

CHARACTERIZATION OF THE SPATIO-TEMPORAL DYNAMIC OF FLUORESCENT DISSOLVED ORGANIC MATTER IN THE PONDS OF BRENNE (FRANCE) USING EEM-PARAFAC MODEL

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ABSTRACT

This article analyzes the spatio-temporal dynamics of fluorescent dissolved organic matter (FDOM) in three ponds in the Brenne National Park (France): Thomas, Pifaudière and Neuf. A total of 216 water samples were collected monthly between October 2017 and October 2019. The collected data were then processed using 3D fluorescence spectroscopy and a PARAFAC (parallel factorial analysis) model. The PARAFAC analysis identified three main fluorophore components: component M (λ ex/em = 290-325 / 370-430 nm), corresponding to low-molecular-weight neoformed humic substances (UVA humic-like); component C (λ ex/em = 320-360 / 420-460 nm) and component A $(\lambda \text{ ex/cm} = 250{\text -}260 / 448{\text -}480 \text{ nm})$, which are representing terrestrial aromatic humic compounds (UVC humic-like). Fluorescence indices indicated HIX values ranging from 5.21 to 16.78, typical of terrestrial origin of dissolved organic matter, particularly prominent in Pifaudière pond during spring (max $= 16.78$). On the other hand, BIX indices, which measure autochthonous biological activity, remained below 1, fluctuating between 0.46 and 0.77, suggesting a low proportion of freshly produced organic matter.

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The results show that Pond Neuf has the highest concentration of dissolved organic matter (DOM), with fluorescence intensities almost twice those of the other ponds, reaching maximum values of 15,000 A.U. for component M in the fall. Pond Pifaudière is characterized by a high content of terrestrial humic compounds, particularly component C. Seasonal analysis reveals that fluorescence intensities are relatively higher in autumn, probably due to the decomposition of surrounding vegetation. In winter, a significant decrease is observed, attributed to MOD dilution and precipitation; for example, fluorescence in Thomas Pond decreased from 8,000 A.U. in autumn to 5,000 A.U. during winter. In spring and summer, a notable increase in fluorescence intensities was recorded in Thomas and Neuf ponds, probably due to anthropogenic inputs linked to agricultural activities. Overall, this study highlights the homogeneity of biogeochemical processes between ponds, with predominantly terrestrial MOD inputs and spatio-temporal variations. These results provide essential information for the appropriate and sustainable management of these aquatic ecosystems.

Keywords: Dissolved organic matter, Ponds, Fluorescence, PARAFAC, 3D spectroscopy*.*

INTRODUCTION

Aquatic systems are complex environments where organic matter (OM) plays a crucial role (Bacha and Achour, 2023; Aw et al., 2016). This OM, a collection of hydrocarbon compounds of both natural and anthropogenic origins, exists in surface waters in various forms: particulate, colloidal, and dissolved (Thurman, 1985; Cawley et al., 2012; Laghzal and Salmoun, 2014; Riopel et al., 2016). Its ubiquitous presence (Labanowski, 2004; Zhao, 2011) and complexity make it a central element in the functioning of aquatic ecosystems. OM originates from allochthonous sources, such as plant debris and terrestrial soils, as well as autochthonous sources resulting from biogeochemical processes like photosynthesis and biomass degradation (Artifon et al., 2019; Cammack et al., 2004; Chen et al., 2003).

The composition and concentration of OM are influenced by multiple factors, including the surrounding watershed, land use/land cover, and the hydrological connectivity between the water body and its surroundings (Besemer et al., 2009). These characteristics affect not only the reactivity of OM within ecosystems but also its role in various environmental processes, such as speciation, solubility, and the transport of trace metals (Buffle, 1988; Campbell, 1995). Furthermore, OM impacts the dynamics of aquatic ecosystems by influencing key processes like light attenuation within the water column (Markager et al., 2000) and nutrient availability (Holbrook et al., 2006).

It is now well accepted that natural organic matter, and in particular humic substances (HS) present in all surface waters, constitute the majority precursors of potentially mutagenic and carcinogenic disinfection by-products (Harrat and Achour, 2010; Achour and Chabbi, 2014; Achour et al., 2018). In addition, studies were carried out to determine the conditions and treatments suitable for the optimal elimination of organic constituents from water. The substances considered are compounds responsible for long-term toxic

effects, initially present in the water or generated during a treatment step (Achour et al., 2002). Other studies have observed the impact of oxidative treatments using chlorine or potassium permanganate on the elimination of organic matter by coagulation-flocculation (Afoufou and Achour, 2003; Hecini and Achour, 2008; Bacha and Achour, 2015).

A notable feature of dissolved organic matter (DOM) is its ability to fluoresce when excited by UV light, a property that has been exploited to characterize and quantify OM in aquatic environments (Valencia et al., 2014; Chen et al., 1993; Coble, 1996). The study of this fluorescence, through excitation-emission matrices (EEMs), provides valuable insights into the structure, composition, and concentration of fluorescent compounds in OM (Coble, 1996; Parlanti et al., 2000). These analyses are particularly important as the composition and physicochemical properties of OM can influence the metabolism of water bodies and the biogeochemical interactions within aquatic ecosystems (Stedmon et al., 2003).

However, the inherent complexity of OM makes its characterization challenging, leading to the increasing use of multivariate techniques such as the PARAFAC model to analyze EEMs. This model allows for the decomposition of fluorescence spectra into their individual components, thus facilitating a better understanding of the present fluorophores (Bro, 1997; Stedmon et al., 2008). The application of PARAFAC in various studies has demonstrated its effectiveness in describing DOM in different aquatic environments (Holbrook et al., 2006; Stedmon et al., 2005; Henderson et al., 2009).

Studies on fluorescent organic matter have already been conducted in several aquatic systems, as mentioned above. However, in fish farming ponds, such research is still largely non-existent, giving this study a particular originality.

The aim of this research is to fill this gap by providing new knowledge on the dynamic of dissolved organic matter in fish farming ponds. It focuses on identifying potential sources of DOM (using 3D fluorescence spectroscopy techniques), monitoring its degradation and transformation processes (exploring its spatio-temporal variability), and its role as an indicator of water quality.

MATERIAL AND METHODS

Study Area

Brenne, one of the largest wetland areas in France, is home to over 3,000 anthropogenic ponds, some dating back to the 7th century. Initially designed for land drainage to facilitate agriculture, livestock farming, and fish farming, these ponds are now primarily maintained for aquaculture. Our study area is located within the Brenne Regional Natural Park, located at the center of this wetland, approximately 40 km west of Châteauroux, in the southwestern part of the Indre department, in the Centre-Val de Loire region. The region is characterized by relatively flat topography with small and flat-bottomed basins. It is dominated by hard clay-sandstone formations known as "grison," along with claysandy soils (Rasplus et al., 1989). The climate is of a degraded continental type with strong oceanic influences, featuring an annual average of 700 mm of precipitation and temperatures ranging from 6.9°C to 16.08°C.

For our study, three sampling sites within the park were selected: Thomas, Pifaudière, and Neuf ponds, located in the Massé-Foucault Regional Nature Reserve (Fig. 1). These ponds were chosen as sentinel ponds to gather extensive information about their general dynamics and management.

Figure 1: Locations and Sampling Points of the Studied Ponds

The ponds are an integral part of the Claise watershed, connected via the Blizon stream, which then flows into the Cinq Bondes stream. To the west of the Thomas Pond watershed, forests and shrubby vegetation dominate, while agricultural land extends to the east. Further south, a deciduous forest, primarily composed of pedunculate oaks, intermingles with heathland. Around the Pifaudière Pond, agricultural meadows stretch to the west, while dense forests cover the north and south. Finally, the Neuf Pond is mainly surrounded by agricultural meadows, with a deciduous forest to the south. Table 1 provides additional characteristics of the studied ponds.

Sampling

Monthly sampling campaigns were conducted over a two-year period, from October 2017 to October 2019, resulting in a total of 216 water samples collected from the shores of the three ponds at specific points called "bondes" (Figure 1). The sampling points correspond to the artificial outlets of each of the ponds, in addition to being easily accessible. Samples were collected using a Niskin bottle (WildCo, Beta-plus-horizontal Water Bottle) and immediately filtered using syringes equipped with 0.45 μm Magma filters. The filtered samples were then transferred into glass tubes and stored in the dark at a cool temperature (4°C). Sample analysis was carried out within 24 hours of collection.

Fluorescence Spectroscopy Analysis

The characterization of DOM from the samples was performed by exploiting its fluorescence properties. This technique is notable for its sensitivity, speed, and noninvasive nature, requiring no sample pre-treatment. Fluorescence measurements were conducted at a controlled temperature of 20 $^{\circ}$ C, using a 10 mm path length quartz cell and a HITACHI F-2500 spectrofluorometer equipped with a pulsed xenon lamp as the light source. Excitation wavelengths were scanned from 220 to 500 nm in 10 nm increments, while emission wavelengths were recorded between 230 and 550 nm with a 1 nm step size and a scanning speed of 1500 nm/min. Data integration was performed with a response time of 0.1 s in signal/reference mode, with a bandwidth of 5 nm for both excitation and emission monochromators. Emission signal normalization was based on the intensity of the excitation light. The resulting fluorescence spectra were represented as three-dimensional excitation-emission matrices (EEMs) (Fellman et al., 2010).

PARAFAC Model

Fluorescence components were identified by constructing a Parallel Factor Analysis (PARAFAC) model and applying a deconvolution algorithm to the fluorescence spectra. This methodology follows the protocol established by Murphy et al. (2013). PARAFAC allows the decomposition of fluorescence data, enhancing our understanding of the contributions from different fluorophores present in a mixture, based on the maximum

intensity peaks corresponding to specific pairs of excitation and emission wavelengths (λ) excitation $\ell \lambda$ emission). In our study, the PARAFAC analysis was performed using the "DrEEM" toolbox in Matlab 8.2 (R2013b). The identification of components was conducted by comparing them to existing literature data, with the main fluorophores summarized in Table 2.

RESULTS AND DISCUSSION

Identification of PARAFAC Components

In this study, several excitation spectra of the components exhibited a bimodal structure. Burdige et al. (2004) proposed a correlation between peaks A and C, which is confirmed here by the PARAFAC analysis. To facilitate interpretation and presentation of the results, we focused on the fluorophores representing the main excitations. A preliminary identification of the PARAFAC components was conducted on the entire dataset of 216 samples, leading to the identification of two components (Fig. 2 and Table 3):

- Component 1 (λ ex-em = 300/410 nm): This corresponds to the fluorophore of neoformed humic substances of low molecular weight, UVA humic-like (peak M/β), associated with biological activity but also detectable in wetlands and agricultural environments (Aiken et al., 1985; Fellman et al., 2010; Coble et al., 2014).
- Component 2 (λ ex-em = 360/456 nm): This corresponds to peak C/ α and is associated with high molecular weight terrestrial aromatic humic compounds (UVC humic-like) of vascular plant origin, particularly prevalent in wetland and forest environments (Fellman et al., 2010).

Figure 2: Identified PARAFAC Components from the Overall Treatment and Pond-Specific Treatment of Water Samples

A second identification of the PARAFAC components was performed on the samples from the three studied ponds. The results are illustrated in Table 3 and Fig. 2.

For Thomas Pond, we distinguished two components:

- Component 1: Located at λ ex-em = 270 (370)/456 nm, associated with terrestrial humic substances (UVC humic-like, peak A/α') in a reduced form (Stedmon et al., 2003; Cory and McKnight, 2005).
- Component 2: Located at $\lambda_{\text{ex-em}} = 300/399$ nm, associated with UVA humic-like compounds (peak M/β), which are neoformed low molecular weight humic substances frequently found in marine environments related to biological activity. However, it can also be found in wetlands and agricultural environments (Aiken et al., 1985; Fellman et al., 2010; Coble et al., 2014).

Two components were also identified for Pifaudière Pond:

- Component 1 (λ ex-em = 310/423 nm): Corresponds to the fluorophore of low molecular weight neoformed humic substances associated with biological activity, with peak M/β (Aiken et al., 1985; Fellman et al., 2010; Coble et al., 2014).
- Component 2 (λ ex-em = 370/462 nm): Associated with peak C/ α and high molecular weight terrestrial aromatic humic compounds (UVC humic-like) of vascular plant origin, particularly present in wetlands and forest environments (Fellman et al., 2010).

Similarly, two components were identified for Neuf Pond:

- Component 1 (λ ex-em = 350 (260)/454 nm): Corresponds to peak C/ α , associated with high molecular weight terrestrial aromatic humic compounds (UVC humiclike) of vascular plant origin, typically found in wetlands and forest environments (Fellman et al., 2010).
- Component 2 (λ ex-em = 300/400 nm): Corresponds to the fluorophore of neoformed low molecular weight humic substances related to biological activity, with peak M/β (Aiken et al., 1985; Fellman et al., 2010; Coble et al., 2014).

The results presented in Fig. 3 and Table 4 correspond to a third identification of the PARAFAC components, conducted on samples collected from the three ponds during different seasons.

Figure 3: PARAFAC components identified for samples taken from the 3 ponds at different seasons during DOM monitoring in Brenne ponds, from October 2017 to October 2019.

Table 4: Identification of PARAFAC components for seasonal treatment

We observe that during the dry season (spring and summer), certain components with identical origins are observed across the ponds. Specifically, components A and M from Thomas Pond in spring and M and C in summer, as well as components M and C from Pifaudière Pond and components A and M from Neuf Pond in spring, and M and C in summer, consist of peaks C (UVC humic-like), A (UVC humic-like) corresponding to high molecular weight terrestrial aromatic humic substances, and peak M (UVA humiclike) corresponding to neoformed biological humic substances (Coble, 1996; Fellman et al., 2010). During the rainy season (fall and winter), humic substances (both terrestrial and neoformed) are also commonly found in all three ponds.

Analysis of Fluorescence Indices (HIX, BIX)

In addition to identifying the different fluorophores present, several fluorescence indices are used to obtain information about the origin and degree of humification of fluorescent dissolved organic matter (FDOM).

The HIX values for this study are presented in figure 4, where a maximum value of 16.78 is recorded in Pifaudière Pond during spring. In contrast, the other ponds show values ranging between 5.21 and 12.87 across the four seasons. HIX fluctuates between 5.21 (Neuf Pond) and 10.45 (Pifaudière Pond) during the fall season, between 6.05 (Thomas Pond) and 9.12 (Pifaudière Pond) in winter, between 9.46 (Thomas Pond) and 16.78 (Pifaudière Pond) in spring, and between 7.55 (Neuf Pond) and 14.99 (Pifaudière Pond) in summer.

Figure 4: Spatio-temporal variation of the HIX and BIX indices

High HIX values (between 10 and 16) indicate that the DOM is heavily humified and of terrestrial origin, while low values $\left($ < 4) suggest that the DOM primarily originates from autochthonous production (Fellman et al., 2010). Therefore, the HIX values obtained from our samples reveal a moderate to high level of humification, suggesting a predominantly terrestrial origin of the DOM.

For the BIX index (Fig. 4), the differences are less pronounced. The maximum value is recorded in Neuf Pond (0.773) during the fall. In all other cases, BIX values range from 0.59 (Pifaudière Pond) to 0.69 (Thomas Pond) in autumn, between 0.64 (Thomas Pond) and 0.65 (Neuf Pond) in winter, between 0.46 (Pifaudière Pond) and 0.59 (Neuf Pond) in spring, and between 0.48 (Pifaudière Pond) and 0.69 (Neuf Pond) in summer.

Generally, BIX values range from 0.6 to 0.7 in natural waters. High BIX values (> 1) suggest a dominance of DOM of bacterial origin, associated with a significant proportion of young organic matter (Ferretto, 2014). For all the studied ponds, BIX values remain below 1, indicating a low presence of autochthonous organic matter and a negligible amount of freshly produced DOM.

Seasonal spatio-temporal variations of fluorescence intensities of different compounds in each pond

Fig. 5 illustrates the seasonal spatio-temporal variations in fluorescence intensities of the identified compounds in each studied pond. The comparative analysis of the ponds shows that Neuf Pond has the highest concentration of DOM, with fluorescence intensities nearly twice as high as those observed in the other two ponds. Additionally, this pond exhibits a similar trend in fluorescence intensities for both components, C and M. On the other hand, DOM in Thomas Pond is primarily influenced by allochthonous humic substances, accompanied by recent production of UVC humic-like material (Component A) and Component M. The influence of UVC humic-like substances (Component C) is particularly pronounced in Pifaudière Pond (Fig. 5), which correlates well with the high turbidity observed in this pond.

Seasonal analysis reveals that during the fall season, fluorescence intensities remain relatively high. This observation can be explained by the decomposition of plant material (notably due to the proximity of forests and shrubby vegetation) and the significant accumulation of organic matter during Spring and Summer. These factors seem to justify the increased fluorescence of humic compounds (Thurman, 1985).

Figure 5: Seasonal spatio-temporal variations in fluorescence intensities of compounds in each pond

A marked decrease in fluorescence is observed in Winter for all three ponds. This could be attributed to:

- Precipitation of DOM: Lower temperatures may lead to changes in the solubility of certain dissolved organic materials, particularly those of larger molecular size. This can cause their precipitation or aggregation, making them less available (Prairie and Kalff, 1988).
- Effects of dilution and hydrological cycles: In Winter, the hydrological regime of the ponds changes. Winter precipitation can lead to dilution of DOM in the water. Furthermore, the lack of significant evaporation in Winter amplifies this dilution effect (Seekell et al., 2018).

In Spring and Summer, a significant increase in intensities is observed across all three ponds*.* This rise may be attributed to substantial anthropogenic inputs, particularly related to agricultural activities such as fertilization and irrigation (Thurman, 1985). These observations are consistent with the identified components and their descriptions in the literature (Table 4).

CONCLUSION

This study focused on characterizing the spatio-temporal dynamics of fluorescent dissolved organic matter (FDOM) in the ponds of Brenne in France. Monthly monitoring using 3D fluorescence spectroscopy was conducted across three sampling sites (three ponds), resulting in a total of 216 samples collected between October 2017 and October 2019.

A PARAFAC model was developed for all studied ponds, enabling detailed characterization of the quality of fluorescent DOM. The analysis of the model allowed for the decomposition of the 3D fluorescence spectra of dissolved organic matter into three distinct components: two components related to the fluorescence of humic substances of varying maturity (UVC humic-like "C" and UVC humic-like "A") and one component associated with humic substances resulting from recent biological activity (UVA humic-like).

The results revealed similar profiles in terms of components covering the same spectral ranges, regardless of the sampling point or monitoring period. This indicates that the DOM in these ponds exhibits uniform chemical structures associated with similar origins and degradation processes.

From a spatial perspective, homogeneity was observed among the different studied ponds. All ponds are subject to anthropogenic disturbances and show very low concentrations of components linked to microbial activity, suggesting a predominantly allochthonous origin of the organic matter. The HIX and BIX indices confirmed these trends.

While general trends were observed, specific fluorescence characteristics were identified for each pond. Notably, Pifaudière Pond stands out with a higher terrestrial humic fluorescence signature (C) compared to the other ponds, while Neuf Pond exhibits the

highest concentration of dissolved organic matter (DOM). These results highlight the importance of considering the unique features of each pond in the analysis of fluorescence properties.

Seasonal analysis of fluorescence intensities reveals significant variations, with high peaks observed in fall. This increase is followed by a decrease in winter, mainly due to dilution and precipitation of organic matter. In spring and summer, a marked resurgence in fluorescence levels is noted, correlated with anthropogenic inputs linked to agricultural activities. These results underline the influence of seasons and human intervention on the dynamics of organic compounds in these limnic entities. In particular, a high concentration of fluorescent organic matter may indicate pollution of agricultural origin, reflecting a deterioration in water quality.

This study sheds light on the biogeochemical processes governing the ponds of Brenne. The information obtained can be used to inform water resource management strategies, particularly in terms of protecting and restoring fish farming ponds. In addition, it highlights the importance of integrated management adapted to seasonality and local characteristics, to maintain the water quality and biodiversity of these ecosystems. These perspectives are essential for the development of effective environmental policies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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