Characterization of natural organic matter from Mediterranean coastal seawater

Ywann Penru, F. Xavier Simon, Andrea R. Guastalli, Santiago Esplugas, Joan Llorens and Sylvie Baig

ABSTRACT

Natural organic matter (NOM) from seawater is a complex mixture of compounds that has only been roughly characterized. In this study, advanced techniques such as fractionation based on adsorption–desorption dynamics (on XAD® resins), liquid chromatography–organic carbon detection (LC-OCD), excitation–emission matrix (EEM) fluorescence and membrane separation techniques were applied to the characterization of seawater NOM. Conventional analyses have shown that Mediterranean coastal seawater has a low NOM content, slightly aromatic and poorly biodegradability. Advanced analysis permitted better characterization by separating the NOM into different fractions. The NOM was found to be mainly hydrophilic (HPI) (70%) and slightly transphilic (TPI) (24%) from XAD fractionation. LC-OCD showed the seawater to be composed mainly of humic substances (HSs) and low molecular weight (LMW) neutrals that accounted for 37 and 40% of the dissolved organic carbon, respectively. The HSs contained hydrophobic, TPI and HPI compounds, revealing advanced hydrolysis, whereas the LMW neutrals were HPI compounds. Membrane separation revealed that most of the seawater NOM had a MW of <1 kDa, confirming that the HSs were in an advanced state of degradation. We have also shown that seawater NOM with a MW of >1 kDa contains mainly aromatic proteins, which are more biodegradable than whole seawater NOM.

Key words | biodegradability, fractionation, LC-OCD, organic matter, seawater, XAD® resin

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INTRODUCTION

Natural organic matter (NOM) is a complex mixture of organic compounds present particularly in surface water. It consists of compounds ranging from largely aliphatic compounds to highly coloured aromatics. Some of this organic matter (OM) is negatively charged and contains a wide variety of compounds with a wide range of molecular weight (MW; Ogawa & Tanoue 2008; Matilainen *et al.* 2011). Thus, NOM in water has both hydrophobic (HPO) and hydrophilic (HPI) components. In surface water, HPO acids, rich in aromatic carbon, are described as humic substances (HSs) and constitute the major fraction of NOM (more than half of the dissolved OM (DOM); Martin-Mousset *et al.* 1997; Matilainen *et al.* 2011). HPI matter contains less refractory components, such as carbohydrates, proteins, sugars and amino acids (Huber & Frimmel 1994). NOM is usually quantified using different conventional techniques such as quantification of total and dissolved organic carbon (TOC and DOC), UV at 254 nm (A_{254}) or chemical oxygen demand and biochemical oxygen demand at 7 days (COD and BOD_7). However, these techniques allow only an approximate characterization of the NOM.

Increasing levels of NOM and changes in its composition present major challenges to the drinking water industry. Its presence can create problems for the quality of drinking water and for its treatment, via processes such as the formation of disinfection by-products and bio-reactive compounds (Richardson *et al.* 2007). The diversity of components that constitute NOM and their relatively low

doi: 10.2166/aqua.2013.113

concentration often make characterization difficult. Thus, methods that can either accurately characterize NOM in such dilute solution, or alternatively can either isolate or concentrate NOM, are essential.

For years, researchers have worked to develop new tools for better characterization of NOM, especially in surface water and wastewater. Some of the newer techniques, namely fractionation on XAD® resins, liquid chromatography combined with organic carbon detection (LC-OCD) and excitation–emission matrix (EEM) fluorescence have been used in this study to improve knowledge of seawater NOM.

Fractionation by way of XAD resins has been used to characterize and concentrate NOM from surface water (Aiken *et al.* 1979; Leenheer 1981) and seawater (Amador *et al.* 1990; Lara & Thomas 1994; Lepane 1999). A more specific protocol using adsorption onto XAD8 and XAD4 resins has been described by Martin-Mousset *et al.* (1997) to fractionate DOM into three categories of polarity, namely: HPOs, transphilics (TPIs) with intermediate or transitional polarity and HPIs. The method has also been used recently to evaluate the impact of oxidative treatment on OM conversion (Croué *et al.* 2003; Gong *et al.* 2008).

LC-OCD is a combination of size exclusion chromatography (SEC) separation and continuous analysis to quantify OC, nitrogen and UV absorbance at 254 nm. It has been used successfully to characterize and classify DOM into five fractions, based on molecular size and chemical properties: biopolymers, humics, building blocks, low MW (LMW) neutrals and LMW acids (Huber & Frimmel 1994; Huber *et al.* 2011). Few uses for characterizing marine water have been reported (Dittmar & Kattner 2003; Baghoth *et al.* 2008).

EEM fluorescence can allow division of NOM into several fractions according to nature and origin. It has been employed for the characterization of surface water and waste water DOM (Chen *et al.* 2003; Hudson *et al.* 2008) and also NOM from seawater (Coble 1996; Sierra *et al.* 2005). Chen *et al.* (2003) described a method for the interpretation of EEM fluorescence spectra that classifies NOM into five different groups: aromatic proteins (regions I and II), fulvic acid (FA)-like and humic acid (HA)-like compounds (regions III and V) and microbial by-products (region IV). They devised the classification through measuring the EEM fluorescence of several target compounds (tyrosine, tryptophan, etc.) and NOM of various origins before and after fractionation via XAD resins.

Improvements in membrane separation technology have also helped in the study of NOM, e.g., concentration via ultra-filtration and/or reverse osmosis (Croué 2004; Lankes *et al.* 2008). The use of several membranes with different MW cut-off (MWCO) allows separation of OM via molecular size. This permits NOM to be divided into different size fractions and their properties and (bio-)chemical reactivity to be studied (Amon & Benner 1996; Chiang *et al.* 2002; Gang *et al.* 2003; Kaiser & Benner 2009). In this study, seawater was filtered over a 1-kDa membrane to distribute the NOM over two fractions: high and low molecular size compounds (HMS and LMS), respectively. We then compared the fractions using conventional parameters: TOC, UV absorption at 254 nm (A_{254}) and BOD_7.

The objective was to characterize the coastal seawater NOM in the northwest Mediterranean Sea. Molecular size, hydrophobicity and biodegradability were studied through the use of several techniques, individually or in combination.

MATERIALS AND METHODS

Cleaning protocol

Because of the very low concentration of OM to be measured, all glass material was cleaned by soaking (24 h, 10% HCl) and rinsing with large amounts of milli-Q water before covering it with Al foil and heating it (450 °C, 4 h).

Conventional analysis

Seawater samples were taken from the northwest Mediterranean Sea, 2 km from the Barcelona shore and at a depth of 25 m. The NOM was first characterized using conventional analysis: A_{254}, TOC and BOD_7. Due to the absorption of double bonds and aromatic rings at 254 nm, A_{254} values reflect the aromatic fraction. Samples were filtered and the absorbance measured (Perkin-Elmer UV-Vis lambda 20 spectrophotometer; 10 cm long quartz cell). The results were normalized to 1-m length cell (m^{-1}). TOC was determined

by way of high-temperature catalytic oxidation (TOC-V_{CSH}, Shimadzu) and expressed in mg C L^{-1}. Samples were acidified to pH 2–3 (50 µL 2 M H_3PO_4 in 10 mL glass vials), hermetically sealed and stored in consumable glass vials. The ratio of A_{254} to TOC, i.e., specific UV absorbance at 254 nm ($SUVA_{254}$; l m^{-1} mg C^{-1}) allows the water to be classified according to the proportion of aromatic OM (Edzwald & Tobiason 1999; Weishaar *et al.* 2003). BOD_7 is a well-known measurement of the amount of oxygen required by aerobes to decompose the OM in water and wastewater (Eaton & Franson 2005; EN 1899-2 1998). However, there are few reports of seawater BOD_7. The method used for BOD_7 determination was adapted here from the closed bottle method (EPA 1998) and standard methods 5210 protocol (Eaton & Franson 2005), with concentrated autochthonous microorganisms used as the seeding material (Simon *et al.* 2011). Samples were incubated (7 days, 20 ± 1 °C; incubator Medilow Selecta, Spain) in 250 mL ISO bottles (Schott, Germany) with GL45 stoppers and polytetrafluoroethylene (PTFE) seals to ensure air tightness. The dissolved oxygen was measured using an HQ40d meter integrated with an IntelliCAL™ LDO sensor (Hach, USA). Each sample was analysed in triplicate and results are expressed in mg O_2 L^{-1}. Detection limit was 0.2 mg O_2 L^{-1} and standard deviation of the analysis was around 0.1–0.2 O_2 L^{-1}. NOM biodegradability was determined from the ratio of BOD_7 to TOC, expressed in mg O_2 mg C^{-1}.

XAD fractionation

Originally, XAD resins were used to isolate and characterize DOM from soil and surface water (Aiken *et al.* 1979; Leenheer 1981). Fractionation with XAD resins has since been adapted and applied to both surface water (Malcolm & MacCarthy 1992; Martin-Mousset *et al.* 1997; Croué *et al.* 2003) and wastewater (Gong *et al.* 2008) to separate DOM into three groups, according to polarity (HPO/TPI/HPI). The aim of fractionation via XAD resins in the context of seawater was not to separate the DOM, but more to concentrate OM (Amador *et al.* 1990; Lara & Thomas 1994; Lepane 1999). The possible effect of salinity on the XAD resin adsorption was studied by Esteves *et al.* (1995). These authors concluded that retention on XAD resins is mainly related to the nature of DOM in the water and the ionic strength has little effect on this adsorption.

In this study, fractionation using XAD resins was performed via continuous adsorption on XAD8 (50 g; Rohm and Hass Amberlite XAD, non-ionic acrylic ester resin) and XAD4 (50 g; Rohm and Hass Amberlite XAD, non-ionic macroporous styrene-divinylbenzene resin) in columns (each 250 mm × 25 mm diam.) connected in series. Before use, an extensive cleaning of the resins was performed using 20 L milli-Q water, checking that the DOC in the eluted water was <0.1 mg L^{-1}. Adsorption was performed at 20 mL min^{-1}. Samples were first filtered through 0.45 µm filters of cellulose acetate and acidified to pH 2 with HCl. XAD8 retains the HPO fraction, while XAD4 adsorbs the TPI fraction. The remaining OM after filtration through the two columns (non-adsorbed on XAD8 or XAD4) is the HPI fraction. In order to characterize each fraction, TOC and A_{254} were measured at the inlet to the XAD8 column (S_0), the outlet from the XAD8 column (S_1) and the outlet from the XAD4 column (S_2). The TOC and A_{254} of each fraction were calculated as follows:

$$TOC_{HPO} = TOC_{S0} - TOC_{S1} \quad (1)$$

$$TOC_{TPI} = TOC_{S1} - TOC_{S2} \quad (2)$$

$$TOC_{HPI} = TOC_{S2} \quad (3)$$

LC-OCD

LC-OCD is a new method developed at DOC-Labor (Karlsruhe, Germany) for NOM characterization. The SEC fractionation separates compounds according to molecular size; smaller molecules being retained for longer in the packed column due to diffusion into the smaller pores, so they elute after larger molecules. Polarity interference in the SEC column also affects the retention time for HPO compounds, in a similar way to the packing of the column. The outlet stream is furnished with three online detectors: C, N and UV absorbance at 254 nm. These provide complementary information for OM characterization of the five different fractions: biopolymers, humics, building

blocks, LMW neutrals and LMW acids. Each group is defined in connection with its properties and origin (Table 1).

EEM fluorescence

The fluorescence measurements allowed us to classify the DOM into five distinct types (Chen *et al*. 2003) that correspond to specific origins of the OM. Excitation was measured between 220 and 470 nm and between 280 and 580 nm. Data analysis was performed by splitting the EEM into the five regions (Table 2).

Table 1 | LC-OCD fraction description (Baghoth *et al*. 2008; Huber *et al*. 2011)

Fraction	MW range (Da)	Properties	Description
Biopolymers	>20,000	Non UV-absorbable, hydrophilic	Polysaccharides and proteins, biogenic OM
Humic substances	800–1,000	Highly UV-absorbable, hydrophobic	Calibration based on Suwannee River standard from IHSS
Building blocks	350–600	UV-absorbable	Breakdown products of humic substances
LMW acids	<350	Negatively charged	Aliphatic and LMW organic acids, biogenic OM
LMW neutrals	<350	Weakly or uncharged hydrophilic, amphiphilic	Alcohols, aldehydes, ketones, amino acids, biogenic OM

Table 2 | Fluorescence regions and characteristics (from Chen *et al*. (2003))

Region	Excitation	Emission	Description
I	220–250	280–332	Aromatic proteins I
II	220–250	332–380	Aromatic proteins II
III	220–250	380–580	Fulvic acid-like
IV	250–470	280–380	Microbial by-products
V	250–470	380–580	Humic acid-like

Membrane separation

Filtration was carried out in dead end mode (with tangential configuration) with a tubular ceramic membrane (240 mm × 10 mm diam., length 240 mm) with a MWCO of 1 kDa (TAMI, France) until 2 L of permeate was recovered. A stainless steel membrane holder and PTFE tubing were used to prevent contamination. Exhaustive cleaning was applied to the membrane, membrane holder and tubing in order to eliminate any residual OM: (i) alkaline washing (NaOH, 16 g L^{-1}, 80 °C, 30 min), (ii) acid washing (HNO_3 58% v/v, 5 mL L^{-1}, 50 °C, 15 min).

Filtration allowed us to separate the DOM into two fractions, according to molecular size (Amon & Benner 1996; Ogawa & Tanoue 2008; Kaiser & Benner 2009). DOM found to permeate corresponds to the LMS fraction, which contains DOM with MW <1 kDa. The second HMS DOM fraction, found in the retentate, contains the DOM that cannot cross the membrane because the MW is >1 kDa. The TOC, A_{254} and BOD_7 of each fraction were calculated as follows:

$$TOC_{HMS} = TOC_{seawater} - TOC_{permeate} \quad (4)$$

$$TOC_{LMS} = TOC_{permeate} \quad (5)$$

Both ratios, $SUVA_{254}$ and biodegradability, were also calculated for each fraction.

RESULTS

Table 3 shows the results using the conventional analytical techniques. They correspond to the averages of multiple

Table 3 | Conventional analytical parameters for seawater characterization

Analysis	Units	Avg. values (standard deviation)
A_{254}	m^{-1}	0.82 (±0.2)
TOC	mg C L^{-1}	0.88 (±0.15)
BOD_7	mg O_2 L^{-1}	0.75 (±0.5)
$SUVA_{254}$	l mg C^{-1} m^{-1}	0.93 (±0.2)
BOD_7/TOC	mg O_2 mg C^{-1}	0.85 (±0.1)

analyses. The OM content of seawater is very low, as shown by the average TOC close to 1 mg L^{-1}. Also, low A_{254} (<1 m^{-1}) and very low $SUVA_{254}$ values are characteristic of water rich in HPI OM with a low content of aromatic compounds. BOD_7 data fluctuated throughout the study, as shown by the high standard deviation. The average value for BOD_7 (0.75 mg O_2 L^{-1}) is quite low, but is in agreement with reported values (Jiang *et al.* 2006; Lin *et al.* 2006; Jin *et al.* 2009).

XAD fractionation

Figure 1 shows the results of fractionation of DOC and A_{254} with the XAD resins. DOC includes all organic compounds in seawater whereas A_{254} measures the UV-absorbing compounds, and consequently their distributions differ. However, cross checking this information should improve the characterization of each fraction. Seawater is mainly composed of HPI matter (70%) followed by TPI matter (24%) while HPO compounds represent only 6% of the DOC. In contrast, HPO compounds represent 31% of the total seawater A_{254}, with the TPI and HPI fractions in this case being 12 and 57%, respectively. These variations highlight the fact that each polarity group presents different moiety composition. Figure 2 shows $SUVA_{254}$ for the different XAD fractions. The HPO fraction has a high SUVA value (close to 5) characteristic of OM rich in UV-absorbing moieties, while the TPI and HPI fractions have a low value (<1) showing their low content of UV-absorbing compounds. Seawater DOM separated into the XAD fractions

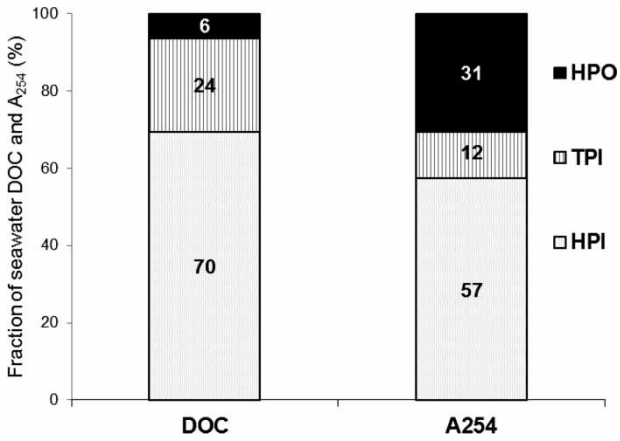

Figure 1 | Distribution of DOC and A_{254} over the three XAD fractions HPO, TPI and HPI.

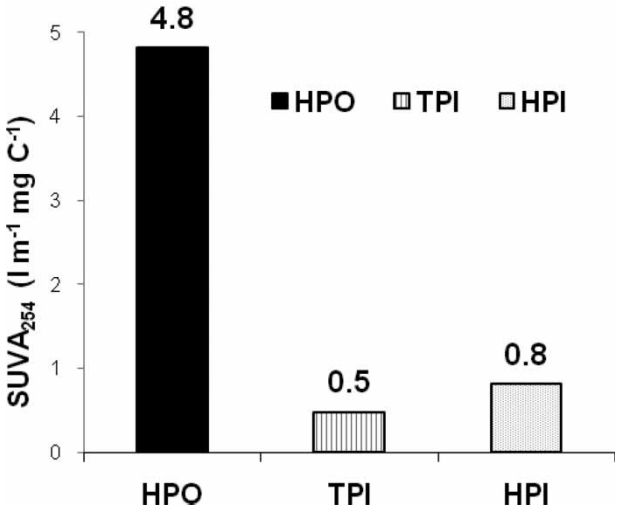

Figure 2 | $SUVA_{254}$ (l mg C^{-1} m^{-1}) of fractions from XAD fractionation of seawater.

gives different results from those for surface water DOM, where HPO is the most important fraction (50%) and is composed of humic-like species (rich in aromatic moieties). In contrast, the TPI and HPI fractions present $SUVA_{254}$ values of <2, typical of biogenic compounds (Martin-Mousset *et al.* 1997; Edzwald & Tobiason 1999; Weishaar *et al.* 2003). Consequently, surface water DOM can be described as HPO, whereas seawater DOM appears to be HPI. The difference may originate in the conversion of HPO OM present in surface water to more HPI compounds via biochemical and/or photochemical oxidation (Miller & Moran 1997; Moran & Zepp 1997; Volk *et al.* 1997).

LC-OCD

LC-OCD was performed on seawater (S_0) and the outflow from the two XAD columns (S_1 and S_2). This provides a better understanding of the nature of the OM retained by the resins. Figure 3 shows the DOC in the LC-OCD fractions of winter and summer seawater samples. The results show that seawater is mainly composed of humics and LMW neutrals (37 and 40% of DOC, respectively), while *building blocks* are the third most important fraction and account for 14%. Biopolymers and LMW acids are very small fractions, representing only 3 and 6%, respectively. Concerning the summer sample, the main difference from the winter sample is the significant increase in biopolymers and LMW

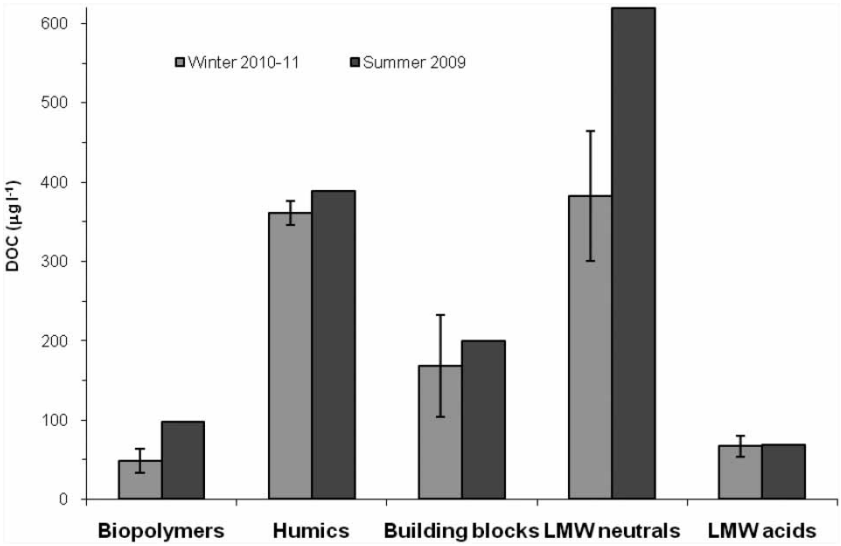

Figure 3 | DOC distribution over the LC-OCD fractions of seawater from winter and summer samples.

neutrals. This phenomenon could be linked to increased biological activity in the summer as a result of the higher temperature (25 °C vs. 10 °C). Dittmar & Kattner (2003) described the LMW neutrals as amphiphilic DOM recalcitrant with respect to biodegradation, such as metabolic intermediates and bacterial membrane moieties, which is consistent with their increase in summer.

Figure 4 shows the LC-OCD fractions of the XAD8 and XAD4 outflow. HPI represents ca. 70–80% of total DOC as seen above, while LMW neutrals and biopolymers are exclusively HPI. HPO and TPI are mainly humics and LMW acids. Humics, usually classified as HPO, fall in this case mainly into the HPI fraction with some tailing into the TPI fraction. This phenomenon can be explained by the gradual and continuous biodegradation of HSs, which would lead to more HPI products. The hypothesis is supported by the fact that there are no HPO building blocks (Baghoth *et al*. 2008).

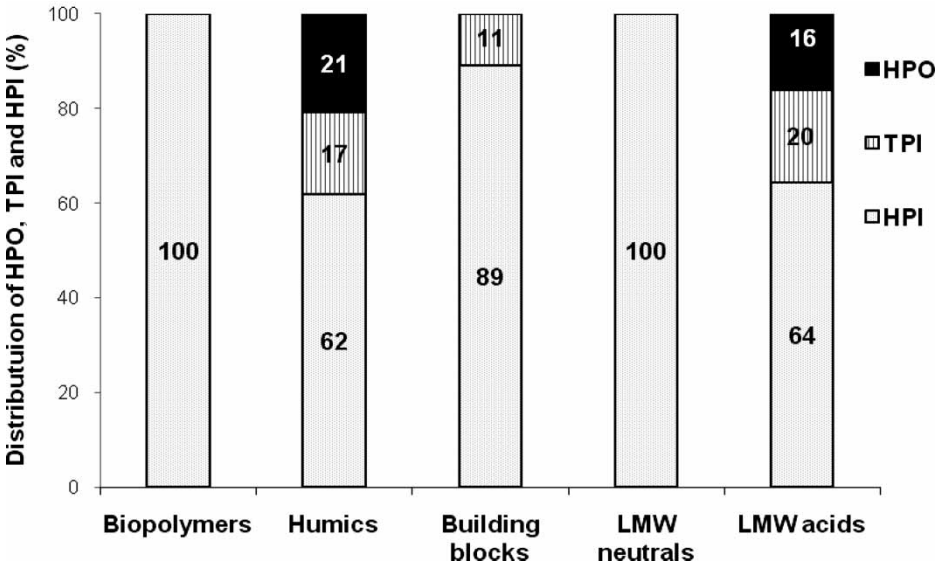

Figure 4 | DOC distribution of XAD fractions (HPO, TPI and HPI) for each LC-OCD fraction.

Fluorescence analysis

Figure 5 shows the relative fluorescence of DOM in seawater. The salinity increases the fluorescence intensities measured in 13–20% (Drioli 2011). However, in this study we focused on the relative distribution of the fluorescence intensities measured in the different groups described by various authors. The most intense fluorescence corresponds to the FA-like region (region III, 30%) and, to a lesser extent, the aromatic proteins (regions I and II, 22 and 17%, respectively). According to seawater fluorescence results reported by Parlanti *et al.* (2000), part of the aromatic protein fluorescence (regions I and II) is related to biological activity in coastal seawater, mainly algae. Moreover, Shimotori *et al.* (2009) demonstrated that humic-like compounds were formed during algal activity and decomposition. This is consistent with humic-like substances from seawater being less aromatic than those from surface water. Unfortunately, fluorescence analysis does not take into account all the OM in seawater but only components that absorb in the UV range (220–470 nm). Consequently, the fluorescence distribution is quite different from the LC-OCD distribution which does take into account the whole NOM fraction (expressed in carbon). Notwithstanding, the FA- and HA-like substances (regions III and V) represent 47% of the total fluorescence, close to the 51% of humics and building blocks that the LC-OCD analysis yields.

Membrane separation

The distributions of conventional parameters over the two OM size fractions (HMS and LMS) vary from one parameter to another. As shown in Figure 6, the major content of the NOM has a molecular size corresponding to <1 kDa, representing 93% of seawater TOC, which is consistent with the findings of Amon & Benner (1996) and Kaiser & Benner (2009). UV-absorbing compounds are mainly in the LMS fraction (84%) vs. three-quarters of BOD_7 (75%) which is found in this fraction. These distributions clearly indicate that each fraction contains OM with specific properties and reactivity, confirmed by the $SUVA_{254}$ and biodegradability ratios in Figure 7. HMS has a higher BOD_7/TOC ratio than seawater (2.9 vs. 0.8) and the same holds for $SUVA_{254}$ (2.1 vs. 0.9). Consequently, HMS substances are richer in

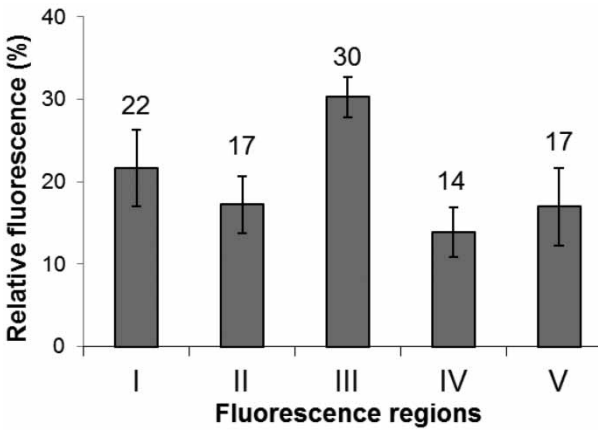

Figure 5 | Relative fluorescence of each fluorescence region for OM in seawater.

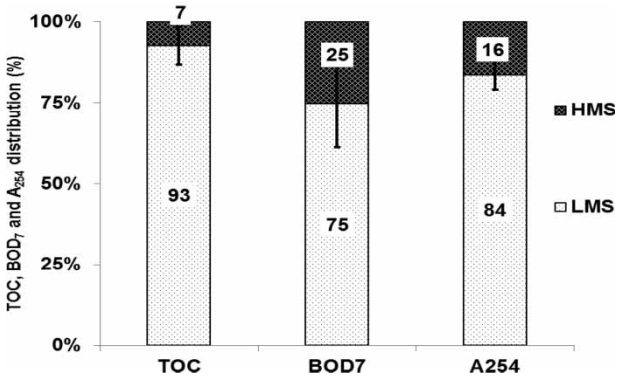

Figure 6 | Distribution of total seawater TOC, BOD_7 and A_{254} over HMS and LMS.

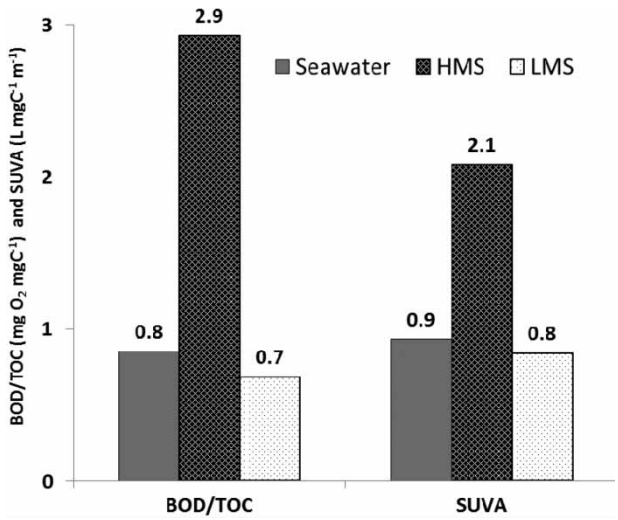

Figure 7 | BOD_7/TOC and $SUVA_{254}$ ratios of seawater, HMS and LMS fractions.

biodegradable and aromatic DOM than the whole seawater DOM content.

In Figure 8, the distributions between LMS and HMS for each fluorescence region provide information on the nature of the DOM with MW >1 kDa. Aromatic proteins are composed mainly of HMS (70 and 44% for regions I and II, respectively) but the HMS fraction is also found in the other fluorescence regions in lower proportion (1, 10 and 10% for regions III, IV and V, respectively). These results support the conclusions from the TOC, BOD_7 and A_{254} distributions. The high biodegradability observed for HMS (Figure 7) correlates with the presence of HMS aromatic proteins that are described as highly biodegradable DOM. These findings reinforce the correlation between the biopolymers, the LC-OCD fraction composed of protein and polysaccharide-like molecules with high and very high MW, and the two EEM regions I and II, corresponding to proteins containing one of the fluorescent amino acids. They remain two different fractions but they both contain HMW aromatic proteins. The higher SUVA for HMS than for whole seawater DOM (Figure 7) is due to the presence of DOM with UV-absorbing moieties which could be aromatic proteins. Moreover, a small part of the HA-like region (V) is present in the HMS DOM. In principle, this is the part of the HA-like region that has the highest MW that undergoes a low extent biodegradation, as demonstrated previously. Consequently, it should contain a higher proportion of aromatic moieties than the LMS HA-like substances (and also the FA-like substances).

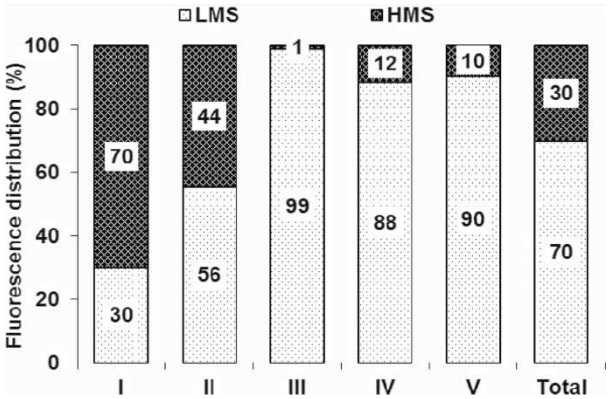

Figure 8 | Fluorescence distribution between HMS and LMS for each fluorescence region.

This should be a complementary explanation for the high SUVA of the HMS fraction.

DISCUSSION

The results allow us to describe seawater DOM with increased precision. LC-OCD results were confirmed by membrane fractionation which shows most seawater DOM to have a MW <1 kDa. The HMS fraction (DOM with MW >1 kDa) contains only a small part of seawater DOM (<10% of TOC), which has been identified as consisting mainly of biopolymers (among them, aromatic proteins) with a HPI character and greater biodegradability than the entire seawater DOM fraction (Baghoth *et al.* 2008) and some HMS HA-like substances with very HPO character (high SUVA value). In contrast, the LMS fraction is a more complex mixture that contains mainly HPI DOM, but also HPO and TPI DOM. Although DOM from HMS contains highly biodegradable biopolymers, the LMS fraction contains most of the biodegradable DOM in seawater (75% of BOD_7); this is consistent with the observations of Donderski *et al.* (1998) that monomers such as sugars, amino acids and organic acids are suitable sources of carbon and energy for bacteria. HPO DOM (determined via XAD fractionation) represents a very small part of total DOM (5% of TOC) and is essentially composed of HSs, whereas HPI matter dominates in seawater (70% of TOC). This distribution, which is reversed relative to surface water (where there is more HPO than HPI DOM), together with the fact that building blocks were partially TPI and HPI DOM, suggests that the originally HPO HSs underwent advanced degradation, leading to smaller and more HPI compounds. This is consistent with the presence of HPI and biodegradable monomers resulting from DOM oxidation through microbial and/or photochemical reaction (photobleaching, etc.; Miller & Moran 1997; Moran & Zepp 1997; Volk *et al.* 1997). Moreover, the proportion of HPO HSs in seawater is reduced due to the *in situ* production of less aromatic HA-like compounds by autochtonous algae (Shimotori *et al.* 2009).

CONCLUSIONS

Mediterranean coastal seawater has a low content of NOM, which is only slightly aromatic and has low biodegradability. We have established the NOM as being mainly HPI (70%) and slightly TPI (24%). The small HPO fraction has a high $SUVA_{254}$ (almost 5), characteristic of HSs. From the LC-OCD results, the seawater appears to be composed mainly of humics and LMW neutrals (37 and 40% of DOC, respectively). The LMW neutrals are HPI, whereas the humics contain HPO and TPI compounds. Fluorescence confirms the higher content of FA-like vs. HA-like substances. These observations confirm the HPI character of seawater OM and may be explained by the advanced hydrolysis in the seawater of the humics of terrestrial origin and by the production of less aromatic HA-like matter by seawater algae. Membrane separation confirms that most of the NOM has a molecular size corresponding to <1 kDa (LMS). However, the NOM with molecular size corresponding to >1 kDa (HMS) is highly biodegradable, suggesting it is composed of biopolymers, being aromatic proteins and polysaccharides.

The techniques used appear to improve on previous OM characterization. Although their respective focuses differ, when applied together, they are complementary and helpful in the analysis of the impact of water treatment processes on DOM.

ACKNOWLEDGEMENTS

The authors are grateful to the Spanish Ministry of Industry (CDTI) for financial support within the framework of the Project CENIT: CEN20071039. They would like to acknowledge N. Cortes and J. Caixach from the CSIC-IDAEA, Mass Spectrometry Laboratory (Spain), M. Abad and E. Berdalet from CSIC-CMIMA (Spain) and M. Philibert, Suez Environnement (France) for their kind contribution to the work.

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First received 17 February 2012; accepted in revised form 26 November 2012