Featured Article

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Aerosol inputs affect the optical signatures of dissolved organic matter in NW Mediterranean coastal waters

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Summary: Aeolian inputs of organic and inorganic nutrients to the ocean are important as they can enhance biological production in surface waters, especially in oligotrophic areas like the Mediterranean. The Mediterranean littoral is particularly exposed to both anthropogenic and Saharan aerosol depositions on a more or less regular basis. During the last few decades experimental studies have been devoted to examining the effect of inorganic nutrient inputs from dust on microbial activity. In this study, we performed experiments at two different locations of the NW Mediterranean, where we evaluated the changes in the quality and quantity of dissolved organic matter due to atmospheric inputs of different origin (Saharan and anthropogenic) and its subsequent transformations mediated by microbial activities. In both experiments the humic-like and protein-like substances, and the fluorescence quantum yield increased after addition. In general, these changes in the quality of dissolved organic matter did not significantly affect the prokaryotes. The recalcitrant character of the fluorescent dissolved organic matter (FDOM) associated with aerosols was confirmed, as we found negligible utilization of chromophoric compounds over the experimental period. We framed these experiments within a two-year time series data set of atmospheric deposition and coastal surface water analyses. These observations showed that both Saharan and anthropogenic inputs induced changes in the quality of organic matter, increasing the proportion of FDOM substances. This increase was larger during Saharan dust events than in the absence of Saharan influence.

Keywords: FDOM; aerosol deposition; DOC; Mediterranean Sea.

Los aportes de aerosoles afectan las propiedades ópticas de la materia orgánica disuelta en las aguas costeras del Mediterráneo Noroccidental

Resumen: Los aportes atmosféricos de nutrientes orgánicos e inorgánicos al océano son importantes ya que pueden aumentar la producción biológica en aguas superficiales, especialmente en las zonas oligotróficas como el Mediterráneo. El litoral del Mediterráneo está particularmente expuesto a aportes de origen antropogénico y a deposiciones de polvo sahariano de forma más o menos regular. Durante las últimas décadas los estudios experimentales se han dedicado, sobre todo, a examinar el efecto de la entrada de nutrientes inorgánicos atmosféricos sobre la actividad microbiana. En este estudio, se realizaron experimentos con comunidades microbianas procedentes de dos zonas del Mediterráneo noroccidental. Se evaluaron los cambios en la calidad y cantidad de la materia orgánica disuelta debido a aportes atmosféricos de distinto origen y sus posteriores transformaciones mediadas por actividades microbianas. En ambos experimentos las sustancias orgánicas fluorescentes y el rendimiento cuántico de fluorescencia aumentaron después de la adición de material atmosférico. En general, estos cambios en la calidad de la materia orgánica no afectaron significativamente a los organismos procariotas. El carácter recalcitrante de la materia orgánica disuelta fluorescente (FDOM) contenida en los aerosoles se confirmó ya que la utilización de compuestos cromóforos durante el período experimental fue insignificante. Los resultados obtenidos se contextualizan en relación con una serie temporal de dos años de datos adquiridos de deposición atmosférica y análisis de agua superficial costera. La variabilidad temporal de estas dos variables mostró que tanto los aportes saharianos como antropogénicos provocaron cambios en la calidad de la materia orgánica disuelta en aguas superficiales, incrementando la fracción fluorescente. Éste aumento resultó ser mayor durante eventos de polvo sahariano que en ausencia de ellos.

Palabras clave: FDOM; deposición de aerosoles; DOC; mar Mediterráneo.

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INTRODUCTION

The Mediterranean Sea, due to its low nutrient and chlorophyll concentration, is considered one of the most oligotrophic marine systems (McGill 1965, Krom et al. 1991, Lucea et al. 2003). During the stratification period, a severe nutrient depletion causes both phytoplankton and bacterioplankton to be strongly limited in phosphorus or nitrogen (Béthoux et al. 1998, Thingstad et al. 1998, Sala et al. 2002). However, climatic conditions and the geographic location of the Mediterranean favour the reception of nutrients due to a noticeable dust flux from the Saharan desert (Guieu et al. 2014b). Around 20 to 50 10^6 t y^{-1} (Guerzoni et al. 1999) of dust from the Sahara are transported to the Atlantic ocean through the predominant westerly winds and towards the Mediterranean basin influenced by the presence of cyclones (Moulin et al. 1997).

Large Saharan dust transport events over the Mediterranean Sea occur commonly in spring and summer (Volpe et al. 2009). Saharan dust provides soluble nutrients (e.g. nitrogen, phosphorus and iron) to surface waters, so its deposition in marine waters can favour plankton productivity in the ocean (Gallisai et al. 2014). During the last few years, the effort to understand the impact of dust deposition on the biogeochemistry of the ocean has increased (Jickells et al. 2005, Suárez et al. 2008). In fact, field and experimental studies in several aquatic ecosystems of the Mediterranean region have shown that Saharan dust may stimulate both phytoplankton and bacterioplankton growth (Pulido-Villena et al. 2008, Romero et al. 2011, Guieu et al. 2014a). However, little attention has been paid to the effect of anthropogenic-derived particles, which have a mainly European origin in the NW Mediterranean (e.g. Guerzoni et al. 1999). Anthropogenic aerosols in the Barcelona area can be a major source of nitrogen and phosphorous to the atmosphere (Querol et al. 2004, Izquierdo et al. 2012). Furthermore, they are much richer than Saharan particles in organic carbon, particularly in black carbon produced by high temperature combustion processes (Querol et al. 2001, Pateraki et al. 2012). On the other hand, anthropogenic aerosols tend to contain high amounts of copper, lead and other trace metals, which are known to be toxic to microbiota at high concentrations (Paytan et al. 2009, Jordi et al. 2012). Thus, one way or another, an effect of anthropogenic aerosols on marine production is also expected. Much less attention has been paid to the potential impact of aerosols on the quality of dissolved organic matter (DOM), and in particular on the quality of the optically active fraction (CDOM). This fraction is a key parameter regulating the penetration of ultraviolet radiation in the water column, so changes in its concentration can alter both primary and secondary production (Smith and Cullen 1995). A sub-fraction of CDOM that emits light when excited by UV radiation is called fluorescent dissolved organic matter (FDOM). Fluorometric analyses can be used to characterize this sub-fraction. Emission fluorescence spectra can be collected at different excitation wavelengths represented in excitation-emission matrices (EEMs), and different peaks of humic- and protein-like fluorophores can be distinguished (Coble 1996, 2007). Usually, peak C and M are associated with humic-like substances, while peak T corresponds to protein-like substances. The fluorescence intensity of these peaks can be used as indicators of biological (Chen and Bada 1992) and photochemical processes (Moran 2000) of the DOM pool.

The optical properties of CDOM are sensitive to biological and physical processes and thus provide valuable information not only of the biogeochemical processes in aquatic environments, but also of the origin of organic matter (OM). Mladenov et al. (2011) determined that the organic carbon associated with dust inputs can contribute to the DOM pool in alpine lakes and that the fraction of airborne water-soluble OM can contain chromophoric groups similar to humic-like substances. More recently, de Vicente et al. (2012) reported that the chromophoric components related to the dust inputs significantly affected water transparency to ultraviolet radiation. The study of aerosol influx as a potential source of CDOM is of particular interest in the Mediterranean Sea because these waters are peculiar for having unusually high values of CDOM to chlorophyll ratio in comparison with other marine systems. Our study aimed to 1) quantify the impact of aerosols of different origin (Saharan and non-Saharan) on the CDOM deposition in the surface waters of a NW Mediterranean coastal system and 2) evaluate the posterior chemical transformations in surface waters by means of CDOM optical signatures. To carry out these objectives, we collected weekly to biweekly samples of atmospheric deposition during 23 months for FDOM analyses concurrently with surface water samples in the Barcelona coastal area. Within this time frame we also conducted two aerosol addition experiments with NW Mediterranean coastal waters, in which we evaluated prokaryote and FDOM dynamics in response to both Saharan dust and anthropogenic aerosols.

MATERIALS AND METHODS

Time series sampling

We collected samples for atmospheric deposition and seawater analyses over a two-year period (September 2012 - July 2014). For atmospheric deposition, one high-density polyethylene (HDPE) container was filled with 500 mL of sterile artificial seawater and left open on the roof of the Institute of Marine Sciences (ICM-CSIC, Barcelona, 41°23′08″N, 2°11′45.5″E) for about one week in summer and two in winter. Upon collection, subsamples for FDOM were analysed after filtering them through Whatman GF/F filters. The fluo-

rescence intensities measured for sterile seawater at time 0 were subtracted from those measured at the end of the exposure period. Seawater samples were taken monthly 0.5 km offshore of Barcelona (NW Mediterranean, 41°22′55″N, 2°11′58″E). Surface water was collected in 2-L acid-cleaned polycarbonate bottles and subsamples for FDOM were analysed freshly.

Aerosol collection for experiments

The aerosols used in the experiments were collected on Munktell quartz filters (quality 360) using an MCV CAV-A/mb high-volume air sampler. The sampler operated for 24 h at 30 m^3 h^{-1}. Filter samples for experimental amendments were obtained at different times in January and March 2014 on the roof of the Institute of Marine Sciences in Barcelona (41°23′08″N, 2°11′45.5″E) and on the roof of the Centre for Advanced Studies of Blanes (CEAB, Blanes, 41°40′59.5″N, 2°48′2.6″E). After collection, half of the filters were used for chemical analyses and the other half were employed for the amendment experiments. Collected aerosols tend to be a mix from different sources. The aerosols were classified according to the relative percentage of Saharan dust versus inputs of anthropogenic origin with previous knowledge of the presence of Saharan events based on transport and deposition models and forecasts (www.calima.ws) and on the chemical analyses of the filters. Aerosols of anthropogenic origin tend to have a higher proportion of non-mineral carbon, nitrogen species and phosphorus, while Saharan dust has a higher proportion of silicate and aluminium oxide (Table 1).

Table 1. – Relative percentage of the composition of the different aerosols used in the BLSp and BCNSp experiments, respectively, analysed from filtered air. Abbreviations: A, anthropogenic; S, Saharan; OC, organic carbon; CO_3, carbon trioxide; SiO_2, silicate oxide; Al_2O_3, aluminium oxide; NO_3^-, nitrate; NH_4^+, ammonium; and P, phosphorus.

	Blanes (BLSp)		Barcelona (BCNSp)	
	A	S	A	S
OC	31.95%	4.93%	26.38%	6.75%
SiO_2	4.75%	40.64%	13.56%	27.88%
Al_2O_3	1.58%	13.55%	4.51%	9.29%
NO_3^-	11.01%	2.48%	7.81%	2.11%
NH_4^+	2.12%	0.37%	1.47%	0.52%
P	0.10%	0.08%	0.13%	0.07%

Water sampling and experimental design

Our experiments were conducted with water from two locations that differed in the degree of oligotrophy. The water was collected at the Blanes Bay Microbial Observatory (41°40′0″N, 2°48′0″E) on 8 April 2014 and on the Barcelona coast (41°22′55″N, 2°11′58″E) on 12 May 2014. Blanes Bay Microbial Observatory is characterized as an oligotrophic area with an annual mean of 0.63±0.05 μg L^{-1} of chlorophyll (Guadayol et al. 2009). The Barcelona coastal area is less oligotrophic as it receives nutrients from the discharge of two rivers, the Besòs River located in the north of the city and the Llobregat River in the south. The annual average chlorophyll concentration at the Barcelona station is 1.58±1.09 μg L^{-1} (Romero et al. 2014). Both experiments were conducted in mid-spring. This season appears to be the ideal period for testing the impact of dust in surface waters of the Mediterranean Sea, because it is a time interval of the year with frequent dust events (Guerzoni et al. 1997, Gkikas et al. 2009, Gallisai et al. 2014).

The experiments were termed BLSp and BCNSp for Blanes and Barcelona, respectively. For both, the water was collected from the surface layer (approximately 0.5 m depth) and pre-filtered through a 150-μm nylon mesh to remove the larger zooplankton. The water was then transported to the laboratory in 50-L carboys, which had previously been washed with a dilute solution of sodium hypochlorite and exhaustively rinsed with Milli-Q water and sample water.

In the laboratory, the water was distributed in 15-L cylindrical methacrylate containers, which were subjected to experimental conditions in a light and temperature controlled chamber for 7 days for BLSp and for 5 days for BCNSp. Conditions, in duplicate, were anthropogenic particles enrichment (A), Saharan dust enrichment (S) and control (C) without enrichment. Aerosol concentration added in each container was 0.8 mg L^{-1}. Light conditions were set to 225 μmol photons m^{-2} s^{-1} inside the containers and the light:dark cycle (13 h:11 h) and temperature (17.5°C) were adjusted to natural conditions. After placing the containers in the experimental chamber, we left them for acclimation before starting the experiment. Because an in situ Saharan event occurred the day before BLSp water collection, we increased the acclimation period (it lasted 45 hours in BLSp in contrast to 19 hours in BCNSp) to prevent the experimental treatment from being masked by a possible response to the in situ input that occurred in the field. An initial sample was taken and aerosols were subsequently added as single doses.

Samples for DOM analyses (CDOM, FDOM and dissolved organic carbon (DOC)) were filtered by glass fibre Whatman GF/F (combusted at 450°C for 4 hours) prior to analysis. Samples were taken at 0, 4, 49, 97, and 144 h for BLSp and at 0, 4, 49 and 97 h for BCNSp. Samples for chlorophyll *a* (Chl *a*) and bacteria were taken daily.

Analytical procedures

CDOM measurements

CDOM absorption was measured in 10-cm quartz cuvettes using a Varian Cary UV-VIS spectrophotometer equipped with a 10-cm quartz cell. Absorbance was performed between 250 and 750 nm at a constant room temperature of 20°C. Milli-Q water was used as blank. The residual backscattering (colloidal material, fine size particle fractions present in the sample) was corrected by subtracting the mean absorbance calculated in the spectral range 600-750 nm. The absorption coefficient ($a_{CDOM}(\lambda)$ in m^{-1}) was calculated as

$$a_{CDOM}(\lambda)=2.303\ A(\lambda) / L$$

where A is the absorbance at wavelength λ, L is the optical path length in m, and 2.303 is the factor that transforms decimal logarithms to natural logarithms.

FDOM measurements

The samples for FDOM were measured immediately after temperature acclimation according to Nieto-Cid et al. (2006). Single measurements and emission-excitation matrices were performed with an LS-55 Perkin Elmer luminescence spectrometer equipped with a xenon discharge lamp, equivalent to 20 kW. Slit widths were set to 10 nm for emission and excitation wavelengths and scan speed was 250 nm min^{-1}. Measurements were performed in a 1-cm quartz cell. The EEMs were generated by concatenating 21 synchronous spectra over excitation wavelengths of 250 to 450 nm and emission wavelengths of 300 to 650 nm with an offset between the excitation and emission wavelengths of 50 nm in the first scan and 250 nm in the last scan. Milli-Q water was used as a blank and Raman scattering was corrected by subtracting the Milli-Q water signal. The samples were converted into quinine sulphate units (QSU). The excitation-emission (Ex/Em) wavelengths used for single measurements were described by Coble (1996): Peak C (Ex/Em 340/440 nm) as an indicator of terrestrial-like substances, peak M (EX/Em 320/410 nm) as an indicator of marine-like substances and peak T (Ex/Em 280/420 nm) as an indicator of protein-like substances.

Finally, the fluorescence quantum yield at 340 nm, defined as the portion of light absorbed at 340 nm that is re-emitted as fluorescence [Φ(340)], was determined using the ratio of the absorption coefficient at 340 nm and the corresponding fluorescence emission between 400 and 600 nm of the water sample and referred to the quinine sulphate standard (QS) ratio (Green and Blough 1994) :

$$\Phi(340) = \frac{F(400-600)}{a_{CDOM}(340)} \cdot \frac{a_{CDOM}(340)_{QS}}{F(400-600)_{QS}} \Phi(340)_{QS}$$

where $a_{CDOM}(340)_{QS}$ is the absorption coefficient of the QS standard at 340 nm (in m^{-1}); F(400-600) and F(400-600)$_{QS}$ are the average integrated fluorescence spectra between 400 and 600 nm at a fixed excitation wavelength of 340 nm (in QS units) obtained for each sample and the QS standard, respectively (Romera-Castillo et al. 2011); $\Phi(340)_{QS}$ is the dimensionless fluorescence quantum yield of the QS standard and equals 0.54 (Melhuish 1961); and $a_{CDOM}(340)$ is the absorption coefficient of each sample at 340 nm. In this study, the ratio Φ(340) was calculated to add another descriptor of the coloured dissolved organic matter. It has been shown that this ratio increases when microbial transformations dominate in comparison with photobleaching and vice versa (De Haan 1993, Lønborg et al. 2010).

DOC analysis

Samples for DOC were filtered through Whatman GF/F filters using an acid-cleaned glass filtration system. Approximately 10 mL of water was collected in pre-combusted (450°C for 12 h) glass flasks for DOC determination. After acidification with H_3PO_4 (50 μL) to pH<2 the ampoules were heat-sealed and stored in the dark until analysis. DOC was analysed following the high temperature catalytic oxidation (HTCO) technique (Cauwet 1994, Sugimura and Suzuki 1998, Cauwet 1999) using a Shimadzu TOC-L analyser. The system was calibrated daily with a solution of acetanilide (C_8H_9NO MW=135.17). The DOC concentration was determined by subtracting the blank samples.

Prokaryotic abundance and Chl a *determination*

Heterotrophic prokaryotic cells were quantified by flow cytometry according to the method of Gasol and del Giorgio (2000). Hereafter the term "prokaryote" will be used as synonym of "heterotrophic prokaryote". Samples (1.8 mL) were fixed with 0.18 mL of a 10% paraformaldehyde and 0.5% glutaraldehyde mixture. Subsamples of 400 μL were stained with SybrGreen deoxyribonucleic acid fluorochrome and left to stain for 15 min in the dark and then ran at low speed (ca. 30 μL min^{-1}) through a Becton Dickinson FACSCalibur flow cytometer with a laser emitting at 488 nm. As standard, 10 μL per sample of a 10^6 mL^{-1} solution of yellow-green 0.92-μm latex beads was added.

For total Chl *a*, a 30-mL sample was filtered through Whatman GF/F glass fibre filters and subsequently extracted with acetone (90%) for 24 h at 4°C in the dark. The fluorescence of the extract was measured with a Turner Designs fluorometer (Yentsch and Menzel 1963).

RESULTS

FDOM time series

In order to evaluate the potential role of atmospheric deposition on the dynamics of coastal FDOM, we calculated its proportion with respect to the in situ seawater concentration for 23 months (September 2012 to July 2014). High contributions to the DOC pool could be found at all times of the year, although the highest was in summer (July 2013). The results revealed that the deposition of humic-like compounds (peak C and M) and protein-like compounds contributed to an increase in FDOM in surface waters that represented between 0.2% and 3% per m^3 per day (Fig. 1). The highest increases (>1% per m^3 per day) tended to coincide with Saharan dust events, although not always. The annual amount of these compounds deposited per m^2 ranged from 1.9 to 2.3 times the average in situ concentration per m^3. Thus, for a 10 m water column, the annual atmospheric input is between 19% and 23% of the standing stock.

Microcosm experiments

Prokaryotic abundance and Chl a

The initial abundance of heterotrophic prokaryotic cells was 8.06×10^5 cells mL^{-1} in BLSp and 1.34×10^6 cells mL^{-1} in BCNSp (Fig. 2A, B). In BLSp, we observed a small increase in cell abundance after aerosol addition that continued to a peak after 1 d. This peak was somewhat larger for A and S than in the control. After this peak, prokaryotes decreased over time, more

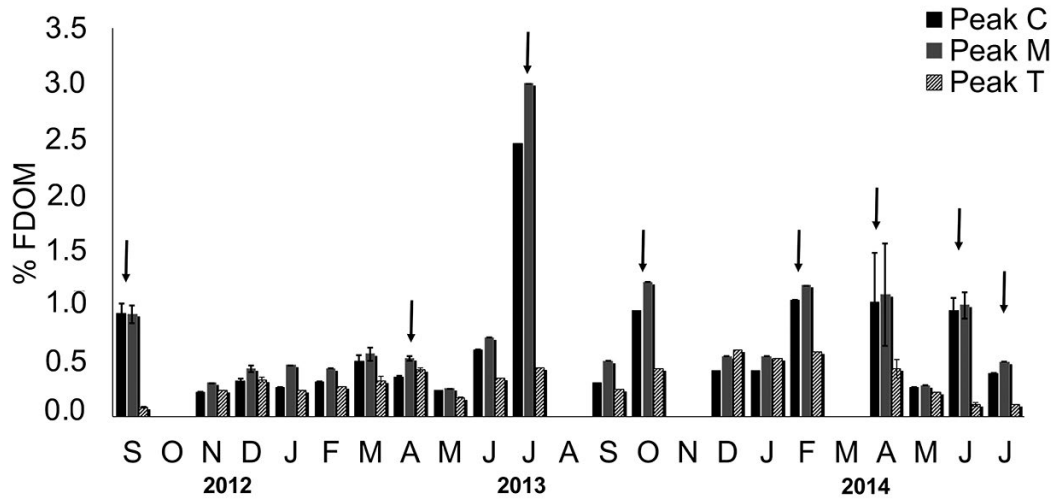

Fig. 1. – Daily aerosol deposition-derived FDOM flow to the sea surface as a percentage of concentration in Barcelona coastal waters. Humic-like (peak C and peak M) and protein-like (peak T) substances. The arrows indicate the Saharan dust events. The percentages were calculated as the daily average of the deposition considering five different time frames (one to five days).

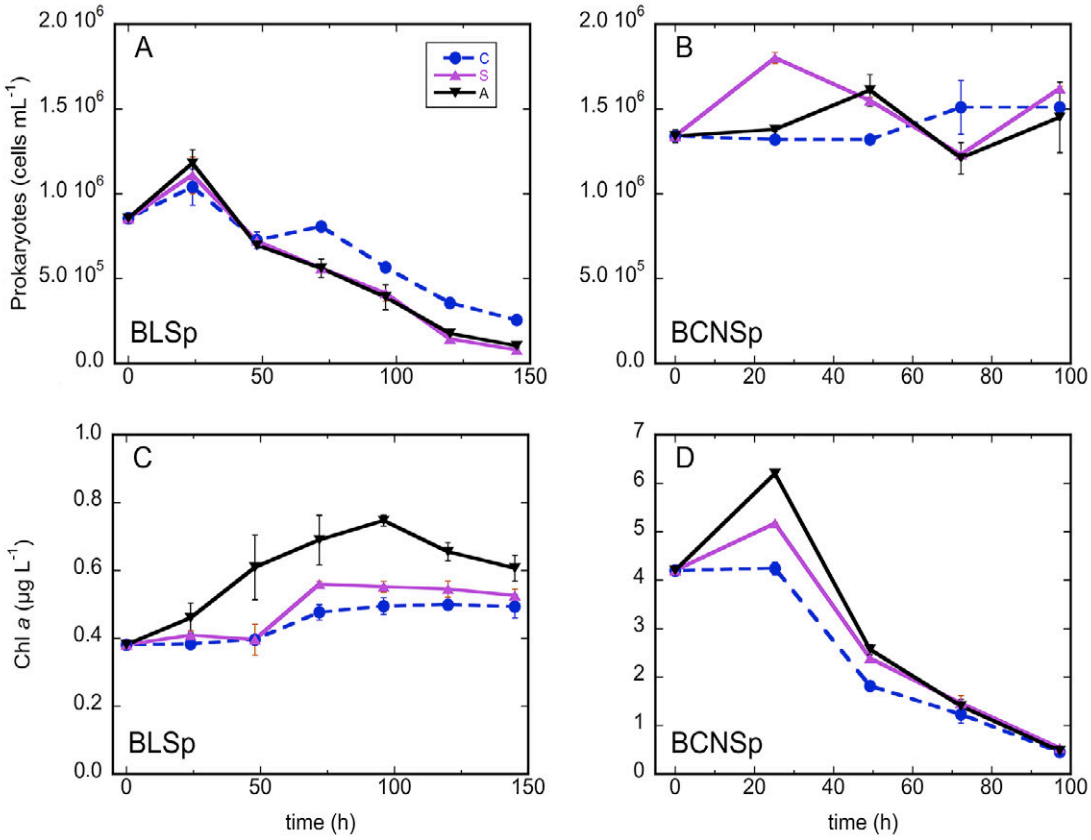

Fig. 2. – Heterotrophic prokaryotic abundance over time for the three treatments of BLSp (A) and BCNSp (B) experiments. Aerosols were added at t=4 h in both experiments.

so in aerosol-amended containers than in the control (Fig. 2A). BCNSp also showed a small initial increase in heterotrophic bacteria abundance following aerosol addition in all the treatments. After that, bacteria peaked at day 1 in S and at day 2 in A and showed some fluctuations. Bacteria in C showed more steady concentrations over time. Unlike in BLSp, bacteria showed no clear decreasing trend after peaking and fluctuated around $1.5 \cdot 10^6$ cells mL^{-1} (Fig. 2B).

During BLSp, Chl *a*, which is a proxy for phytoplankton biomass, increased more in A than in S and C, reaching values of 0.51±0.02, 0.74±0.02 and 0.56±0.01 µg L^{-1} for C, A and S, respectively (Fig. 2C). In BCNSp, a peak in S and A was observed at day 1 (higher in A) and then Chl *a* declined to the end of the experiment, reaching a common value of ca. 0.45 µg L^{-1} on the last day in all containers, including C (Fig. 2D).

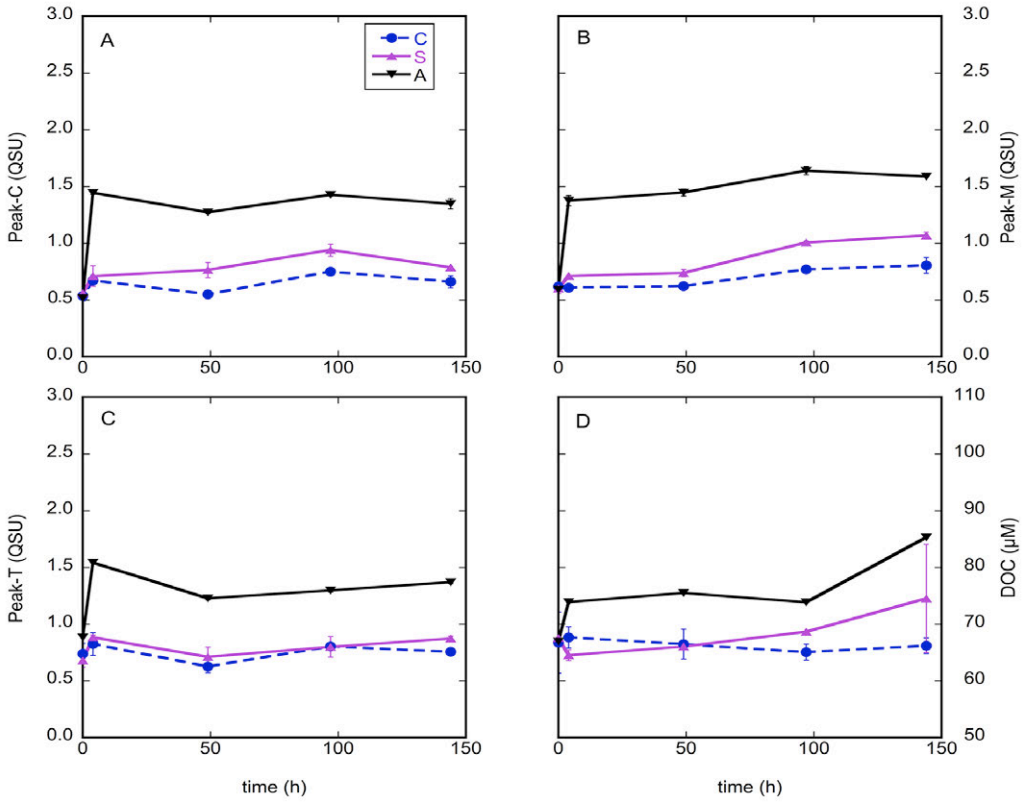

Fig. 3. – Fluorescence intensities of FDOM peaks during the course of incubation in the BLSp experiment. (A) peak C, (B) peak M, (C) peak T and (D) dissolved organic carbon (DOC). The FDOM peaks are in quinine sulphate units (QSU) and DOC is in µM. The bars indicate the standard deviation.

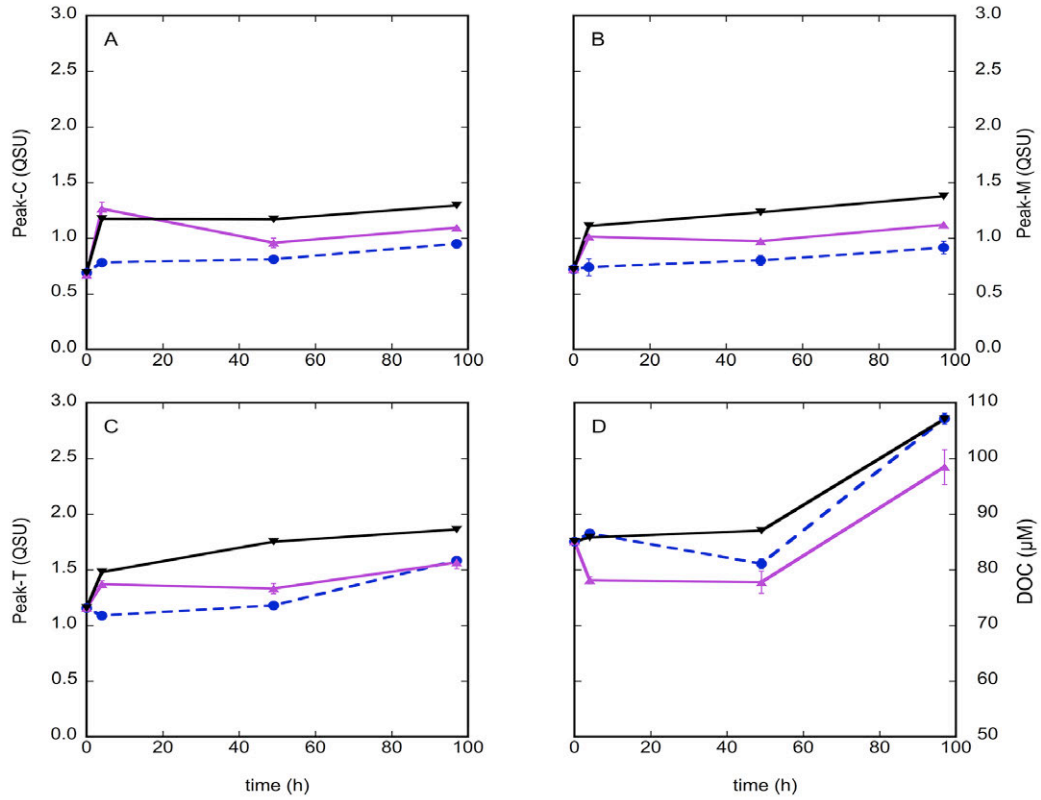

Fig. 4. – Fluorescence intensities of FDOM peaks during the course of incubation in the BCNSp experiment. (A) peak C, (B) peak M, (C) peak T and (D) dissolved organic carbon (DOC). The FDOM peaks are in quinine sulphate units (QSU) and DOC is in µM. The bars indicate the standard deviation.

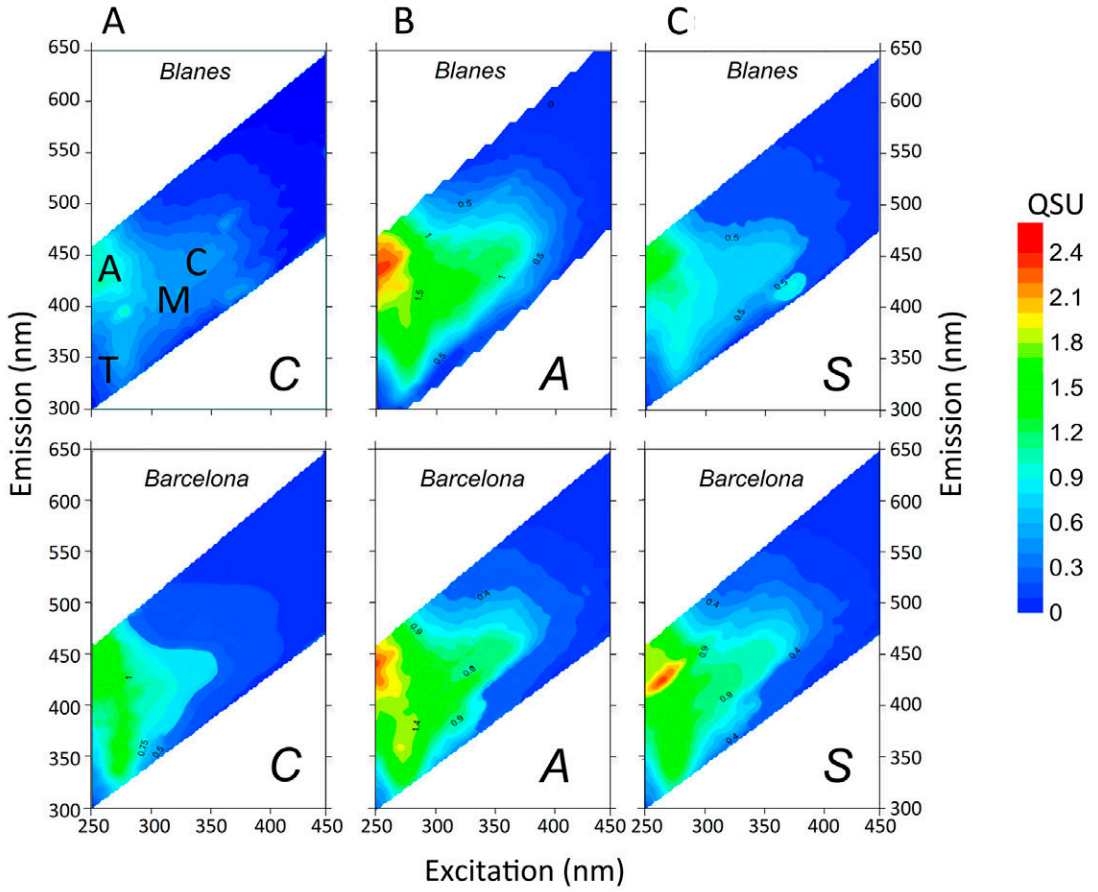

Fig. 5. – Changes in the excitation-emission matrix (EEM) of FDOM after dust addition in the two experiments. Before addition (*C*) in column A, and after additions of anthropogenic aerosols (*A*) in column B and Saharan dust (*S*) in column C. The different peaks are indicated. The fluorescence is expressed in quinine sulphate units (QSU).

Dynamics of DOC and FDOM

Aerosols did not significantly alter the DOC concentration (Figs 3D and 4D). The largest difference was found after anthropogenic addition in BLSp (Fig. 3D), where it accounted for an increase of about 10% in the total DOC concentration. After the enrichment, DOC concentrations were maintained fairly constant in all conditions of the BLSp experiment. In BCNSp, DOC values did not increase immediately after addition, but an increase at the end of the experiment occurred in all the containers (Fig. 4D). In both experiments, the increase in humic-like (Figs 3A, 3B, 4A and 4B) and protein-like (Figs 3C and 4C) substances was higher in the treatment enriched with anthropogenic particles than in the one enriched with Saharan dust. Humic substances reached values of about 1.5 QSU in A conditions, while in treatments C and S the maximum values were about 0.74 and 0.94 QSU, respectively. Although the increase in humic compounds after addition in treatment A was higher in BLSp, the different groups of OM followed similar patterns in all treatments in both BLSp and BCNSp (Figs 3A, 3B, 3C, 4A, 4B and 4C). After the initial increase due to dust addition, FDOM values were maintained fairly constant during the course of incubation (Figs 3 and 4).

We compared the FDOM matrices before addition (C) with the changes in FDOM that occurred in each experiment after the addition (Fig. 5). In BLSp, the EEM in treatment A showed marked fluorescence peaks in the humic-like and protein-like areas after addition (reaching concentrations around 1.5 QSU), whereas in the S microcosm the increase in fluorescence intensity was minimal (about 0.2-0.3 QSU) and with no defined peak. In BCNSp, the EEM of the water before addition (C condition, Fig. 5) showed two peaks at Ex/Em 280/350 nm and 250/435 nm, corresponding to peak T and peak A (2.0 and 1.3 QSU, respectively). In contrast, the fluorescence in the treatment A after the addition showed two peaks within the range of the marine and terrestrial humic-like substances (peak M and peak C, respectively). These increases were small (about 0.5 to 0.75 QSU) in comparison with BLSp. Finally, the fluorescence alteration after additions in treatment S was low in comparison with initial fluorescence intensities (values only increased by about 0.1-0.3 QSU in the humic-like area, and by about 0.4 QSU in the protein-like area).

DISCUSSION

Atmospheric deposition influence on surface waters

Our two-year data set on deposition constitutes the first time series that evidences the atmospheric impact on FDOM dynamics in Mediterranean surface waters.

Interestingly, several of the highest FDOM deposition values coincided with the Saharan dust events (Fig. 1). Although CDOM is present in low concentrations in the Mediterranean (Romera-Castillo et al. 2013, Xing et al. 2014), the CDOM/chlorophyll ratio is exceptionally high in comparison with other areas (Morel and Gentili 2009, Organelli et al. 2014, Pérez et al. 2016). Because FDOM is a part of the CDOM pool, our observations could indicate that atmospheric inputs during Saharan events could significantly contribute to this high CDOM/chlorophyll ratio. Para et al. (2010) pointed out that the humic fluorescent components and the salinity had an exceptionally weak correlation and suggested that other processes could influence CDOM distributions. Thus, our results about FDOM deposition during Saharan events could help to explain these anomalies.

Effects of aerosol additions on prokaryotic abundances

Previous studies have shown that prokaryotic abundances increased in response to Saharan dust inputs in oligotrophic systems (Pulido-Villena et al. 2008, Reche et al. 2009, Marín et al. 2017). In our experiments, the prokaryotic response to aerosol addition was relatively low. BLSp showed more oligotrophic conditions than BCNSp, as can be seen from the initial bacterial and Chl *a* concentrations. In BLSp an initial peak response was seen in all treatments and thus cannot be attributed to aerosol addition, whereas the response of chlorophyll took place much later in time. This is in good agreement with the results obtained by Marañon et al. (2010), they also observed a quicker response of prokaryotes compared with that of chlorophyll when studying oligotrophic areas. The opposite occurred in more eutrophic areas (Teira et al. 2013). In general, the abundance tended to decrease during the course of the incubations, and this decrease was more conspicuous in the enriched treatments than in the control. We attributed this behaviour to the competition for limiting nutrients between phytoplankton and bacteria (Marañon et al. 2010). However, the response in both experiments was lower than expected. Ridame (2001), Marañon et al. (2010) and Herut et al. (2005) also found low prokaryotic stimulation to aerosol inputs in Mediterranean waters. Differences in the microbial responses seemed to be related to the initial environmental conditions (e.g. nutrient availability). Martinez-García et al. (2015) also pointed out the importance of initial conditions to explain the variety of microbial responses when examining the effect of rainwater additions in experiments performed with NW Iberian Peninsula shelf waters. In our experiments, the quality of the added particles could be another factor explaining the differences in microbial response between the two experiments. Even if the A and S aerosols were collected during non-Saharan and Saharan events, respectively, the OM composition of the particles differed between locations-experiments. The aerosols collected at the Blanes site induced a higher increase in FDOM in the experimental waters than in the aerosols collected in Barcelona (especially during non-Saharan events). As the weight of total aerosol added was the same between treatments and experiments, these differences indicated local peculiarities related to the quality of the particles. However, more data are needed to investigate the causes of these differences in quality and whether or not they are persistent.

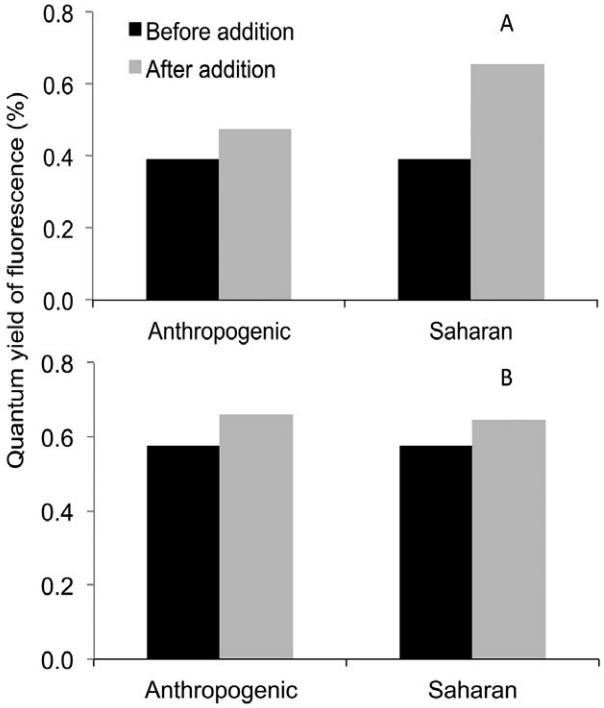

Fig. 6. – Fluorescence quantum yield at 340 nm [Φ(340)] before and after anthropogenic and Saharan dust addition. A, BLSp experiment; B, BCNSp experiment. The [Φ(340)] is expressed in percentage (%).

Transformations of DOM optical properties after dust additions

In BLSp, the humic-like fractions of OM increased after the addition of aerosols, and this increase was more conspicuous in treatment A than in treatment S. In fact, peak C/DOC ratios in QSU/μmol C L^{-1} were 153% higher in A and 33% higher in S than in the control. However, FDOM compounds showed no variation during the incubation time. In BCNSp, FDOM values were also higher after the addition. However the increases in FDOM/DOC ratio after additions did not differ significantly between A and S. In both BLSp and BCNSp, we observed that DOC tended to increase in all treatments during the time of incubation. This increase was larger in BCNSp than in BLSp, which is in accordance with a high activity of phytoplankton (Fig. 2C, D). The EEMs confirmed that both anthropogenic and Saharan aerosols contained fluorescence organic substances (Fig. 5), as has been previously reported by Mladenov et al. (2011).

Regarding the fluorescence quantum yield at 340 nm [Φ(340)], we observed a similar increase in both experiments after the addition was performed, reaching values of about 0.65% (Fig. 6). The quantum yield decreases with light exposure and increases with microbial activity (Romera-Castillo et al. 2011). Therefore,

the increase in quantum yield values observed after the enrichment could indicate a rapid, although low, bacterial response. In fact, we observed that low values of Φ(340) coincided with low bacterial abundance and vice versa. The values of Φ(340) obtained in our experiments were within the range of other data reported previously from field studies in the Mediterranean (Ferrari 2000, Romera-Castillo et al. 2011*)*, thus indicating that our induced changes in optical characteristics of OM were within the range of variations occurring in nature.

CONCLUSIONS

A two-year time series data shows, for the first time, the influence of atmospheric deposition on the temporal dynamics of FDOM in Mediterranean surface waters. This time series data evidences an increase in the FDOM flux during Saharan events. Our experimental results revealed that aerosol deposition induced an increase in the proportion of FDOM in comparison with DOC. The refractory character of the OM added with aerosols was confirmed from the negligible utilization of this fraction within a short time period (days). Thus, considering our in situ results showing how dust deposition increases the coloured DOC content in surface waters, together with the experimental findings that corroborate the low biological utilization of this coloured fraction, we conclude that the atmospheric deposition could help to explain the exceptionally high CDOM/ chlorophyll values found in the Mediterranean Sea.

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