

On the possible origin of the fluorescence properties of humic substances

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Humic substances (fulvic and humic acids) of terrestrial origin and humic-like substances of phytoplankton origin can be identified quite conveniently by fluorescence excitation-emission matrix (EEM) spectroscopy, thanks to their couple of peaks at Ex/Em \sim 220-230/400-450 nm (peak A) and Ex/Em \sim 300-350/400-450 nm (peak C) (Coble, 1996). Despite the widespread use of the fluorescence signals to enable detection, the nature of the fluorescence emission has remained elusive and this contribution has the goal of proposing some potential explanations.

Our studies on the subject started from the finding that the simulated-sunlight irradiation of aromatic amino acids (tryptophan, tyrosine) and phenolic compounds (4-phenoxyphenol, hereafter 4PP) shows evolution from a protein-like or phenolic EEM fluorescence to a humic-like one, with formation of peak C in the case of tryptophan and of peaks A and C with tyrosine and 4PP (Bianco et al., 2014; see also Figure 1). Interestingly, the UV irradiation of phenolic compounds such as tyrosine and 4PP yields phenoxy radicals that tend to dimerise and oligomerise, which prompted for a deeper study into the possible fluorescence emission of phenol oligomers.

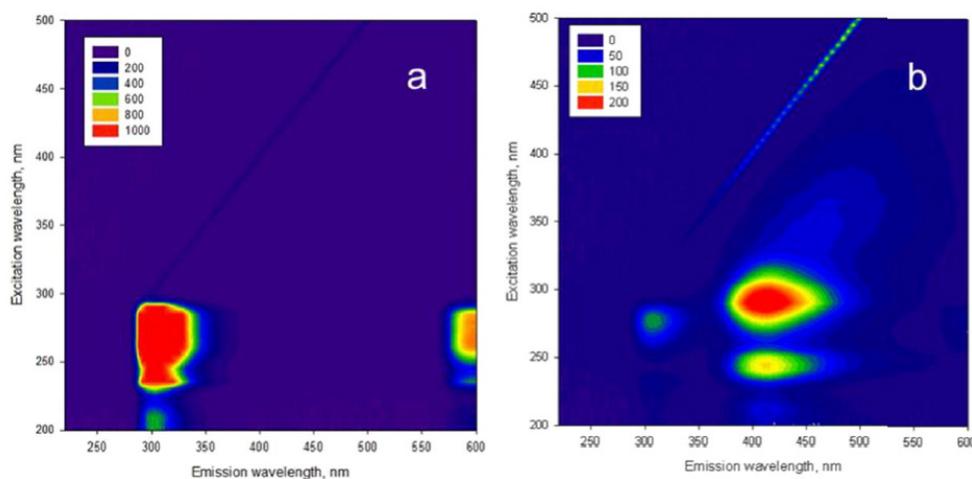


Figure 1. EEM spectra of tyrosine (1 mM initial concentration) before irradiation (left) and after 72-h irradiation under simulated sunlight (Xenon lamp with 300 nm cut-off filter, 28 W m^{-2} UV irradiance).

The oligomer fluorescence was studied computationally due to the lack of authentic standards (except for 4PP), showing the following issues: (i) the EEM fluorescence spectrum of 4PP has two emission peaks that do not follow Kasha's rule, because of the occurrence of different rotational conformers with different emission wavelengths; (ii) there is actually a gradual shift of the fluorescence emission wavelengths from the phenolic region (300 nm) to the humic-like one (450 nm). This shift has a plateau trend, because even the five-ring 4PPPPP is foreseen to emit in the same region. The 450-nm emission is accounted for by an electron transfer from the first aromatic

ring (bearing the OH group) to the third one (Barsotti et al., 2016). Considering that phenols are common in lignin and that the protein-like fluorescence in natural waters is largely (~50%) accounted for by free amino acids, it is possible that the described photoinduced formation processes of humic compounds play an important role in natural waters. Interestingly, the compounds arising from the photodegradation of tyrosine share with humic substances not only the EEM spectra, but also the featureless exponential UV-vis absorption spectrum and several acid-base as well as metal complexation properties (Berto et al., 2016).

The following molecule to be studied has been 4-hydroxybenzophenone (OHBP), which includes functional groups that are quite common in humic substances. The EEM spectrum of OHBP in aprotic solvents overlaps with humic peaks A and C, but this compound does not fluoresce in water. The reason is that the excited states (singlet $^1\text{OHBP}^*$ and triplet $^3\text{OHBP}^*$) are strong acids that quickly deprotonate in protic solvents, causing radiationless inactivation. Moreover, in addition to fluorescence, also the production of $^1\text{O}_2$ by $^3\text{OHBP}^*$ is quenched in water (Barsotti et al., 2015). The fluorescence peak at 450 nm is caused by a $S_1 \rightarrow S_0$ transition, but OHBP also has an emission peak at 300 nm that is due to a $S_2 \rightarrow S_0$ transition and does not follow Kasha's rule (Barsotti et al., 2017). Similar non-Kasha behaviour has been described for other aromatic carbonyl compounds.

It has been found that humic substances yield $^1\text{O}_2$ under irradiation, but singlet oxygen is much more concentrated in the waterless humic core than in the surrounding aqueous phase (Latch and McNeill, 2006). This finding may have two explanations: (i) $^1\text{O}_2$ is quenched in water by collision with the solvent, much more quickly than in the humic cores where it can thus occur at higher levels, and (ii) potentially photoactive molecules such as OHBP are quickly inactivated in aqueous solution, but they could produce $^1\text{O}_2$ rather efficiently in a waterless environment.

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