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Published in:
Science of the Total Environment

Link to article, DOI:
10.1016/j.scitotenv.2019.04.170

Publication date:
2019

Document Version
Peer reviewed version

Citation (APA):
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PII: S0048-9697(19)31699-7
DOI: https://doi.org/10.1016/j.scitotenv.2019.04.170
Reference: STOTEN 31878

To appear in: Science of the Total Environment

Received date: 5 February 2019
Revised date: 1 April 2019
Accepted date: 11 April 2019


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Article Type
Full Paper

Title
Sediment alkaline-extracted organic matter (AEOM) fluorescence: an archive of Holocene
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Author contributions
All authors participated in conceiving the study and writing the manuscript. CAS
conceptualized, CPF conducted the alkaline extraction of the sediments and did the
fluorescence analyses with guidance from CAS. CAS carried out the statistical analysis.

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Abstract
Organic matter (OM) is comprised of a complex mixture of substrates, which are difficult to fully characterize. Therefore a range of analytical approaches is applied to provide a better understanding of the dynamics and biogeochemical cycling of aquatic system. One approach is UV-Visible spectroscopy, which includes measurements of spectral absorption and fluorescence of colored and fluorescent fractions of dissolved OM (DOM, CDOM and FDOM). In this study OM fluorescence is characterized by excitation-emission matrix spectroscopy on alkaline extracted DOM from a Baltic Sea sediment core that spanned 8500 years and fluctuating levels of hypoxia. Our results showed that three underlying fluorescence components had strong correlations with carbon, nitrogen content and δ15N. Our results demonstrate that optical properties of extracted OM from sediments reveal information about OM quality and quantity similar to those of biomarkers, which can be a useful additional tool for investigating OM deposition.

Keywords
organic matter, excitation-emission matrix spectroscopy, fluorescence, burial, Baltic Sea, Holocene
1. Introduction

The Baltic Sea is a semi-enclosed brackish body of water that has experienced dramatic physical and biogeochemical changes during the past 8,500 years (Björck 1995, Gustafsson and Westman 2002, Zillen and Conley 2010). Due to restricted water exchange and fluctuating organic matter (OM) supply, the Baltic Sea has gone through varying degrees of hypoxia over the past 8,500 years. One of the largest hypoxic (dissolved oxygen <2 mg/L) periods identified was during the Littorina Transgression (7,000-4,000 years B.P.), were as a result of deglaciation and subsequent eustatic sea level rise, seawater entered the Ancylus Lake converting it to the Littorina Sea (Björck 1995). The seawater intrusion created a halocline, which reduced vertical mixing and limited the resupply of oxygen to bottom waters. Approximately 4,000 years B.P. the depth of the Danish straits became shallower due to isostatic rebound. This decreased the intrusion of saline water from the North Sea and coupled with declines in temperature at the end of the Holocene Thermal Maximum (Zillen and Conley 2010), weakened the halocline. The second period of widespread hypoxia occurred during the Medieval Climate Anomaly (MCA) (1,400-700 years B.P.) with both the onset and termination of hypoxia influenced by changes in temperature (Papadomanolaki et al. 2018). The third and current hypoxic period started approximately 100 years B.P. This period has been attributed to eutrophication, the increases in OM supply due to high nutrient loadings related to human activity (Gustafsson et al. 2012) and climate change (Kabel et al. 2012).

These switches in hypoxia have had a large influence on the production and cycling of OM in the Baltic Sea. For example the three periods of hypoxia have shown to correlate with increases in cyanobacteria concentrations (Bianchi et al. 2000; Funkey et al. 2014). This is caused by the release of sediment bound phosphate during hypoxic conditions, which can trigger cyanobacteria blooms (Conley et al. 2002), and incidentally correlates with higher concentrations of OM being stored in the sediments. Another factor that affects the quantity and quality of OM stored in sediments is microbial degradation, the extent of which is directly related to the oxygen concentrations. With varying OM degradation at the water-sediment interface and variable contributions of cyanobacteria derived OM one would expect to see fluctuations in the quantity and quality of OM buried in sediments.

Sediment organic matter (OM) represents a complex mixture of molecules ranging in size and complexity from simple monomers to more or less intact remains of organisms. As a result it is currently impossible to fully characterize OM and each measurement technique has its own specific analytical window and limitations. OM analysis can also require the use of
expensive instruments and extensive sample preparation to obtain information on specific biomarkers, distribution of functional groups or potential molecular formula (Minor et al. 2014). UV-visible spectroscopy is focused on the light absorbing and fluorescent fractions of OM and unlike other approaches, this method requires little sample preparation and is very economical and rapid. In this light these measurements can be considered as an optical-marker allowing us to tracer the supply and turnover of different fraction of OM. (Coble 2007; Stedmon and Cory 2014; Spencer et al. 2012).

Excitation-emission matrix (EEM) spectroscopy uses a combination of multiple emission spectra collected over a range of excitations collated into a matrix, representing a comprehensive mapping of the fluorescence properties of an OM sample (Coble 2007). Often two overarching features are apparent: a broad visible wavelength fluorescence (i.e. emission wavelength >400 nm) often referred to as humic like due to its association with microbial processing of OM; and a more constrained/narrow fluorescence peaks in the UVA region (i.e. emission wavelength <400 nm) with characteristics similar to that of monomeric aromatic acids (Coble 2007; Wünsch et al. 2015). EEMs actually represent a more complex combination of independent overlapping fluorescence signals which can be isolated using parallel factor analysis (PARAFAC) (Murphy et al. 2013), offering a sensitive approach to trace subtle changes in underlying OM fractions that are often not apparent in bulk measurements or involve comprehensive sample treatment and characterization.

The majority of studies employing EEM spectroscopy have focused on measurements of DOM omnipresent in natural waters (Stedmon and Cory, 2014). However a few studies have expanded this approach to study particulate OM (POM) after an initial base extraction (Santín et al. 2009; Osburn et al. 2012; Brym et al. 2014). For example Brym and colleagues (2014) have shown that the fluorescence characteristics of base extracted OM is closely linked to its’ source (terrestrial or aquatic) and can be used to decipher the importance of DOM release from the dissolution of sediment OM. Recently a few studies have analyzed the fluorescence of alkaline-extractable OM (AEOM) in sediments (He et al. 2016; Chen et al. 2017). He et al. (2016) study compared the spectroscopic features of AEOM in sediments with that from pore waters. They concluded that tyrosine-like (UVA fluorescent) components were found in higher intensities in pore water than in the AEOM and humic-like visible wavelength fluorescence was greater in AEOM than in pore water. Chen et al. (2017) examined AEOM in a 240 meters sediment core and found that visible fluorescent components increased with depth as opposed to the UVA fluorescent components. These findings suggest that we can expect the spectroscopic properties of sediment OM to reflect...
the environmental conditions (OM characteristics, OM quantity and local oxic conditions) at the time of burial. Insights from these two studies show that visible wavelength fluorescence signals are likely older material and sediment bound, whereas UVA fluorescence signals are associated with newer material and mostly found in pore water and surface sediments.

In this study we measured fluorescence of AEOM in sediments to evaluate the quantity and quality of OM and compare it to the trends in other paleo-markers already documented for a sediment core from the Gotland Basin in the Baltic Sea (Funkey et al. 2014; Jilbert and Slomp 2013). This sediment core is particular interesting since it documents switches in oxic conditions over a span of 8,500 years that, have had a large influence on the production and cycling of OM. Combining fluorescence EEM spectroscopy and PARAFAC has shown to be a powerful tool to obtain more information about different fraction of dissolved organic matter in aquatic systems (Stedmon and Cory 2014). The aim of this study is to examine if the AEOM fluorescence intensity and characteristics show any correlation with biochemical biomarkers and to start to explore the potential application of its use as a paleo-tool in sediments.

2. Materials and Methods
2.1 Sediment core collection
Fluorescence was measured on AEOM from sediment samples (n=112) from the LL19 core, obtained from the Northern Gotland Basin (58.8807°N, 20.3108°E, 169 m water depth) (Funkey et al. 2014). The sediment samples were freeze-dried, homogenized and have a sample resolution that varies between 1-5 cm in thickness.

2.2 Sediment core analysis
We extracted organic matter using the method used for extracting biogenic silica from sediments (Conley and Schelske 2001), to investigate if additional measurements can be made on the remaining extract, and provide more biogeochemical information limited additional sampling and preparation. 30 mg of sediment for each section was added into 40 mL of 1% sodium carbonate in a water bath at 85°C for 3 hours, (5 mL of the basic solution was filtered through a combusted 0.45-mm Whatman GF/F filter. The pH was approximately 11 for all samples. The samples were stored in a glass container prior to analysis. Fluorescence spectra were measured on a Horiba Scientific Aqualog fluorescence spectrophotometer. The instrument simultaneously measures spectra fluorescence and absorption across wavelength from 240 to 600 nm. Measurements were carried out in a 1 cm
cuvette with Millipore water as a reference. The fluorescence spectra were inner filter corrected, calibrated and subsequently characterized with PARAFAC analysis using the drEEM toolbox following the recommendations in (Murphy et al. 2013) and a four component model was validated using randomized split-half validation. The spectral properties of each component are shown in Figure 1. The measured samples all had the same pH and therefore pH-induced changes between samples down the core can be assumed to be negligible.

2.2 Age Model
The age model for this core was determined by using both $^{210}$Pb dating for the multi-core and converting the gravity core organic C profiles to the loss on ignition profile of core 32740-3 from the Gotland deep. Core 372740-3 was independently dated by identification of two Pb pollution isochrones and 10 paleomagnetic secular variation features (more detailed information can be found in the Supporting Information sections of Funkey et al. (2014).

2.3 Biomarkers
Samples were collected and analyzed for sediment carbon and nitrogen content, as well as phytoplankton pigments and nitrogen isotopes as described in Funkey et al. (2014). For δ$^{13}$C measurements, samples were analyzed using a Carlo Erba NC2500 analyzer connected to a Finnigan MAT Delta V mass spectrometer. The reproducibility was better than 0.15‰ for δ$^{13}$C.

Sediment organic matter source was fractionated into three groups: phytoplankton, cyanobacteria and terrestrial; based on molar N/C ratios, δ$^{13}$C and δ$^{15}$N (Perdue and Koprivnjak 2007). Appropriate estimates of end member characteristics were collected from the literature (Cloern et al. 2002): phytoplankton, 0.1509, -22, 7.5; cyanobacteria 0.1509, -28, -4; terrestrial 0.05, -27, 4.5; for N/C ratios, δ$^{13}$C and δ$^{15}$N respectively.

2.4 Statistical Analysis
Regression analysis was carried out in MATLAB. As data from an independent core was not available, the regressions were evaluated using split-half analysis. The regression coefficients from models on two independent halves of the dataset were compared. If the coefficient values were within one standard error the models were deemed identical and data combined to derive the final model coefficients (Table 1).
3. Results and Discussion

3.1 AEOM fluorescence components

EEMs of two contrasting samples of AEOM are shown in Figure 1. In comparison to natural water DOM fluorescence, the EEMs of the extracts have fluorescence maxima at shorter emission wavelengths due to the higher pH (~11) (Brym et al. 2014). The overall fluorescence intensity varied down the core and there were also subtle changes in fluorescence characteristics down core. These are visible in the EEMs as apparent shifts in the position of shoulders and peaks in the EEMs (Figure 1) but are due to changes in the relative proportions of underlying fluorescent fractions. As pH was kept constant, we can attribute these changes to alterations in the composition of fluorescent organic matter rather than pH induced. Four AEOM fluorescence components were identified using PARAFAC and they are numbered according to the position of their emission maxima (Figure 1). Two components had relatively constrained UVA wavelength fluorescence peaks (C₁³₇₉ and C₁₃₃₇) and two with broad visible wavelength fluorescence (C₁₄₉₆ and C₁₄₂₁). Comparison of the spectral properties of the components with spectra available in Openfluor.org (Murphy et al. 2014) revealed numerous matches (TCC>0.97) for C₁₃₃₇, C₁₄₂₁ and C₁₄₉₆ with previous studies. C₁₃₃₇ was similar with a commonly found peak attributed to the amino acid tryptophan and indoles in general (Wünsch et al. 2015). It has also been identified in Baltic Sea sediment pore waters (Reader et al. 2019) and represents relatively fresh organic matter. No identical matches were found for C₁³₇₉ in Openfluor. However, several studies have identified a component with a similar emission spectrum but with emission maxima approximately 20 nm higher, at 400 nm. It is possible that the higher pH of these samples could be responsible for this slight dissimilarity. Fluorescence at these wavelengths has previously been correlated with plankton productivity (Coble et al. 1998, M-peak). C₁₄₂₁ and C₁₄₉₆ are similar to components found in other studies and are ubiquitous across a range of aquatic environments including sediment OM studies (Brym et al. 2014; Wünsch et al. 2019). In the vicinity of rivers these fluorescence signals often trace terrestrial organic material as they are a dominant source. However these fluorescence signals have also been identified, albeit at much lower intensities, in waters not influenced by run off (Stedmon and Cory 2014), where organic matter originates from aquatic primary production. It is likely that they represent a common metabolite of organic matter degradation in general.

4.2 Summary of the Biomarkers
The core profile is characterized by a period of low carbon preservation indicative of oxic bottom waters before 7000 years B.P. and from 3750-1400 years ago (Figure 2). In contrast there are periods of repeated hypoxia evident from the Mo/Al ratios and elevated carbon content during the transition to the Littorina Sea, the MCA and from modern eutrophication. The OM that reached the sediment surface during these periods is also characterized as having a higher contribution from cyanobacteria as indicated by biomarkers zeaxanthin, echinenone and δ15N (Funkey et al. 2014). The C/N molar ratios for this period are between 6.7-15.8 and δ13C between -28 to -24 ‰ indicates that it is primarily of autochthonous origin (Figure 2 C & E). The changes in organic matter provenance reflected in N/C, δ13C and δ15N in the core are summarized in the fractionation of the contribution of the three sources modeled (Figure 2F). There is also an abrupt increase in C/N across the transition from Ancylus Lake to Littorina Sea. This shift, which persists for the rest of the core, likely reflects a slight increase in contribution of terrestrial OM to the system (Figure 2F). During the Holocene Thermal Maximum, starting 10,000 years B.P, the rise in temperatures melted some of the Scandinavian ice sheet (Borzenkova et al. 2015), which would have also induced permafrost to melt and erosion, leading to leaching to the increase terrestrial signal shown in Figure 2C and F. Despite this, the persistent low C/N ratios indicate that much of the OM preserved is nitrogen rich and representative of carbon fixed by aquatic algae (Meyers 1994). The slight increase in terrestrial organic matter fraction towards the top off the core is likely explained by the increase in land erosion due to human impact (Gaillard et al. 2010).

4.3 Linear Regression Analysis
The intensity of each of the isolated fluorescence components generally followed the trend in OM content down core. A stepwise linear regression analysis revealed that the down core variability in sediment carbon and nitrogen content could be modeled by a linear combination of two UVA fluorescent components, C379 and C337 (Figure 3). The two components were not inter-correlated and the derived coefficients were significant (Table 1). The combination of these components also had the best predictive value, capturing the variability in carbon and nitrogen over the 8500 year period sampled (Figure 3). As described above, organic matter fluorescence with spectral characteristics similar to these (C337 and C379) components are indicative of comparatively fresh OM originating from aquatic production (Stedmon and Cory 2014). These correlations are also compatible with previous findings (Funkey et al. 2014) showing that the increase and variability in OM concentrations throughout the core
was largely driven by the variable contribution to sediment OM from phytoplankton blooms during hypoxic periods.

4.4 Fluorescence signals and hypoxic periods during the Baltic Sea

The fluorescence signals normalized to the sediment organic carbon content revealed different vertical trends reflecting periods with changes in OM quality (Figure 2). These were associated with documented periods of change in the Baltic Sea. The carbon specific fluorescence of C\textsubscript{421} is highly correlated to the δ\textsuperscript{15}N signature (Figure 3, Table 1). Low values of δ\textsuperscript{15}N are indicative of an increased contribution of N-fixing bacteria to OM burial, which also correlates with times of euxinia (high Mo/Al). During hypoxic conditions sediment OM δ\textsuperscript{15}N values are depleted (~2‰) compared to more enriched values (~3‰) δ\textsuperscript{15}N during oxic periods (Funkey et al. 2014). This reflects the shift in the contribution of N-fixing cyanobacteria to sediment organic matter. Hypoxia driven release of sediment bound phosphorus favors predominance of N-fixing cyanobacteria (Conley et al. 2002). As N-fixators use atmospheric N\textsubscript{2} gas, which is lighter (close to 0‰) than the N-species dissolved in water (Ryabenko 2013), the δ\textsuperscript{15}N values in aquatic biomass during times of high fixation are more depleted. The carbon normalized C\textsubscript{421} fluorescence signal is inversely correlated with cyanobacteria contribution, which indicates that the production of the chemical structures responsible for C\textsubscript{421} decreases during hypoxic periods. Degradation of OM in oxic pore waters results in an increase in visible wavelength fluorescence signals (Burdige et al. 2004). We therefore hypothesize that the lower carbon specific C\textsubscript{421} fluorescence during hypoxic periods is a result of greater preservation of OM. This would suggest the linkage to δ\textsuperscript{15}N is not necessarily direct (as a biomarker), but rather an indicator of periods of hypoxia with high OM supply to sediments and enhanced burial and preservation.

The material represented by C\textsubscript{337} is considered to be more labile and recent OM, and these signals are often observed at higher intensities in surface sediments pore waters decreasing down core (e.g. Chen et al. 2017). Signals similar to C\textsubscript{421} are considered “humic-like” and related to older, processed, refractory OM, which are found to increase in abundance down core (Burdige et al. 2004; Chen et al. 2017). However the results presented here to do not follow this diagenetic sequence and indicate that the OM characteristics preserved more likely represent overall changes in OM quality. In this light we conclude that the spectroscopic properties of the alkaline extracted from OM from laminated sediments reflects larger scale changes in environmental conditions in Baltic bottom waters rather than initial processing of OM.
5. Conclusion
This study has shown that optical properties of organic matter stored in sediments correlates with other commonly measured biomarkers in sediment cores, in this case %C, %N and δ^{15}N. Measuring the fluorescence of AEOM has shown potential to be a valuable additional paleo proxy, which can easily be assimilated into existing measurement protocols. For example the samples collected here were part of a routine sample analysis for biogenic Si. Our results indicate that AEOM fluorescence does not only reveal relative concentrations changes of OM throughout the core, but it can also trace qualitative changes which can be linked to depositional conditions and other biomarkers. The approach and correlations warrant further investigation and testing and potentially represent a novel spectroscopic marker for changes in past organic matter quantity and quality supply to sediment.

Conflict of interest statement
The authors declare no conflict of interest.

Acknowledgments
We thank H. Reader for laboratory assistance. DFF-1323-00336 from the Danish Council for Independent Research-Natural Sciences to CS. This research was also partially supported by the BONUS COCOA project (grant agreement 2112932-1) that has received funding from BONUS, the joint Baltic Sea research and development program (Art 185), funded jointly from the European Union’s Seventh Program for research, technological development and demonstration and from The Danish Council for Strategic Research and the Swedish Research Council for Sustainable Development.
Appendix A. Supplementary data

Supplementary data to this article can be found at: Data are available in the Pangaea repository at [https://issues.pangaea.de/browse/PDI-17683].

References


Spencer, R.G.M., Butler, K.D, Aiken, G.R. 2012. Dissolved organic carbon and


Figure 1. The fluorescence properties (excitation- emission matrices) of base extracted organic matter from a sediment core from the Baltic Sea. The top two graphs show excitation-emission matrices (EEMs) for a sample from 765 years before present (B.P.) (A) and from 7028 years B.P. (B). These EEMs show the similarities in excitation and emission but a difference in the intensities. Color bar indicates the fluorescence intensity in Raman Units (R.U.). The bottom row shows the excitation and emission properties of the four components validated identified using PARAFAC, from left to right C$_{379}$ (a marine-humic like), C$_{496}$ and C$_{421}$ (humic like) and C$_{337}$ (amino acid, tryptophan signal).

Figure 2. First row: year B.P vs carbon %, molybdenum/aluminum ratio, δ$^{13}$C vs. PDB (%), δ$^{15}$N vs. air (%), molar C/N ratio (data from Funkey et al. 2014); Second row: organic matter fractions, and fluorescence intensities of each component (Raman Units per mg sediment); Bottom row: Carbon normalized fluorescence intensities for each component (Raman unit per mol C).

Figure 3. Predicting OM properties from fluorescence properties. Top row: Modelled vs measured data for percent carbon, nitrogen and δ$^{15}$N based on regressions in Table 1; Bottom row: measured (blue) and modeled (red) data plotted downcore covering the 8500 year period. See Table 1 for the model regression coefficients and parameters.
Table 1. Linear regression coefficients from the models predicting total percent carbon and nitrogen content, $\delta^{15}$N vs. air (‰) versus fluorescence intensities $C_{379}$, $C_{337}$ and carbon normalized $C_{421}$ fluorescence ($C_{421}^*$) in sediment samples (n=115). All coefficients are significant (p<0.01) except where marked with * (p=0.01). Carbon and nitrogen intercepts have units of % C and % N respectively. Slopes %C (RU)$^{-1}$ and %N (RU)$^{-1}$. $\delta^{15}$N intercept is ppm and slope is ppm (RU)$^{-1}$ mgC$^{-1}$ L.

<table>
<thead>
<tr>
<th></th>
<th>Total % Carbon</th>
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<th>$\delta^{15}$N vs air (%)</th>
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<tr>
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Highlights

- Linking alkaline extracted sediment organic matter to provenance
- Organic matter characterized by fluorescence excitation-emission spectroscopy
- Fluorescence signature was correlated with biomarkers and offer a proxy of source