high CDOM solution, as seen in Figures 18 and 16, is thought to be responsible for the stabilization of the Fe(II) in rainwater-seawater mixtures.

(a)



(b)

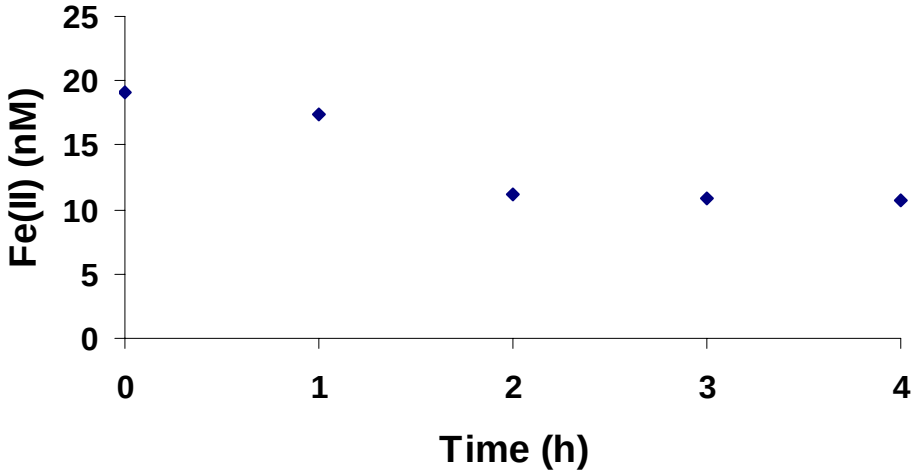


Figure 18. Concentration of Fe(II) (nM) as a function of time (h) in a 1:1 mixture of SRW and Gulf Coast seawater in the presence of extracted CDOM from (a) event 570 (b) event 589.

Table 4. Physical parameters (pH, H_2O_2, and salinity) of reconcentrated CDOM in a 1:1 mixture of SRW and Gulf Coast seawater before and after mixing for e570 and after reconcentration mixing e589.

	pH	H_2O_2 (μM)	Salinity (ppt)	$Fe(II)_{initial}$ (nM)
Seawater	8.22	0.03	33	0.06
e570 initial	4.48	8.45	0	10.89
e570 CDOM mix	8.205	10.7	15	7.91
e589 CDOM mix	8.225	9.67	15	19.05

In summary, the length of time that atmospherically deposited Fe remains dissolved in surface seawater is critical to its role as a phytoplankton nutrient because it is predominantly the soluble form of Fe which is bioavailable (Rich and Morel 1990). Iron(II) deposited via rainwater is protected from rapid oxidation after mixing with seawater. Theses studies show that Fe(II) is stable in the presence of extracted CDOM suggesting that the mechanism by which Fe(II) is stabilized is the CDOM ligand.