FLUORESCENCE CHARACTERIZATION OF THE EXTRACELLULAR ORGANIC MATTER (EOM) RELEASED BY THE MARINE DIATOM *CHAETOCEROS SP.*

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Abstract
Fluorescence spectroscopy was used to characterize the extracellular organic matter (EOM) released by laboratory cultures of the marine diatom *Chaetoceros.* The accumulation trend of the mixture of chromatophoric compounds into the extracellular medium, as measured on stationary growing cultures, provided a natural system of sample pre-concentration, which revealed just effective for a complete EOM characterization by fluorescence analysis over a wide wavelength range.
Keywords : Organic Matter, Phytoplankton, Mucus Aggregates.

The extracellular organic matter (EOM) produced by phytoplankton represents a significant contribution to the marine DOM on a global scale. Field-based investigations using fluorescence properties of DOM to distinguish water masses from various sources are generally suggesting the UV band (protein-type fluorescence) as a potential tracer of the organic material freshly transferred from phytoplankton to the DOM pool. By contrast, the humic-type fluorescence is typically used as a tracer of riverine inputs of terrestrial humic substances in estuarine and coastal zones [1-3]. Nevertheless, a number of laboratory investigations on different algal species provided experimental evidence that both the protein and humic-type DOM components are released simultaneously by algae, the humic-type fluorescence having species-specific spectral features [4-6].
Since the low concentration, as well as the complexity of the mixture, makes difficult the analysis of EOM in ambient water, the biological procedure based on the monitoring of the extracellular medium from laboratory algal cultures was adopted in this work. The aim was to combine fluorescence analysis with biological assays to provide a complete and distinctive characterization of *Chaetoceros* EOM.
The marine diatom *Chaetoceros sp.*, isolated from North Adriatic coastal waters, was aseptically cultured in laboratory. Controlled conditions supporting a long-lasting stationary growth phase of living cells were provided to favor the natural accumulation of the released EOM compounds [5]. After an incubation time of 7 months, a set of filtered EOM-samples from differently aged cultures was simultaneously sampled and immediately analyzed by synchronous fluorescence spectroscopy in a wavelength range of 250-500 nm using a Dl =25 nm [5,6].

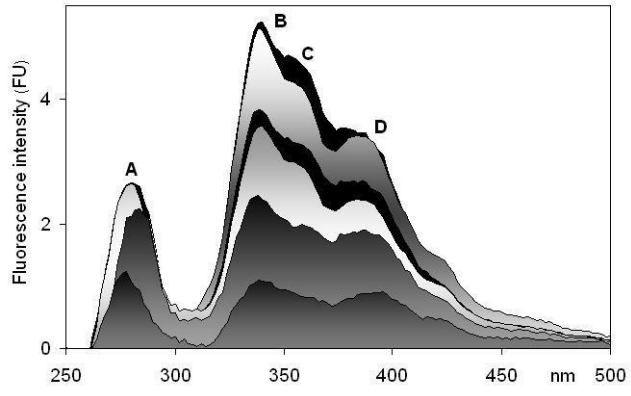

Fig. 1. Synchronous spectra of EOM accumulated during 7 months

As shown in Fig.1, both the fluorescing components are present in each of the 6 spectra of the EOM released by *Chaetoceros*. The two, UV and visible excitation-maxima A and B, were recorded at 280 and 340 nm, respectively. As shown in Fig. 2, their fluorescence intensity follows a different evolution trend over time.
The protein-type peak A, which is typically produced by algae only in exponential growth [5,6], maintains an intensity of about 2 FU all over the sampling period. By contrast, the humic-type peak B, which is also present ever since the first sample, increases progressively with ageing of cultures, becoming the main EOM component in the most aged samples. The accumulation trend of the humic-type EOM into the extracellular medium was just working as a sample-preconcentration system, effective for a spectral fingerprint of the mixture of chromatophoric compounds.

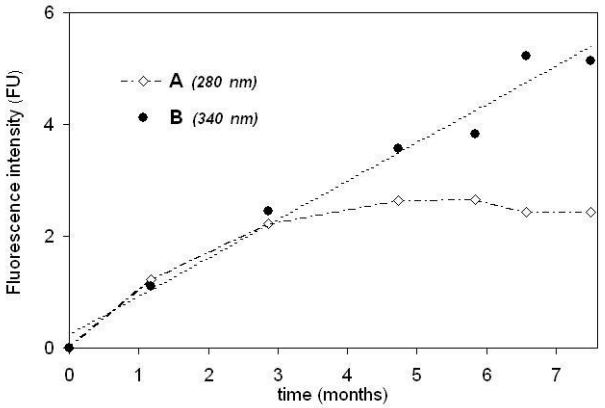

Fig. 2. Trends of the fluorescence-intensity maxima A and B

When spectra of Fig.1 are compared with previously analyzed EOM from different algal species [5,6], the humic-type peak position (B,C,D) as well as the maximum location (B) in the range 300 to 450 nm, contrary to the aspecific UV-peak A, are clearly distinctive of the *Chaetoceros* EOM. The results suggest that the complete spectral fingerprint of EOM released by phytoplankton could greatly help field-based studies on DOM sources in marine environments.

References

1 - Coble P. G., 1996. Characterization of marine and terrestrial DOM in seawater using EEM spectroscopy. *Mar. Chem*., 51: 325-346.
2 - Mayer L.M., Schick L.L. and Loder III T.C., 1999. Dissolved protein fluorescence in two Maine estuaries. *Mar. Chem*., 64: 171-179.
3 - Parlanti E., Worz K., Geoffroy L. and Lamotte M., 2000. DOM fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochem*., 31: 1765-1781.
4 - Mingazzini M., Colombo S. and Ferrari G.M., 1995. Application of spectrofluorimetric techniques to the study of mucilages in Adriatic Sea. *Sci. Total Environ.*, 165: 133-144.
5 - Mingazzini M., 2001. Synchronous fluorescence spectra as chemical tracers to monitor the DOM in North Adriatic waters. *In*: Faranda F. M. et al. (Eds.), Mediterranean Ecosystems. Springer Verlag, 16: pp. 115-122.
6 - Mingazzini M., Palumbo M.T. and Senesi N., 2006. A combined biological and fluorescence approach for the characterization of the humic-type EOM released by phytoplankton. 13th IHSS Meeting, Karlsruhe, Jul. 2006 . Proc. Vol. 45-I: 401-404.