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Supporting Information

ABSTRACT: Fluorescence spectroscopy coupled with parallel factor analysis (PARAFAC) has been widely used to characterize dissolved organic matter (DOM). Characterization is based on the intensity and location of independent fluorescent components identified in models constructed from excitation–emission matrices (EEMs). Similar fluorescent components have been identified in PARAFAC studies across a wide range of systems; however, there is a lack of discussion regarding the consistency with which these similar components behave. The overall goal of this critical review is to compare results for PARAFAC studies published since the year 2000 which include one or more of three reoccurring humic-like components. Components are compared and characterized based on EEM location, characteristic ecosystems, and behavior in natural and engineered systems. This synthesis allows PARAFAC users to more confidently infer DOM characteristics based on identified components. Additionally, behavioral inconsistencies between similar components help elucidate DOM properties for which fluorescence spectroscopy with PARAFAC may be a weak predictive tool.

INTRODUCTION

There is an urgent need for methodologies that allow for frequent and informative monitoring of dissolved organic matter (DOM) due to its integral role in natural and engineered systems. In the environment, the variable concentration and composition of DOM have a substantial effect on the health of ecosystems through processes such as light attenuation,1,2 nutrient availability,3 and contaminant transport.4,5 By tracking DOM, one can better predict the impact DOM will have on natural processes. Fluctuations in DOM also help elucidate factors, such as land use and climate change, that cause variations in DOM and thus, the status of an ecosystem.6−8

DOM also presents challenges in engineered systems, as all water treatment processes are affected by DOM. DOM concentration and composition control coagulation efficiency, disinfection byproduct formation, membrane fouling, oxidant demand, microbial growth, and contaminant mobility.9−16 As a result, water treatment facilities require DOM monitoring tools to predict the influence and removal of DOM during treatment. Such methodologies allow utilities to modify their processes in response to fluctuations in raw water DOM and increase their ability to treat water effectively. The impact of DOM on water treatment and the need for DOM monitoring is expected to become more prevalent due to the combined effect of increasing DOM concentrations in surface waters and the pressing need to consider DOM-rich waters as alternative sources of drinking water.17,18

In accordance with the need to track DOM, fluorescence spectroscopy has shown great promise. Fluorescence data are presented in three-dimensional excitation–emission matrices (EEMs) in which fluorescence intensities are given as a function of excitation and emission wavelengths. EEMs for DOM characterization are commonly evaluated by visual inspection;19−21 however, multivariate data analysis techniques are increasingly used for quantitative comparison of EEMs. The most widely used technique, parallel factor analysis (PARAFAC), deconvolutes complex EEMs into independent fluorescent components which represent groups of similar fluorophores.22 Fluorescence spectroscopy combined with PARAFAC has been applied to a variety of ecosystems.23−26 Jaffe et al. (ref 26) determined that DOM concentration and composition vary widely, both temporally and spatially, and that changes in DOM composition could not be predicted with DOM concentration. Applications of fluorescence spectroscopy with PARAFAC are also evident in engineered systems. Fluorescence spectroscopy, both with and without PARAFAC, has been used as an indicator of water treatment efficiency and finished water quality.27−33

Despite the wide breadth of applications belonging to fluorescence spectroscopy and PARAFAC, considerable uncertainty still remains regarding the relevance of a component.

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Researchers often relate the location and shape of PARAFAC components to previously identified components in order to validate results, but there is a lack of discussion regarding how the characteristics of similar components compare across studies. An investigation of the ubiquity of certain PARAFAC components and the consistency with which these components behave is needed. The wealth of information in previously conducted PARAFAC has the potential to identify and answer key questions that would extend the usefulness of fluorescence spectroscopy and PARAFAC.

The overall goal of this Review is to evaluate PARAFAC results for a wide range of studies in order to better understand the chemistry of reoccurring fluorescent components. This was accomplished by comparing PARAFAC studies published since

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### Table 1. EEM Locations, Representative EEMs, and Spectral Loadings for Reoccurring PARAFAC Components; Excitation Curves in Spectral Loadings Are to the Left of Emission Curves (EEMs and Spectral Loadings Taken from Kowalczuk et al.51)

<table>
<thead>
<tr>
<th>Component Label (this study)</th>
<th>Approximate EEM location</th>
<th>EEM</th>
<th>Spectral Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ex: &lt;230-260 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Em: 400-500 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ex: &lt;240-275(339-420) nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Em: 434-520 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ex: &lt;240-260(295-380) nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Em: 374-450 nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Table 2. Traditional Peak Classifications of Components and EEM Location Information

<table>
<thead>
<tr>
<th>Component label</th>
<th>traditional classification</th>
<th>description based on EEM location</th>
</tr>
</thead>
</table>
| Component 1     | A (UVC humic-like)        | Absorbs light primarily in the UVC region  
Terrestrial UV light is lacking in UVC  
Expected to be photoresistant due to limited exposure to UVC light  
Short excitation wavelengths  
Expected to consist of small molecular size compounds |
| Component 2     | A + C (UVC humic-like + UVA humic-like) | Absorbs light in the UVC and UVA region  
UVA light constitutes the majority of terrestrial UV radiation  
Expected to be photodegraded by UVA light  
Long excitation and emission wavelengths  
Expected to consist of large molecular size, hydrophobic compounds |
| Component 3     | A + M (UVC humic-like + UVA marine humic-like) | Absorbs light in the UVC, UVB, and UVA region  
Absorption band extends less into the UVA light region than that of Component 2  
Expected to be photodegraded by UVA light, but to a lesser extent than Component 2  
Intermediate excitation and emission wavelengths  
Expected to consist of compounds with molecular sizes that fall between Component 1 and Component 2 |

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1 Excitation wavelengths given in parentheses represent secondary peaks.
Critical Review

the behavior of reoccurring components in a variety of settings

characterization, but rather to synthesize an incredibly diverse

making UVC light poorly distributed in aquatic ecosystems.34

light (290 nm or less) is sparse in terrestrial sunlight, thus

this component occurs in this region of light. However, UVC

(UVC) light, as the peak excitation (absorption) wavelength for

expected to be most easily photodegraded by ultraviolet C

wavelength ranges beginning around 250 nm. Component 1 is

partially represented in an EEM due to typical excitation

400 nm. Component 1 oftentimes appears to be only

assumed visually and given traditional classifications.19–21 This paper is not intended to review the applications of fluorescence spectroscopy for dissolved organic compound characterization, but rather to synthesize an incredibly diverse set of PARAFAC studies. This synthesis is intended to describe the behavior of reoccurring components in a variety of settings such that PARAFAC users can more confidently infer DOM composition based on identified components.

EEM LOCATIONS

This review article focuses on the behavior of three commonly occurring PARAFAC components. Each component is defined by a range of excitation and emission wavelengths that encompass the variation seen across studies. The three components, arbitrarily labeled 1, 2, and 3, are commonly characterized as humic- or fulvic-like. Table S1 in Supporting Information (SI) lists all the PARAFAC studies and corresponding components that were used in this review. Protein-like components were eliminated from this review because they are consistently well-correlated with pure proteins. Table 1 provides the approximate EEM location of each component and Table S2 in SI provides additional examples of component spectral loadings. Table 2 compares the three reoccurring PARAFAC components to their respective traditional classifications.

Component 1. Component 1 has an excitation peak around 260 nm or less with a broad emission band centered around 400–500 nm. Component 1 oftentimes appears to be only partially represented in an EEM due to typical excitation wavelength ranges beginning around 250 nm. Component 1 is expected to be most easily photodegraded by ultraviolet C (UVC) light, as the peak excitation (absorption) wavelength for this component occurs in this region of light. However, UVC light (290 nm or less) is sparse in terrestrial sunlight, thus making UVC light poorly distributed in aquatic ecosystems.34 As a result of limited exposure to UVC light, Component 1 is expected to be relatively resistant to photodegradation.35

Fluorophores responsible for Component 1 are expected to be relatively small based on short peak excitation wavelengths (<260 nm). Wu et al.36 using size exclusion chromatography, liquid chromatography, and EEMs, determined that molecular size tends to be positively associated with the length of peak excitation and emission wavelengths. Accordingly, Ohno et al.37 found that soil-derived DOM with the highest abundances of Component 1 also had the lowest molecular weight (average highest molecular weight peak ~665 Da).

Component 2. The primary excitation peak wavelength range of Component 2 is similar to that of Component 1; however, Component 2 also has a secondary excitation peak around 339–420 nm. This secondary peak is shown in the EEM and spectral loadings for Component 2 in Table 1, where a location of peak fluorescence is obvious, but at a lesser intensity than the primary peak. In a well-known study conducted by Cory and McKnight,25 PARAFAC components similar to Component 2 were characterized as reduced quinone-like and the spectral loadings of these components were compared to that of the model compound anthrahydroquinone-2,6-disulfonate (AHDS). Cory and McKnight’s successful comparison of PARAFAC components to model compounds is notable, as most PARAFAC components are only compared to previously indentified components. The locations of the primary and secondary excitation peaks indicate that Component 2 is expected to absorb light in the UVC and UVA (wavelengths greater than 320 nm6) regions. UVC light is not likely to cause photodegradation of Component 2 due to the lack of UVC light in the water column, as is the case for Component 1. The majority of UV light (~94%) that reaches the earth’s surface is UVA light, thus leaving Component 2 susceptible to photodegradation.34

The long peak excitation and emission wavelengths belonging to Component 2, 339–420 nm and 434–520 nm, respectively, indicate that this component consists of large molecular size, hydrophobic compounds.36 When studying DOM derived from plant and animal manure, Hunt and Ohno38 found that Component 2 was significantly and positively associated with apparent molecular weight. For example, Component 2 was one of the three most intense components in 9 out of the 13 DOM types with apparent molecular weights greater than 1000 Da. Cook et al.39 found that Component 2 was more dominant in the hydrophobic acid fraction of DOM from the Atchafalaya Basin, Louisiana, USA, than the transphilic acid fraction.

Component 3. Component 3 has a primary and secondary excitation peak, occurring around 260 nm or less and 295–380 nm, respectively, and a single emission peak around 374–450 nm.

Table 3. Summary of Component Occurrence in Different Aquatic Systems

<table>
<thead>
<tr>
<th>ecosystem</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>terrestrial</td>
<td>dominant source, e.g., forested regions, wetlands, thermokarsts</td>
<td>potential source</td>
<td>widespread and highly variable</td>
</tr>
<tr>
<td>microbial</td>
<td>NA</td>
<td>nonlinear negative association with salinity</td>
<td>widespread and highly variable</td>
</tr>
<tr>
<td>freshwater</td>
<td>negatively correlated with salinity from freshwater to seawater</td>
<td>freshwater to seawater variation affected by internal production and degradation</td>
<td>nonlinear negative association with salinity</td>
</tr>
<tr>
<td>marine</td>
<td>conservative representative of terrestrially derived humic acids in ocean water</td>
<td>evidence of marine production</td>
<td>evidence of marine production</td>
</tr>
<tr>
<td>photic zone</td>
<td>positively associated with exposure to sunlight</td>
<td>negatively associated with exposure to sunlight</td>
<td>negatively associated with sunlight, but more resistant to photodegradation than Component 2</td>
</tr>
</tbody>
</table>

highest concentrations near water surface | highest concentrations reported below photic zone or in groundwater | | |
nm. PARAFAC components similar to Component 3 have been characterized as oxidized quinone-like and compared to the model compound anthraquinone-2,6-disulphonate (AQDS), which has a blue-shifted emission peak compared to AHDS.\textsuperscript{53} This component is characterized by absorption of UVC and UVB light, most of which is attenuated prior to distribution in the water column, and UVA light. Component 3 is expected to be photodegraded by UVA light, but to a lesser extent than Component 2 due to an excitation band that extends less into the UVA region. Compared to Component 2, Component 3 is expected to consist of compounds that are less hydrophobic and smaller in molecular size, as indicated by the shorter peak excitation and emission wavelengths.\textsuperscript{36} In agreement, Cook et al.\textsuperscript{39} found that the transphilic acid fraction of Atchafalaya Basin DOM was dominated by Component 3, whereas the hydrophobic acid fraction was dominated by Component 2.

\section*{CHARACTERISTIC ECOSYSTEMS}

This section discusses the ecosystems in which each of the components are commonly found, including freshwater to seawater transects, surface water, groundwater, deep ocean water, springs, rivers, and soils. A summary of component occurrence in different ecosystems is provided in Table 3.

\textbf{Component 1.} Component 1 is most commonly abundant in DOM dominated by terrestrial precursor material, such as DOM from soil extractions, soil solution, forested streams, wetlands, thermokarsts, and tree leaves, especially during warmer months.\textsuperscript{23,35,40−44} Stedmon and Markager (ref 35) found that Component 1 was generally absent from wastewater and only present at low levels after storm events, during which terrestrial DOM was likely flushed into the wastewater collection system. Lapierre and Frenette (ref 45) found that Component 1 in Lake Saint-Pierre, located in Quebec, Canada, was mostly a result of terrestrial inputs, and also that Component 1 contributed minimal amounts to the fluorescent DOM leached from submerged and emergent macrophytes. Rainfall generally results in an increase in Component 1, likely due to increased terrestrial inputs from runoff.\textsuperscript{35,46} Another study, however, conducted by Luciani et al. (ref 47), found that Component 1 was dominant in samples taken from the open Sepetiba Bay in Brazil and nearly absent in areas of the bay that were heavily influenced by riverine inputs.

In accordance with sources of Component 1 being predominantly terrestrial, several studies have found that Component 1 is negatively correlated with salinity. This linear relationship indicates conservative mixing of Component 1 as aquatic systems transition from freshwater to seawater.\textsuperscript{42,48−53} Kowalczuk et al. (ref 51) deemed Component 1 "the most important representative of humic acids in the oceanic water." Osburn and Stedmon (ref 48) identified Component 1 as having the strongest positive correlation with dissolved lignin concentrations along the mixing zone between the Baltic Sea and North Sea. Conservative mixing of Component 1 suggests that contributing fluorophores are largely resistant to internal processes of degradation and production along the freshwater to seawater continuum.

Additionally, Component 1 in fluorescent DOM appears to be associated with exposure to sunlight, thus making the photic zone and shallow surface waters characteristic ecosystems of Component 1. In a study conducted by Yamashita et al. (ref 50), fluorescence intensities for Component 1 were highest at the surface of the Ise Bay in Japan and decreased with depth. Fulton et al. (ref 54) found that the relative abundance of Component 1 in an ice-covered Antarctic lake decreased from 30–40% in surface waters to 10–20% in bottomwaters. When comparing groundwater to surface water in the Florida coastal Everglades, two systems with significantly different light availability, Component 1 was sparse in groundwater and more abundant in surface waters.\textsuperscript{58} Additionally, Balcarczyk et al. (ref 42) found that thermokarst water samples had a higher proportion of Component 1 than did stream and spring samples from a watershed in interior Alaska, and noted the increased exposure to sunlight in thermokarst systems due to consistently shallow waters and a higher incidence of irradiation.

\textbf{Component 2.} Component 2 has been identified in DOM from a wide variety of aquatic systems, including those dominated by terrestrial and microbial inputs. For example, Stedmon and Markager (ref 35) identified Component 2 in DOM from a forest stream, an agricultural stream, wastewater, and seawater. Ohno and Bro (ref 40) found that Component 2 was dominant in DOM extracted from soils, wetland plants, manures, tree leaves, and crops. Lapierre and Frenette (ref 45), however, showed that Component 2 was sparse in DOM leached from submerged and emergent macrophytes. As a result of the widespread identification of Component 2, Williams et al. (ref 7) found that this component relates poorly to land use.

Similar to Component 1, several studies have established an overall negative relationship between Component 2 and salinity at the freshwater−seawater interface, thus suggesting the dominance of terrestrial sources of Component 2.\textsuperscript{47,49−53,56−60} However, the distribution of Component 2 cannot be explained by simple conservative mixing. Markager and Stedmon (ref 49) found that variation in Component 2 in the Horsens Estuary, Denmark, was affected by internal processes of degradation or removal, thus falling below the theoretical conservative mixing line, as did Fellman et al. (ref 53) in three estuaries in coastal Alaska. Yamashita et al. (ref 50) found that the data fell above the conservative mixing line, indicating off-shore sources of Component 2 in the Ise Bay, Japan. Murphy et al. (ref 56) found that the fluorescence intensity of Component 2 increased within a short distance offshore in the Pacific Ocean and Atlantic Ocean and then decreased to baseline levels in open ocean. These findings suggest that Component 2 fluorophores may be intermediate products and not just a result of terrestrial inputs, as discussed in a later section. In fact, Osburn and Stedmon (ref 48) identified Component 2 as having the worst correlation with dissolved lignin content out of all the identified humic-like components.

Characteristic ecosystems of Component 2 also include those that experience minimal exposure to sunlight. Stedmon and Markager (ref 35) found that Component 2 covaried with Component 1 in times and locations of low irradiation; however, when subject to high levels of sunlight, Component 1 would dominate Component 2. Balcarczyk et al. (ref 42) found that the relative abundances of Component 2 were greater in streams and springs than in thermokarsts and Chen et al. (ref 55) found that groundwater was more enriched with Component 2 than surface water in the Florida Everglades. Gueguen et al. (ref 61) recorded the lowest intensities of Component 2 from 0 to 10 m below the water surface and the highest intensities at 30–200 m below surface. In the deep ocean waters, Yamashita et al. and Jorgensen et al. (refs 62 and 58) reported the highest fluorescence intensities for Compo-
Table 4. Summary of Component Behavior during Physiochemical Processes in Natural Systems

<table>
<thead>
<tr>
<th>process</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixing</td>
<td>conservatively mixed at freshwater–seawater interface</td>
<td>negatively associated with salinity, but not linearly</td>
<td>negatively associated with salinity, but not linearly</td>
</tr>
<tr>
<td>biodegradation and production</td>
<td>biologically unavailable</td>
<td>biologically degraded and produced</td>
<td>biologically degraded and produced</td>
</tr>
<tr>
<td>photochemical degradation and production</td>
<td>product of photodegradation</td>
<td>subject to photodegradation</td>
<td>subject to photodegradation</td>
</tr>
<tr>
<td>sorption</td>
<td>constant intensities with distance downstream</td>
<td>photochemically produced from terrestrial precursor material</td>
<td>no evidence of photochemical production</td>
</tr>
<tr>
<td>metal complexation</td>
<td>weakly and similarly quenched by Hg and Cu</td>
<td>similarly quenched by Hg and Cu</td>
<td>greater quenching by Cu than Hg</td>
</tr>
<tr>
<td></td>
<td>greater Cu quenching in freshwater than brackish water</td>
<td>greater quenching by Hg in freshwater than brackish water</td>
<td>greater quenching by Cu and Hg in freshwater than brackish water</td>
</tr>
<tr>
<td></td>
<td>slightly enhanced by Al</td>
<td>moderately enhanced by Al</td>
<td>inconsistent behavior with Al (quenching and enhancement)</td>
</tr>
<tr>
<td></td>
<td>slightly quenched by Fe</td>
<td>moderately quenched by Fe and positively, linearly associated with Fe solubility</td>
<td>low complexation capacity for Fe</td>
</tr>
</tbody>
</table>

Component 3. Components in previously conducted PARAFAC studies that fall within the range of Component 3 are widespread and there is considerable variability in the reported occurrence and behavior of these similar components. Studies suggest that Component 3 fluorophores are similar to those with terrestrial and marine precursors. 

Component 3 models identified by Chen et al. (ref 55) to describe DOM in the Florida coastal Everglades included two components similar to Component 3, one of which was sparse in groundwater, while the other was prevalent in groundwater. Two components identified in a PARAFAC model used by Fellman et al. (ref 53) were also within the range of Component 3, one of which increased with salinity until a middle range between freshwater and seawater and then sharply decreased with salinity thereafter, whereas the other gradually decreased with salinity around the theoretical conservative mixing line. In a study conducted in Ontario, Canada, two components, both deemed Component 3 in this review, were actually inversely related. One of the components was most abundant in wetland areas with low bacterial production rates, while the other component was positively associated with bacterial production rates and agricultural land use. In contrast, two Component 3 components identified by Lapierre and Frenette (ref 45) behaved similarly; both components were strongly correlated with dissolved organic carbon (DOC) and terrestrial sources of DOM, as well as more abundant in DOM from natural lake water, followed by DOM leached from emergent and submerged macrophytes, respectively.

Fulton et al. (ref 54) found that the combined contribution of Components 2 and 3 increased from ~12% in the surface waters to ~40% at 18 m deep in Lake Fryxell, Antarctica. When comparing Component 2 to Component 3, Yamashita et al. (ref 62) reported that the ratio of Component 2 to Component 3 was ~1.0 near the sea surface and increased with depth until a final ratio of ~1.5. These results indicate that although Component 2 is more abundant than Component 3 at depth, Component 2 is more quickly depleted than Component 3 near the sea surface, thus suggesting that Component 3 tends to be more resistant to degradation by sunlight. Gueguen et al. (ref 61) also found lower ratios of Component 2 to Component 3 at the water surface compared to deeper in the water column. Additionally, Jørgensen et al. (ref 58) found that fluorescence intensities of Component 3 increased with depth for a shorter distance than Component 2. Further evidence of component photoreactivity is discussed in a later section.

Physiochemical Processes in Natural Systems

This section discusses the role of reoccurring PARAFAC components in physiochemical processes in natural systems, including biological and photochemical degradation and production, sorption, and metal complexation. Table 4 provides a summary of component behavior during these processes.

2010

Critical Review

Component 1. Several studies have shown that the biodegradability of Component 1 is minimal. Fellman et al. (ref 41) evaluated DOM from bog, forested wetland, fen, and upland forest soil solution samples collected near Juneau, Alaska. It was concluded that the contribution of Component 1 to overall fluorescence was significantly and negatively correlated with soil solution BDOC. Similarly, thermokarst DOM had the highest proportion of Component 1 and was also the most resistant to biodegradation.\(^ {42}\) Hunt and Ohno (ref 38) concluded that Component 1 intensities in DOM derived from plant biomass only increased by \( \sim 5\% \) following decomposition and that there was no significant difference in DOM derived from animal manure after decomposition.

With regard to photoinduced processes, Component 1 appears to be a product of photodegradation that is resistant to further photodegradation, as shown by its positive association with sunlight in the environment and its reaction in controlled photoreactivity experiments. Stedmon et al. (ref 66) found that fluorescence intensities of Component 1 increased by 34\% to 2740\% during UVA incubations of marine DOM. In addition to concluding that Component 1 compounds are photochemically produced, Stedmon and co-workers hypothesized that these photoproducts may be solely derived from terrestrial precursor material. This conclusion was based on the fact that Component 1 had not been identified in a previous study which investigated the production and fate of DOM with only marine origins, despite the use of similar experimental and analytical methods.\(^ {25}\)

In a study of in-stream DOM uptake, Fellman et al. (ref 67) found that Component 1 intensities remained relatively constant with distance downstream, even when the headwaters were spiked with different types of DOM. These findings, in combination with previously discussed conservative mixing, suggest that Component 1 is resistant to in-stream removal processes. When using fluorescence quenching titrations to investigate complexation of copper and mercury with DOM, Yamashita and Jaffe (ref 68) found that the fraction of Component 1 binding fluorophores was relatively low compared to the other PARAFAC components. Copper and mercury binding fluorophores accounted for 13\%–42\% and 22\%–33\% of Component 1 fluorescence, respectively, whereas the binding fluorophores pertaining to the other components ranged from 32\% to 54\% for copper and 16\%–42\% for mercury. Component 1 fluorescence was slightly more quenched by copper than mercury, and copper induced quenching was approximately 3 times greater in freshwater samples than brackish water samples. The difference between freshwater and brackish water results may indicate that Component 1 compounds preferentially bind with constituents found in brackish water, e.g., calcium and magnesium, over copper. Mounier et al. (ref 69) also found that Component 1 is quenched by copper, but to a lesser extent than Component 2. In a study of soil derived DOM from coniferous and deciduous forests, Component 1 fluorescence intensity was again found to be the least affected by metal addition; Component 1 was slightly quenched by iron and enhanced by aluminum.\(^ {70}\) The lack of Component 1 complexation with metals as indicated by a change in fluorescence may be indicative of Component 1 compounds having low acidity.

Component 2. Hunt and Ohno (ref 38) found higher intensities of Component 2 in 8 out of 10 DOM types derived from plant biomass, as well as DOM from poultry and swine manure, following decomposition. In DOM derived from dairy manure, decomposition reduced the intensity of Component 2. These results show that in the majority of DOM tested, Component 2 consisted of compounds that were biologically produced. In a study conducted by Zhang et al. (ref 65), Component 2 intensities in DOM from a eutrophic lake increased and then returned to initial values during a 9 day degradation experiment, suggesting that Component 2 compounds were first dominated by biological production and then later dominated by degradation. Beggs and Summers (ref 71) studied the effects of biological treatment on PARAFAC components in DOM from pine needle leachate and found that biological treatment reduced the intensities of Component 2, as well as all other components, but increased the percent contribution of Component 2 to total fluorescence. Borisover et al. (ref 63) suggested biological production of Component 2 in Lake Kinneret with a negative linear correlation between Component 2 and the dissolved oxygen concentration during periods of thermal stratification.

In marine environments, Yamashita et al. (ref 62) concluded that Component 2 was linearly correlated with apparent oxygen utilization from the ocean surface to great depths, thus indicating in situ production of Component 2 in the open ocean. Jorgensen et al. (ref 58) also found a linear correlation between Component 2 and apparent oxygen utilization in most marine environments. An exception was found for samples taken from the North Atlantic; it was assumed that this discrepancy was due to additional terrestrial inputs. Jorgensen and co-workers hypothesized that microbial origins of Component 2 were indirect, i.e., microbial production of Component 2 precursor material followed by extracellular formation of Component 2 compounds. This hypothesis was due to the assumed large molecular weight of Component 2 compounds compared to the molecular weight cutoff of bacterial membranes. Further evidence of biological production of Component 2 was found by Stedmon et al. (ref 72). On average, \(~ 15\% \) of Component 2 fluorescence in Antarctic sea ice was generated internally; this was determined by comparing Component 2 fluorescence in sea ice to the expected value based on conservative behavior during freezing.

The UVA absorption band associated with Component 2, the abundance of UVA wavelengths in terrestrial sunlight, and an increase in Component 2 intensities with depth in the ocean indicate that Component 2 compounds are likely subject to photodegradation (Sections 2 and 3).\(^ {58,62}\) Stedmon et al. (ref 66) suggested that Component 2 consists of intermediate compounds that are susceptible to photochemical degradation, but are also produced by photochemical degradation of terrestrial DOM based on results for UVA incubations of marine DOM samples. A study conducted by Banaitis et al. (ref 73) shows that Component 2 has a strong tendency to sorb onto gibbsite and goethite, as indicated by a reduction in Component 2 fluorescence intensity after contact with the minerals. Banaitis et al. (ref 73) also showed that, although Component 2 fluorescence was consistently reduced by exposure to gibbsite and goethite, the extent of the reduction was dependent on the DOM source. Simon et al. (ref 74) also implied that Component 2 has a strong tendency to sorb onto sediments in a study of karst basins. Component 2 was strongly present in soil extracts, but not in the water that had percolated through the soils, indicating that Component 2 sorbed onto soil particles instead of traveling with the water until the end of the flow path.

Inactive Review

Table 5. Summary of Component Removal and Transformation in Water and Wastewater Treatment Plants

<table>
<thead>
<tr>
<th>process</th>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>reservoir storage</td>
<td>no data</td>
<td>moderate reduction in fluorescence intensity</td>
<td>unaffected</td>
</tr>
<tr>
<td>coagulation</td>
<td>minimal removal expected</td>
<td>preferential removal</td>
<td>preferential removal</td>
</tr>
<tr>
<td>sand filtration</td>
<td>no data</td>
<td>unaffected</td>
<td>unaffected</td>
</tr>
<tr>
<td>ozonation</td>
<td>no data</td>
<td>large reduction in fluorescence intensity</td>
<td>large reduction in fluorescence intensity</td>
</tr>
<tr>
<td>softening</td>
<td>no data</td>
<td>unaffected</td>
<td>unaffected</td>
</tr>
<tr>
<td>BAC filtration</td>
<td>no data</td>
<td>large reduction in fluorescence intensity</td>
<td>large reduction in fluorescence intensity</td>
</tr>
<tr>
<td>ultraviolet disinfection</td>
<td>no data</td>
<td>variable results</td>
<td>variable results</td>
</tr>
<tr>
<td>chlorination</td>
<td>no data</td>
<td>moderate reduction in fluorescence intensity</td>
<td>initial reduction in fluorescence intensity</td>
</tr>
<tr>
<td>finished water</td>
<td>no data</td>
<td>dominant in residual DOM</td>
<td>dominant in residual DOM</td>
</tr>
</tbody>
</table>

Component 2 likely complexes with copper, as indicated by fluorescence quenching; however, Yamashita and Jaffe (ref 68) saw similar quenching by copper in Components 1 and 2, whereas Mounier (ref 69) found that copper more preferentially quenched Component 2 than Component 1. In the study by Yamashita and Jaffe (ref 68), Component 2 was similarly quenched by copper and mercury, with the fraction of fluorophores that bind with these metals ranging from 27 to 42%. Mercury induced quenching was higher in freshwater than brackish water; however, copper induced quenching was similar in freshwater and brackish water samples. These results suggest that Component 2 may preferentially complex with copper, followed by ions found in brackish water, and then mercury. Component 2 is also expected to consist of important ligands for iron. Ohno et al. (ref 70) found that Component 2 was quenched by the addition of iron to a greater extent than Component 1 and Yamashita et al. (ref 62) found a positive correlation between Component 2 and iron solubility in the mesopelagic layer of the North Pacific Ocean. Evidence of metal complexation with Component 2 as indicated by fluorescence quenching suggests the increased acidity of Component 2 relative to Component 1.

Component 3. Hunt and Ohno (ref 38) found that Component 3 had relatively equal intensities in fresh and decomposed DOM derived from plant biomass and dairy manure, but higher intensities in decomposed DOM than fresh DOM from poultry and swine manure. Zhang et al. (ref 65) found that degradation of phytoplankton caused an increase in Component 3 intensity followed by a slight reduction, therefore resulting in an overall factor of increase of 2.2. The percent contribution of Component 3 to total fluorescence also increased after degradation. These results suggest that during the 9 day experiment, Component 3 was biologically produced and then relatively resistant to further biodegradation. Beggs and Summers (ref 71) also found that the percent contribution of Component 3 increased as a result of biodegradation; the extent of the increase was similar to that of Component 2. Singh and Swenson (ref 75) found that high intensities of Component 3 were related to high chlorophyll a concentrations, a water quality parameter often used as a proxy for biological activity. As a result of this relationship, Component 3 was often dominant in samples taken from agricultural catchments. In a study of Lake Kinneti, Borisover et al. (ref 63) made note of the negative linear correlation between Component 3 and the dissolved oxygen concentration during periods of thermal stratification; however, the correlation was stronger for Component 2.

Yamashita et al. (ref 62) concluded that Component 3 is indicative of recent biological production due to a linear correlation between Component 3 and apparent oxygen demand in bathypelagic layer ocean samples; the nonlinear relationship in the mesopelagic layer was attributed to higher levels of preformed Component 3 compounds in this layer from nonmarine sources. Jorgensen et al. (ref 58) found that, although Components 2 and 3 are both linearly related with apparent oxygen demand, Component 3 increased 1.3 times slower than Component 2 with oxygen utilization. In Antarctic sea ice, Component 3 intensities were largely due to the apparent oxygen demand in the original solute, not internal production.72 Regarding photochemical production and degradation, Stedmon et al. (ref 66) found that UVA incubations of marine DOM resulted in a reduction in Component 3 fluorescence. Additionally, as discussed in Sections 2 and 3, absorption bands and reported ratios of Component 2 to 3 with depth in the water column suggest that Component 3 is less vulnerable to photodegradation than Component 2.58,61,62

Regarding sorption and complexation, Guo et al. (ref 60) hypothesized that reduced Component 3 intensities at the convergence of two brooks in southeastern China were due to Component 3 sorption onto sediments. The convergence of the water bodies resulted in a turbid mixing zone, possibly affecting the fate of fluorescent compounds. Simon et al. (ref 74), however, found that Component 3 in karst basins experienced little processing along the cave streamflow path, as indicated by Component 3 being transmitted for long distances underground with limited change. Banakis et al. (ref 73) concluded that Component 3 in several types of DOM did show sorption tendencies in the presence of gibbsite and goethite, but that overall reductions in fluorescence were less than those seen for Component 2. Fluorescence quenching.

results collected by Yamashita and Jaffe (ref 68) suggested that copper complexation with Component 3 was greater than that of other components (41–54% binding fluorophores versus 13–42%), whereas mercury complexation with Component 3 was relatively intermediate. Copper and mercury complexation with Component 3 was higher in freshwater samples than brackish water samples, indicating the competitive advantage of brackish water ions for complexation sites on Component 3 constituents. Ohno et al. (ref 70) saw that aluminum had variable effects on Component 3 fluorescence depending on the source of the DOM and that Component 3 had the lowest complexation capacity for iron out of the three components discussed in this review. In agreement, Yamashita et al. (ref 62) noted that Component 3 was not related to iron solubility in the Okhotsk Sea or the northwestern North Pacific Ocean.

In general, it is important to recall the variability of Component 3. Although the above-mentioned consistencies across studies are notable, there are several contradictory results for Component 3, even within one PARAFAC model.73,35 As discussed in Section 3, the occurrence of Component 3 is widespread and its associated behavior appears to be equally variable.

## TREATABILITY IN ENGINEERED SYSTEMS

This section discusses the effect of common water treatment processes on DOM fluorescence. The majority of PARAFAC research concerns the natural environment and far fewer studies have monitored PARAFAC components throughout engineered systems. As a result, studies involving traditional fluorescent peak classifications are included in this discussion (see Table 2). Briefly, peak A is indicative of Component 1 if peak A behaves independently of other peaks. Co-variation of peak A with peak C suggests the presence of Component 2 and co-variation of peak A with peak M suggests the presence of Component 3. A summary of component treatability in engineered systems is provided in Table 5.

**Component 1.** Literature concerning the treatability of Component 1 in engineered systems is markedly limited. In a study of surface water DOM, Baker et al. (ref 76) found significant correlations between fluorescence intensities and functional properties of DOM that were unique to peak A. The fluorescence intensity of peak A was significantly and positively correlated with hydrophilicity, as well as significantly and negatively correlated with benz[a]pyrene binding and alumina adsorption. Although these functional assays do not directly represent treatment processes, the results suggest peak A, and thus Component 1, tendencies that would undoubtedly affect its treatability in engineered systems. The above-mentioned correlations for peak A indicate that Component 1 compounds are expected to be relatively resistant to coagulation, adsorption, and filtration processes.

**Component 2.** The following treatment processes are expected to reduce the fluorescence intensity of Component 2: storage in a surface water reservoir, coagulation, ozonation, biologically activated carbon (BAC) filtration, membrane filtration, UV disinfection, and chlorination.28,29,33,71 The reduction in Component 2 fluorescence intensity during reservoir storage may be attributed to physical, biological, or photochemical reactions. Regarding coagulation, studies have shown preferential removal of UV (254 nm) absorbing DOM; although all three components absorb light in this region, Baghoth et al. (ref 29) found that Component 2 was preferentially removed relative to the other components. Following coagulation, Beggs et al. (ref 71) saw Component 2 decrease by a factor of 2, and Gone et al. (ref 77) saw preferential and equal removal of peaks A and C. The reduction in Component 2 by coagulation agrees with research by Bieroza et al. (ref 78), in which clarification most efficiently removed organic matter with long emission wavelengths. Baghoth et al. (ref 29) found that the greatest reductions in Component 2 fluorescence intensity throughout two full-scale treatment trains resulted from ozonation and BAC filtration. Henderson et al. (ref 28) attributed the removal of peak C, and therefore Component 2, by ultrafiltration, nanofiltration, and reverse osmosis to the hydrophobic nature of Component 2 compounds.

The extent to which UV irradiation and chlorination reduce Component 2 fluorescence intensity depends on treatment process order.33 Murphy et al. (ref 33) found that if chlorination preceded UV disinfection, Component 2 was reduced by chlorination, but relatively unaffected by UV light; without chlorination, UV light reduced Component 2 intensities. Outside of water treatment, Seredynska-Sobecka (ref 32) also found that chlorination reduced Component 2, as Component 2 was specific to wastewater and anthropogenic contamination of swimming pool water, but did not accumulate in swimming pools throughout the day. Component 2 is also highly predictive of trihalomethane (THM), and to a slightly lesser extent, haloacetic acid (HAA) formation upon chlorination.71,79 Bieroza et al. (ref 79) attributed the correlation between THMs and Component 2 (peak C) intensity to the fluorescent signal also being highly correlated with the total amount of organic precursor material. Beggs and Summers (ref 71) explained that the positive correlation between Component 2 and disinfection byproduct formation was likely due to Component 2 representing the most aromatic fluorophores, as previous work has shown that disinfection byproduct yields are highly dependent on DOM aromaticity.80 Tan et al. (ref 81) found that the precursor material for THMs is largely aromatic, whereas HAA precursor material also includes aliphatic DOM. The correlation between aromaticity and Component 2, as well as the correlation between aromaticity and THM formation, explains why Component 2 is more predictive of THM formation than HAA formation. Despite the susceptibility of Component 2 to several DOM removal processes, Baghoth et al. and Murphy et al. (refs 29 and 33) found Component 2 to be dominant in finished water.

**Component 3.** Baghoth et al. (ref 29) found that Component 3 was preferentially removed by coagulation, but to a slightly lesser extent than Component 2. Beggs et al. (ref 71) also found that coagulation of fresh DOM reduced the percent contribution of Component 3 to the total fluorescence; however, coagulation of biodegraded DOM increased the percent contribution of Component 3, although the intensity was reduced. In two full-scale water treatment facilities, the greatest percent reductions in Component 3 resulted from BAC filtration and ozonation. Unlike Component 2, Component 3 was relatively unaffected by storage in a surface water reservoir.29 Murphy et al. (ref 33) found variable results regarding the effect of UV disinfection on Component 3. When considering three reclaimed water treatment plants, UV disinfection reduced the fluorescence intensity of Component 3 in two of the plants, the extent of which was greater if not preceded by chlorination, but in one of the plants, UV disinfection resulted in a slight increase in Component 3 fluorescence intensity. The

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authors offer no explanation as to why UV disinfection had variable effects on the fluorescence intensity of Component 3 at the different water treatment facilities. In all three plants, the first instance of chlorination decreased the fluorescence intensity of Component 3, but secondary chlorination resulted in a slight increase. Research conducted by Seredyńska-Sobecka et al. (ref 32) suggests that Component 3 is more resistant to chlorination than Component 2. Similar to Component 2, Component 3 was found to be specific to wastewater and not in swimming pool water by itself. Component 3 accumulated in pool water throughout the day as anthropogenic contamination increased, despite chlorination of pool water. Potential resistance to chlorination is further confirmed by Murphy et al. (ref 33), as Component 3, in combination with Component 2, commonly dominated finished water fluorescence. Baghoth et al. (ref 82) found that one Component 3-like component increased in fluorescence intensity following chlorination at two water treatment facilities, whereas another Component 3-like component in the same study decreased in fluorescence intensity.

**FUTURE RESEARCH NEEDS**

Table 6 provides a summary of reoccurring PARAFAC component behavior. The “trend” shows the relative pertinence of a “category” to a component. For example, the first category (terrestrial origin) and the first trend (1 > 3 > 2) indicate that DOM of terrestrial origin most contributes to Component 1, followed by Component 3, and then Component 2. Tables 6 and S3 in SI show that several overall tendencies can be established for reoccurring components. As a result, spatial and temporal component variability help predict changes in DOM behavior. However, inconsistencies between similar components across studies are also prevalent, thus indicating that caution should be exercised when drawing conclusions for one PARAFAC model based off of another model. Furthermore, changes in molecular size, polarity, and physicochemical properties of DOM, and thus DOM fluorescence, under varying conditions must be acknowledged when interpreting PARAFAC results. Water sample conditions, including pH, ionic strength, dissolved oxygen, temperature, and metals content, should be held constant or accounted for when attempting to characterize DOM based on PARAFAC component behavior, as these parameters undoubtedly affect DOM fluorescence.

The majority of PARAFAC studies have attempted to describe the temporal and spatial variability of DOM as indicated by component behavior; however, existing research has failed to recognize whether this variability in DOM fluorescence is due to a chemical transformation, a physical transformation, or a change in the source of the DOM. PARAFAC users must recognize that fluctuations in DOM fluorescence over time and space may be due to variability in the sources of organic carbon, as well as environmental conditions that also change over time and space, potentially causing a change in the three-dimensional structure or constitution of DOM. For example, Component 1 was found to be negatively correlated with salinity. Is this reduction in Component 1 fluorescence a result of decreasing sources of DOM compounds along the freshwater to seawater continuum or perhaps a result of DOM contracting under high ionic strength conditions, thus inhibiting its fluorescence? A deeper understanding of the relationship between DOM fluorescence and environmental conditions is greatly needed to accurately explain the spatial and temporal variability of PARAFAC components.

Further studies are also needed to strengthen the link between natural and engineered systems in order to extend the usefulness of fluorescence spectroscopy with PARAFAC. PARAFAC models spanning both system types would be able to address the environmental conditions that affect certain components and the water treatment processes that most effectively mitigate those effects. Research regarding the effects of water treatment processes on PARAFAC component fluorescence is minimal and additional work is needed to further establish treatment process–DOM fluorescence relationships. It must also be determined whether these relationships are based on fluorescence quenching, DOM removal, or a transformation of DOM and if the transformation is physical or chemical. Treatment processes that serve as alternatives to chlorination, such as UV disinfection and ozonation, should be a primary focus. After establishing treatment process–fluorescence relationships, DOM monitoring in raw water could be used to better design and modify water treatment systems throughout the year. Additionally, fluorescence spectroscopy with PARAFAC should be combined with other experimental methods to determine if fluorescence quenching is truly indicative of complexation. If Component 2 is quenched by mercury, are the transport and fate of Component 2 compounds and mercury then coupled in the environment? If Component 2 removal is achieved during water treatment, will mercury have also been removed? A more systematic approach to fluorescence spectroscopy with PARAFAC, in which systems are linked and hypotheses based on fluorescence are verified, would allow the results from individual studies to be more effectively applied.

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**Table 6. Component Comparisons**

<table>
<thead>
<tr>
<th>category</th>
<th>trend</th>
<th>category</th>
<th>trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>terrestrial origin</td>
<td>1 &gt; 3 &gt; 2</td>
<td>mercury complexation</td>
<td>2 &gt; 3 ~ 1</td>
</tr>
<tr>
<td>conservatively mixed</td>
<td>1 &gt; 3 &gt; 2</td>
<td>removal by reservoir storage</td>
<td>2 &gt; 1 ~ 3</td>
</tr>
<tr>
<td>biologically degraded</td>
<td>2 &gt; 3 &gt; 1</td>
<td>removal by coagulation</td>
<td>2 &gt; 3 &gt; 1</td>
</tr>
<tr>
<td>biologically produced</td>
<td>2 &gt; 3 &gt; 1</td>
<td>removal by ozonation</td>
<td>2 ~ 3 &gt; 1</td>
</tr>
<tr>
<td>photochemically degraded</td>
<td>2 &gt; 3 &gt; 1</td>
<td>removal by BAC filtration</td>
<td>3 &gt; 2 &gt; 1</td>
</tr>
<tr>
<td>photochemically produced</td>
<td>1 &gt; 2 &gt; 3</td>
<td>removal by sand filtration</td>
<td>1 &gt; 2 &gt; 3</td>
</tr>
<tr>
<td>sediment sorption tendencies</td>
<td>2 &gt; 3 &gt; 1</td>
<td>removal by UV disinfection</td>
<td>3 &gt; 2 &gt; 1</td>
</tr>
<tr>
<td>iron complexation</td>
<td>2 &gt; 3 &gt; 1</td>
<td>removal by chlorination</td>
<td>2 &gt; 3 &gt; 1</td>
</tr>
<tr>
<td>copper complexation</td>
<td>3 &gt; 2 &gt; 1</td>
<td>presence in finished water</td>
<td>2 ~ 3 &gt; 1</td>
</tr>
<tr>
<td>aluminum complexation</td>
<td>3 &gt; 2 ~ 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

“Category is equally pertinent to components on either side of the “−” symbol.”
Critical Review

Environmental Science & Technology

ASSOCIATED CONTENT

Supporting Information
PARAFAC references with corresponding components, additional examples of component spectral loadings, and trends from Table 6 with traditional peak classifications. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
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REFERENCES


