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## **CAMBIOS TEMPORALES EN EL ORIGEN DE LA MATERIA ORGÁNICA EN LAS MARISMAS DEL RÍO MIÑO (NW PENÍNSULA IBÉRICA) MEDIANTE MARCADORES LIPÍDICOS**

### **TEMPORAL CHANGES IN THE ORGANIC MATTER SOURCES IN THE MINHO RIVER TIDAL-MARSHES (NW IBERIAN PENINSULA): A LIPID BIOMARKER APPROACH**

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#### **Resumen**

Con el objetivo de evaluar las variaciones en las fuentes y distribución de la materia orgánica (MO) durante los últimos siglos, se estudia la distribución de biomarcadores lipídicos en una secuencia sedimentaria de las marismas del río Miño (NW Península Ibérica) mediante Cromatografía de Gases-Espectrometría de Masas (CG/MS). La distribución de biomarcadores terrestres y marinos muestra una mezcla heterogénea de MO con diferente origen. Los fenoles derivados de la lignina, triterpenoides y *n*-alcanos de cadena larga con número impar de carbonos proceden de plantas superiores, indicadores de MO de origen continental, se encuentran con mayor abundancia en las secciones superiores del sondeo. Los lípidos derivados del plancton marino (ej., ácidos grasos y alcoholes de cadena corta, fitosteroles) se encontraron con mayor abundancia relativa en las secciones más profundas del sondeo. Sin embargo, la distribución global de los biomarcadores lipídicos estudiados muestra la complejidad de las fuentes de MO en este estuario, y que incluye las descargas del río y deposiciones de sedimentos marinos. Algunas variaciones observadas pueden deberse a contribuciones de MO terrígena debidas a descargas elevadas procedentes del río Miño. No obstante, a la hora de interpretar los cambios observados en la distribución de determinados biomarcadores en este ambiente geodinámicamente activo, se debe tener en cuenta la posible ocurrencia de fenómenos de degradación preferencial o selectiva en el sedimento.

Palabras clave: biomarcadores, estuario del río Miño, lípidos, materia orgánica sedimentaria.

#### **Abstract**

The lipid biomarker distribution in a sediment core from Minho river tidal-marshes (NW Iberian Peninsula) was studied using gas chromatography-mass spectrometry (GC/MS) in order to evaluate changes in the sources and distribution of organic matter (OM) in the estuary during the last centuries. The distribution of terrestrial and phytoplankton biomarker inventory reflected an heterogeneous mixture of OM from marine and terrestrial sources. Lignin derived phenols, triterpenoids and long chain *n*-alkanes with an odd number of carbons are indicators of soil- and vascular plant-derived continental OM, being more abundant on the topmost sections of the core. In addition, a conspicuous contribution from plankton-derived OM (e.g. short chain fatty acids and alcohols, phytosterols) is observed in the deepest sections of the core. However, in general the lipid biomarker assemblage found indicate the existence of a complex input of organic matter (OM) to this estuarine area, from both river run-off and marine depositions. Temporal variations observed could also be attributed to terrigenous contributions after flooding events from Miño river, although a differential degradation of individual biomarkers should not be neglected when interpreting biomarker assemblages in this geodynamically active environment.

Keywords: biomarkers patterns, lipids, Minho estuary, sedimentary organic matter.

## Introduction

Estuarine environments are key locations for organic matter (OM) burial and play an essential role in the global carbon cycle. It is estimated that more than 90% of the total organic carbon preserved in marine sediments is buried in deposits along the estuarine areas and continental margins in "terrigenous-deltaic" regions near river mouths (Berner et al., 1989). Nevertheless, due to varying OM composition, sedimentological regimes, and geochemical processes, OM accumulation, preservation and remineralization are not fully understood. Common to all estuarine areas is the diversity of OM sources. Terrestrial OM from plants and soils accumulate together with marine and riverine OM derived from autotrophic and heterotrophic organisms. The understanding of biogeochemical processes and environmental conditions that lead to the preservation of sedimentary OM in sediments has been the subject of many studies (e.g. Gofñi et al., 1998; Prahl et al., 1994; Hedges and Keil., 1999; Hedges et al., 1999). Further studies with a comprehensive set of geochemical indicators are needed to obtain a deeper knowledge of the various processes that drive the OM distribution and preservation in estuarine sediments.

Lipids biomarkers have been widely used as tracers (or proxies) for the characterization of the nature and distribution of OM in aquatic systems because of their source specificity (Volkman, 2006). By choosing either lipid classes or specific lipids that represent different sources of OM, it is possible to identify the relative contributions of autochthonous and allochthonous OM inputs to the sedimentary record over a wide range of temporal and spatial scales (Wakeham et al., 1997; McCallister et al., 2006).

The main goal of this research was to use the shifts observed in the lipid assemblage isolated from subsections of a core taken at the Minho river tidal-marsh to obtain information about the origin, dispersal pathways, alteration and transformation processes of OM deposited in this estuary.

## Methodology

### *Study area and Sampling:*

The Minho river originates in Serra da Meira, in the province of Lugo (Spain) and drains into the Atlantic Ocean. It is more than 300km long, the last 70 km of which comprises its international section (the natural border between Portugal and Spain). Its hydrological basin has an area of 17.080 km<sup>2</sup>, 95% of which is located in Spain. The Minho river estuary is located on the Northwest coast of the Iberian Peninsula and has a maximum width of just over 2km (Fig.1). This mesotidal estuary is partially mixed; however, during the period of high floods, it tends to evolve towards a salt wedge estuary.

The influence of spring tides extends approximately 40 km upstream (Bettencourt et al., 2003).

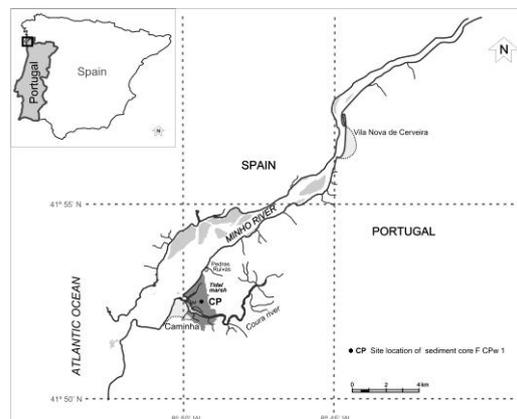


Fig. 1 – Location of the area of study.

In June 2010, several cores (~1m depth) were collected in the high-marsh of Caminha, in the left fringe of Minho estuary, with a manual Auger corer. The core studied here was located at 41° 52' 36,8" N and 8° 49' 28,2" W (Fig. 1). Samples for lipid analysis were wrapped in a clean aluminium foil and transported to the lab. The core was divided in 50 subsections of 2cm using a ZrO<sub>2</sub> knife. Finally, all the sub-samples were stored frozen in glass containers and freeze dried before analysis.

### *Bulk analysis:*

Sediment samples were ground to a fine powder and homogenized in a ball mill. In order to determine total organic carbon (TOC); nitrogen (TN) and sulfur (TS), 1 g of each sediment section was decarbonated with HCl 2M (3x), washed and dried (40 °C; 48 h) prior to analysis in an EA1100 Carlo Erba C/H/N/S elemental analyser. Other aliquot was analysed directly without acid pre-treatment. TOC content was obtained from the instrumental reading for the decarbonated sample and total inorganic carbon (TIC) was calculated by difference with the untreated one (Nieuwenhuize et al., 1994). The instrumental readings were checked against aliquots of the sulfanilamide standard analysed every five samples.

### *Lipid analysis:*

Sediment samples (5g) were Soxhlet-extracted with a solution containing dichloromethane-methanol (3:1) for 16h. In order to remove elemental sulfur, copper curls were added. Experimental details for analysis of this type of samples by gas chromatography and gas chromatography-mass spectrometry (GC-MS) can be found in González-Vila et al. (2003). Briefly, aliquots of lipid extracts are methylated

and silylated in order to improve the resolution of the chromatograms and injected in a gas chromatograph coupled to a mass spectrometer (GC-MS Hewlett-Packard). Identification of evolved compounds was carried out by displaying traces of selected ion characteristics for specific homologous series (SIM mode) and by comparison of mass spectra using libraries (NIST and Wiley libraries).

## Results and discussion

Total organic carbon (TOC) content varied from 13.9 to 1.7 %, whereas TN ranged 1.1-0.1% in bulk sediments. Higher values were found in the upper core sections, being consistent with the effects of diagenesis, which cannot be overlooked when comparing sediment deposited during several centuries. It is known that the C/N ratio for marine phytoplankton is around 6.6, for fresh marine sediment is 7 to 10 and for terrestrial plants is over 20 (Meyers, 1994). Because of this, it is possible to use the C/N ratio as a proxy for an initial inference of sedimentary OM origin. However, diagenetic processes have to be taken in consideration. In the studied core, C/N ratios usually presented values between 13 and 17, indicating a mixed input of land and aquatic OM. Lowest values ( $\leq 11$ ) were obtained in the deepest sections (>70cm depth) correlating with TOC contents. TS ranged from 0.13 to 0.46%. Deeper sections were enriched in S, while the lowest values were found for intermediate sections (20-62cm). Sulphur incorporation into the diagenetic products may be relevant in this estuarine area.

### Biomarker distribution:

The decreasing gradient observed in total lipid concentration is consistent with the reduction in TOC with depth. This is consistent with inputs, which derive in the first instance from the water column and independent from its origin terrigenous or marine.

Figure 2 shows the chromatogram of selected ions (m/z) corresponding to the 0-2cm section of the sediment core, in which we have identified three of the most abundant lipid classes, i.e. a) *n*-alkanes, b) *n*-fatty acid methyl esters (FAMES) and c) *n*-alkan-2-ones.

A homologous series of C<sub>14</sub>-C<sub>34</sub> *n*-alkanes was found in most subsamples studied. In general the distribution was bimodal distribution with maxima at C<sub>29</sub> and C<sub>31</sub>, which is usually related to the occurrence of wax lipids derived from higher plants, particularly angiosperms, although it may also reflect inputs from sea grasses (Jaffé et al., 1995). Lower molecular weight alkanes with maxima at C<sub>15</sub>, C<sub>17</sub> and C<sub>21</sub> are thought to be synthesised by phytoplankton and benthic algae (Cranwell, 1987; Wakeham, 1995). These are less abundant than the higher MW homologues due, presumably, to their

preferential degradation during early diagenesis. The high values of the C<sub>31</sub>/C<sub>17</sub> or C<sub>31</sub>/C<sub>19</sub> ratios (>3) found in this study, can indicate the relative proportions of allochthonous and autochthonous organic matter inputs, suggesting that the main source of organic matter is non-marine. The predominance of *n*-alkane distributions with an odd/even predominance in the C<sub>27</sub>-C<sub>33</sub> range for the topmost sections of the core denotes contributions from higher plants (Simoneit et al., 1977). Gonzalez-Vila et al (2003) identified this behaviour as typical input from a pine assemblage. Specific bacterial biomarkers, branched chain iso- and anteiso- acids were also detected in lipid extracts of deeper sections.

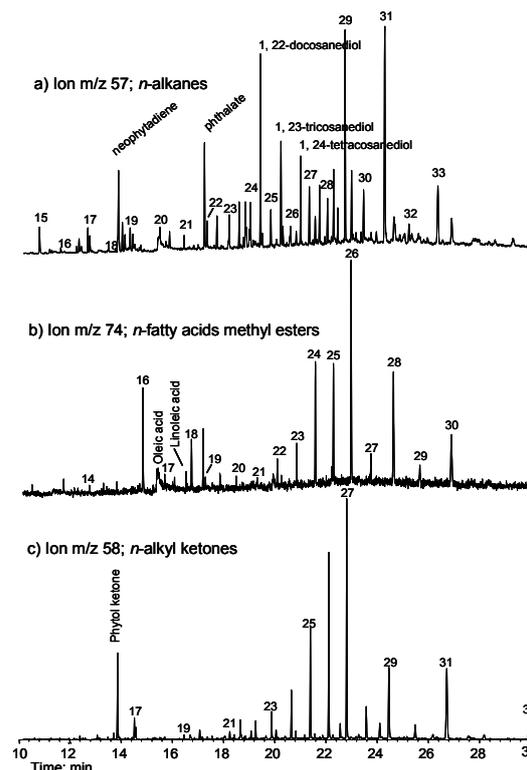


Fig. 2 – Selective ions gas chromatograph/mass spectra chromatograms of the 0-2cm depth sediment fraction's lipid extract. a) m/z 57; b) m/z 74 and c) m/z 58 (numbers over the peaks represent the number of carbons of the *n*-alkyl chain).

In addition, several *n*-alkanediols (C<sub>22</sub>-C<sub>24</sub>) were identified in numerous upper and intermediate sections, and although their source organisms is yet unknown, these compounds have been observed in several oxygen depleted sediments (Nierop et al., 2011).

Fatty acid methyl esters in the core ranged from C<sub>14</sub> to C<sub>30</sub>, with a strong predominance of even carbon numbered long-chain homologues. These molecules are characteristic constituents of surface waxes of higher plants (Kolattukudy, 1976). In general algae contain fatty acids and hydrocarbons in the C<sub>14</sub>-C<sub>18</sub> range while terrestrial vascular plants usually have components in the range C<sub>22</sub>-C<sub>32</sub>. However an

algal origin of the long-chain fatty acids cannot be completely excluded (Volkman et al., 1998).

Series of *n*-alkan-2-ones ranging from C<sub>17</sub> to C<sub>33</sub> with maxima at C<sub>27</sub> were identified in most subsamples. These have been reported in many sedimentary environments, including marine and lacustrine sediments (Meyers and Ishiwatary, 1993), soils, glacier ice and paleosols (Xie et al., 2003), and aerosols (Abas and Simoneit, 1996). The *n*-Alkan-2-ones maximizing at C<sub>27</sub> have been reported to occur in higher plants, microalgae and phytoplankton (Hernandez et al., 2001), suggesting also a direct biological input to soils and sediments. Phytone was also identified in upper sections, is thought to be a photo-induced microbial oxidation product from incorporated phytol and/or of direct input from higher plant waxes.

Sterols were also identified in all samples down the core. A total of 7 different sterols and stanols were identified, being more abundant in the TOC richer upper sections. This may be due to the progression of diagenetic transformations in deeper samples. The major sterols found were sitosterol and stigmasterol, which are usually found in higher plants (Volkman, 1986). Cholesterol was also identified, that can be derived from the faeces and carcasses of fish and zooplankton (Gagosian et al. 1983), however, is also present in phytoplankton including cyanobacteria (Killops and Killops 1993).

## Conclusions

The variability observed in the biomarker distribution in sediments from the Minho river high-marsh suggested that OM inputs mainly derive from soil erosion and from the deposition of continental OM. The predominance of *n*-alkane distributions with an odd/even predominance in the C<sub>27</sub>-C<sub>33</sub> range for the topmost sections of the core suggest a greater contribution from higher plants in recent times.

The presence of phytone and *n*-alkanediols could indicate that oxic and anoxic environmental conditions might have alternated during the last centuries. The exposure of sediment layers to periods with oxic conditions may also exert a selective degradation of the more labile planktonic marine OM.

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