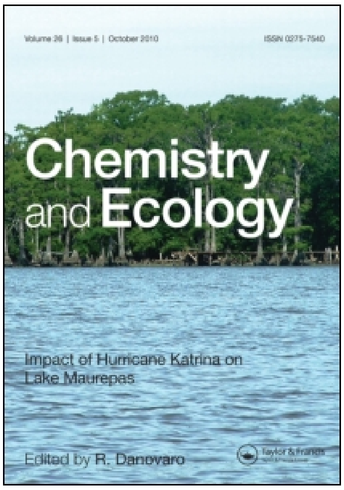

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TRACE ORGANIC MATTER IN THE RIA FORMOSA, PORTUGAL

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The Ria Formosa lagoon, Portugal, receives organic matter from many natural and anthropogenic sources. This paper presents an overview of the contribution these sources made through the use of lipid biomarkers. Sixty-one surface sediment samples were collected and analysed for a range of lipids. Sewage materials were confined to regions within 2 km of discharge points; phytoplankton biomarkers (sterols and fatty acids) suggest production was greatest in the inner parts of the lagoon that also have greatest inorganic nutrient enrichment; terrestrial organic matter was present in relatively low concentrations as shown by both sterol and fatty alcohols; bacteria were widespread although the Sulphate Reducing Bacteria (SRBs, shown by the presence of 3-OH fatty acids) were located near but not adjacent to sewage discharge points.

Keywords: Lagoon; lipid; biomarked sewage; phytoplankton; Ria Formosa; organic pollution; terrestrial sources; bacteria

INTRODUCTION

The Ria Formosa is a tidal lagoon on the Southern Coast of Portugal. The lagoon is a large (55 × 6 km maximum) wetland region of international importance. The mean water depth is only 1.5 m although there are 2 main channels to the ports of Faro and Olhão that are regularly dredged. In total, there is 18×10^3 ha of wetlands of which 10×10^3 are subtidal (Newton and Mudge, in submission). There are five rivers that empty directly into the lagoon, although four of these are seasonal. Most of the rainfall (mean 634 $mm.a^{-1}$) occurs between November and March each year and could be concentrated

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into a few days; this feeds the rivers that bring terrestrially-derived sediment into the system. There are 5 major openings to the Atlantic Ocean. These are located between the barrier islands and their position and importance has changed with the shifting of these islands. The major entrance to Faro has now been confined within concrete walls to prevent such drift with subsequent effects on sand accretion and erosion.

Contamination of the lagoonal system occurs mainly through sewage discharges, industrial processes located close to Faro and Olhão, mariculture and operation of the ports. Metal concentrations have been reviewed by Bebianno (1995) and indicate that cadmium concentrations close to these ports are greater than the EEC standard (EEC, 1983).

There have been relatively few studies of the organic matter in the Ria Formosa. Chlorinated organic compounds have been investigated by Castro and Vale (in press) and sewage derived markers have been studied following a release at Olhão (Mudge and Bebianno, 1997).

This paper briefly indicates some of the results from a major study of the organic matter undertaken in the lagoon in 1995. The principal aim was to identify the sources of organic matter to the system using lipid biomarkers, and to assess the distribution of sewage and oil-derived hydrocarbons. Several in depth papers are in preparation at present.

MATERIALS AND METHODS

Sixty-one surface sediment samples were collected by boat or from the shore in pre-cleaned glass jars with aluminium lined lids. They were kept in a cool-box until extracted (usually during the same day). Extraction consisted of reflux in 6% potassium hydroxide in methanol for four hours. The liquor was separated by centrifugation and the sterols, fatty alcohols, aliphatic and aromatic hydrocarbons were partitioned into hexane after the addition of water to the methanol phase. The methanol fraction was back titrated to pH 6.0 by the addition of hydrochloric acid and the fatty acids were partitioned into 20% dichloromethane in hexane. All samples were concentrated by rotary evaporation and nitrogen blow-down. The dry lipids were then frozen and returned to the laboratories in Menai Bridge for further analysis.

The lipid weight of each sample was determined by drying each sample in small pre-weighed glass vials. At this point, the hexane fraction was divided into two equal portions; one was derivatised with *bis*-trimethylsilyl-trifluoroacetamide (BSTFA) to form the trimethylsilyl ethers of the sterols and fatty alcohols, the other was separated into aliphatic and aromatic hydrocarbons on a combined Si/Al column. Pentane and 20% DCM in pentane were used to elute the two fractions. The fatty acids were derivatised with boron trifluoride in methanol to form the methyl esters.

All compounds were analysed on a Fisons MD-800 Gas Chromatograph – Mass Spectrometry system using the cool on-column injector port. Sterols, fatty alcohols and the hydrocarbons were quantified on a BPX-5 column (50 m × 0.32 mm ID) and the fatty acids were quantified on a BPX-70 column (50 × 0.32 mm ID). The mass spectrometer settings were electron impact ionisation at 70 eV scanning from 45–585 m/z (sterols) or 45–400 m/z (fatty acids).

The wet weight/dry weight was determined by drying the sediments at 70°C for four days and the loss on ignition at 500°C was used as a proxy for the Total Organic Carbon (TOC) content.

RESULTS

General

The Total Organic Carbon expressed as a percentage of the total sediment dry weight (Fig. 1) indicated values of greater than 2% in regions of sewage discharges (Faro and Olhão) and the sheltered ends of the lagoons with restricted water exchange. These regions also had black sediments due to the presence (presumably) of insoluble metal sulphides.

Sterols

A total of 26 different sterols were identified in the lagoonal system. These include classical marine markers (cholesterol), phytoplankton

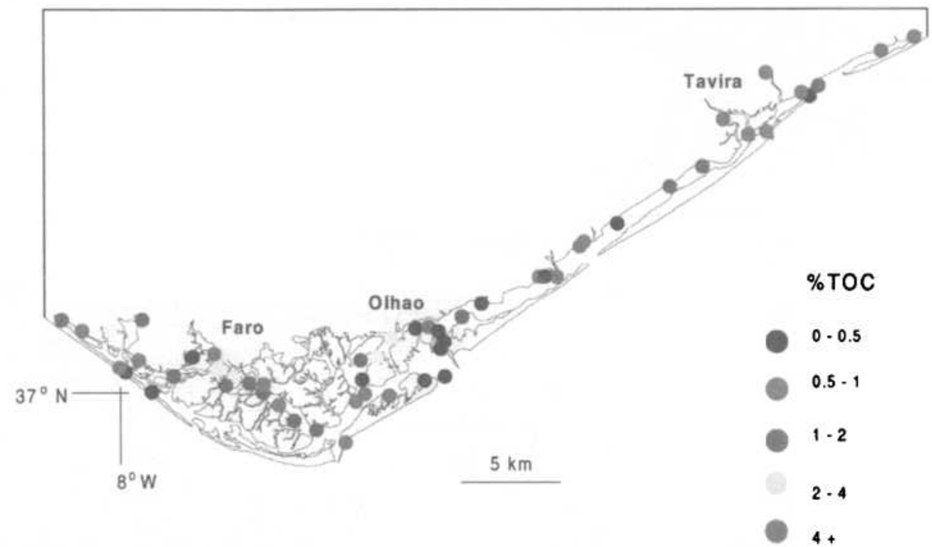

FIGURE 1 The Total Organic Carbon (TOC) content of the sediments samples from the Ria Formosa. (See Color Plate I).

derived compounds (brassicasterol, dinosterol), terrestrial compounds (β-sitosterol) and sewage indicators (coprostanol). Several other compounds were identified including fucosterol and ergosterol.

An example of the distribution of the sewage component of the organic matter (Fig. 2) can be seen with the coprostanol/cholesterol ratio used routinely for indicating the degree of contamination (Nichols and Espey, 1991; Mudge and Bebianno, 1997). Enrichment can be seen close to the sites of domestic sewage discharge, notably around Faro, the Olhão channel and near Tavira in the north east. The majority of the sites had low ratios indicating a small contribution of sewage to these sediments.

The presence of cholest-5,22-dien 3β-ol has been used as an indicator of diatoms in the water column or on/in the sediments (Skerratt *et al.*, 1995). The distribution of this sterol (as percentage of total sterols) can be seen in Figure 3 and suggests that these organisms are present mainly in the waters adjacent to the main sea water inlets. Other studies (Newton, 1995) have shown elevated nutrient concentrations in the lagoon, usually in the inner regions. The temperature and salinity profiles in summer and winter and at low and high water indicate a restricted mixing of the two water bodies (lagoonal and ocean) and algae appear to prosper at the interface between them.

Fatty Alcohols

A large number of different fatty alcohols were identified in the lagoon; straight chain saturated moieties from C_9 to C_{32}, a series of branched chain odd carbon number alcohols, a series of monounsaturated even carbon number alcohols and phytol, a branched unsaturated fatty alcohol. Fatty alcohols can be used to identify both terrestrial organic matter, chlorophyll and bacterial biomass (Mudge and Norris, 1997). The influence of terrestrially derived organic matter in the lagoonal sediments can be assessed using long chain compounds prevalent in plant waxes. Short chain compounds are generally more abundant in marine products, notably from zooplankton. The ratio between long and short fatty alcohols (Fig. 4) indicates that there is relatively little impact from this source in the lagoon, and those regions are confined to the sediments adjacent to the rivers.

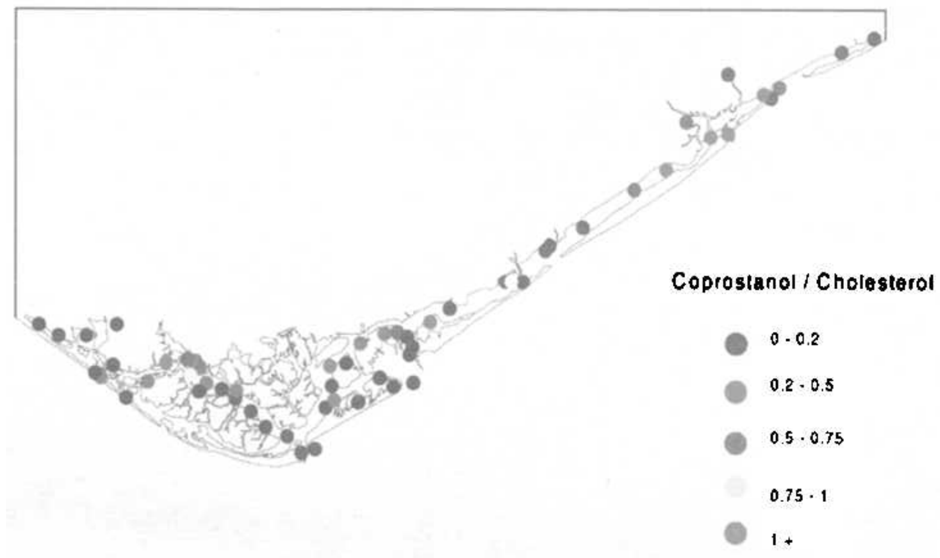

FIGURE 2 The coprostanol/cholesterol ratio in the surface sediments of the Ria Formosa. (See Color Plate II).

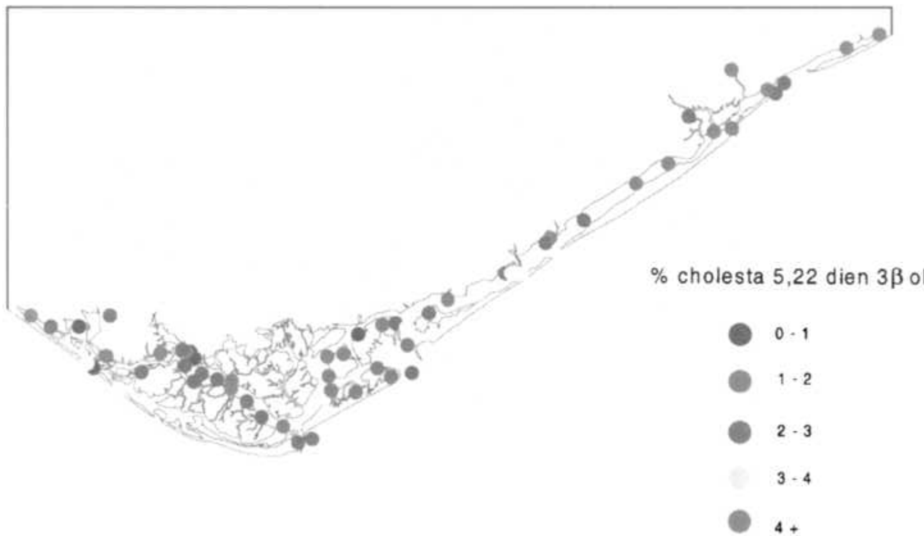

FIGURE 3 Distribution of a sterol (cholest-5,22-dien 3β-ol) biomarker for diatoms in the Ria Formosa. (See Color Plate III).

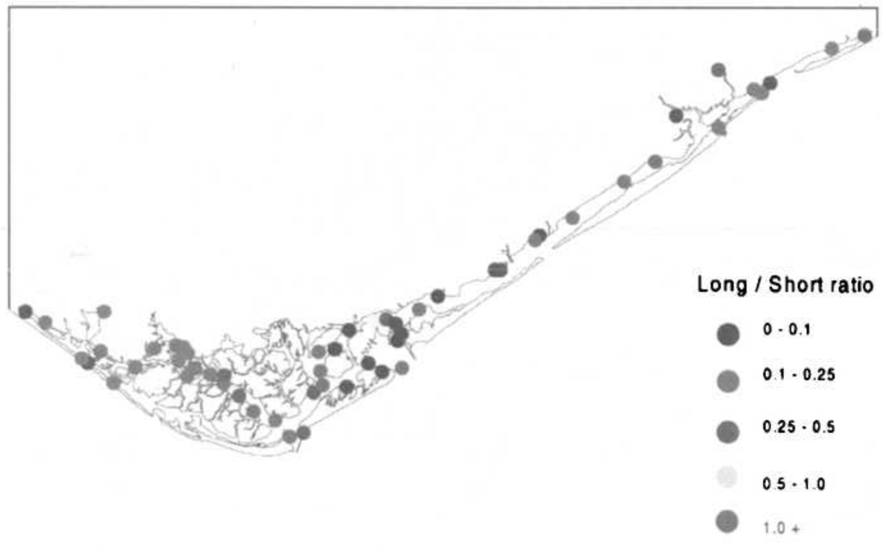

FIGURE 4 The long/short fatty alcohol ratio in the Ria Formosa.. (See Color Plate IV).

The branched chain fatty alcohols were located primarily near to sewage discharge points in the Faro and Olhão regions. These are probably associated with bacteria that were either discharged with the organic materials or *in situ* micro-organisms that are utilising this matter for food. Phytol, on the other hand, was at its greatest concentration in the inner lagoonal areas that have the highest measured nitrate and phosphate concentrations (Newton, 1995). This was similar to the distribution of the phytoplankton sterol biomarkers (e.g. Fig. 3).

Fatty Acids

During this study, 170 different fatty acids were identified and quantified in the sediments. These consisted of saturated compounds from C_{10} to C_{30} as well as mono-and poly-unsaturated fatty acid (PUFAs) species. Significant quantities of branched chain, hydroxy and methyl hydroxyl derivatives were also recorded. Recent reinvestigation of the data suggests that there may also be PUFAs with chain lengths in excess of 22 carbons present.

Plant materials usually contain greater concentrations of PUFAs than animals which exhibit high saturate, especially 16:0 and 14:0, concentrations. This can be demonstrated by observing the percentage of PUFAs as a proportion of the total fatty acids in the sediment (Fig. 5). Regions adjacent to the main sewage outfalls have low (< 5%) PUFAs and regions with greater planktonic production shown by other biomarkers can have values in excess of 30%.

The distribution of the branched chain fatty acids is similar to that of the fatty alcohols and greatest concentrations can be seen near the sewage sources. Several fatty acid biomarkers for phytoplankton (e.g. $16{:}1\omega 7$ for diatoms, Berge *et al.*, 1995) produce very good correlations to other sterol biomarkers. Together, these give credence to the use of those biomarkers for that particular class of organism, and also provide supplementary evidence on the nature of the organic matter in the sediments at any one site.

The distribution of the 3-OH fatty acids, markers of Sulphate Reducing Bacteria (SRBs), indicate that they are only present in the surface sediments at sites some distance from the sewage discharge

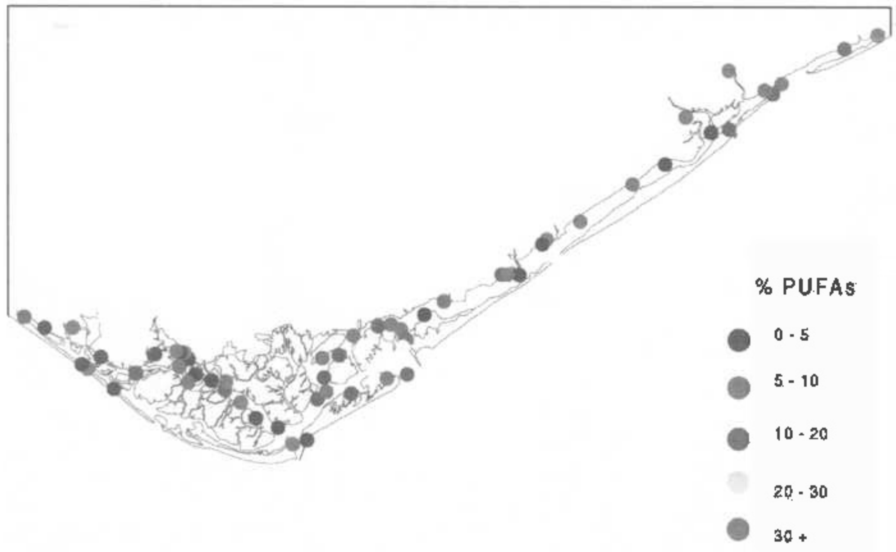

FIGURE 5 The percentage of the total sediment fatty acids comprising PUFAs in the Ria Formosa.. (See Color Plate V).

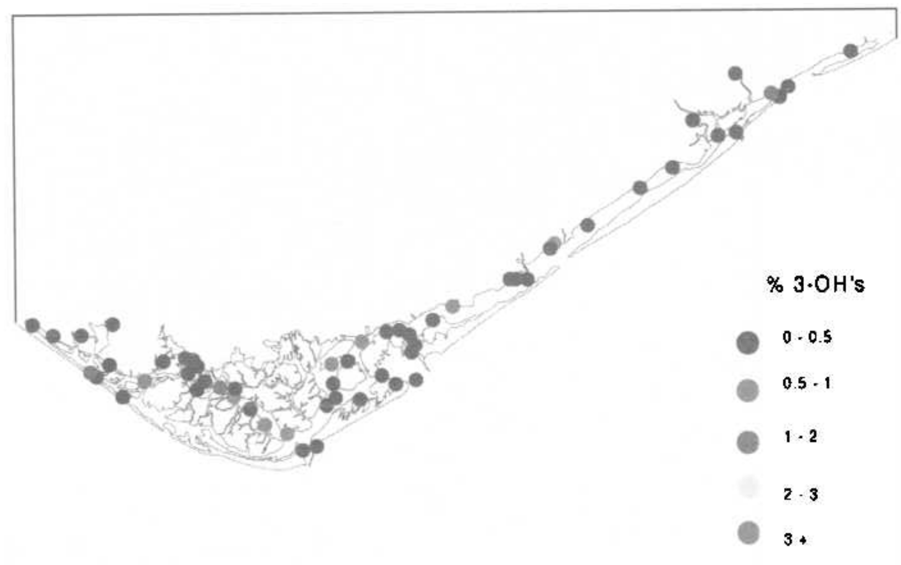

FIGURE 6 The contribution (%) of 3-OH fatty acids to the surface sediments of the Ria Formosa.. (See Color Plate VI).

points (Fig. 6). These bacteria are obligate anaerobes and finding them close to the surface of intertidal sediments suggests a rapid oxygen depletion in those areas. The organic matter supply must be great enough to maintain this oxygen deficit.

Aliphatic and Aromatic Hydrocarbons

The majority of these data is still being assessed but the highest concentrations of aliphatic components can be seen near the ports and also the numerous small harbours where pleasure craft operate. Aromatic hydrocarbons can be seen all over the lagoon but greatest concentrations are near the sewage outlets and sites with road runoff.

Initial Conclusions

1. There are many different lipid biomarkers present within the lagoon indicating a wide range of sources of organic matter to the surface sediments.
2. Sewage derived organic matter can be seen at several sites although the distribution according to the coprostanol/cholesterol ratio was limited. Elevated values were seen only within 2 km of known discharge points.
3. Several biomarkers indicate the presence of phytoplankton derived organic matter, especially diatoms and dinoflagellates. This is confirmed by the occurrence of several blooms of these organisms in the inner lagoon where the inorganic nutrient enrichment is greatest.
4. Several biomarkers highlight the diversity and concentration of micro-organisms in the sediments. Surprisingly, at some sites there was relatively high proportion of SRBs in the surface sediments although these were located at some distance from the sewage discharge points.
5. Relatively little terrestrial organic matter was seen through use of either the sterol or fatty alcohol markers. As expected, only those sediments near the river inputs had elevated concentrations.
6. The distribution of the aliphatic and aromatic hydrocarbons appears to be controlled either by shipping activity or land drainage including sewage.

Acknowledgements

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References

Bebianno, M. J. (1995) Effects of pollutants in the Ria Formosa lagoon. *Sci. Total Env.*, **171**, 107–115.

Berge, J. P., Gouygou, J. P., Dubacq, J. P. and Durand, P. (1995) Reassessment of lipid composition of the diatom, *Skeletonema costatum*. *Phytochemistry*, **39**, 1017–1021.

Castro, O. and Vale, C. (in press) Poluicão na Ria Formosa. *Bull., UCA* **2**.

E. E. C. (1983) Cadmium. Council Directive 83/513/EEC, Brussels.

Mudge, S. M. and Bebianno, M. J. (1997) Sewage contamination following an accidental spillage in the Ria Formosa, Portugal, *Mar. Poll. Bill.*, **34**, 163–170.

Mudge, S. M. and Norris, C. E. (1997) Lipid biomarkers in the Conwy Estuary; a comparison between fatty alcohols and sterols. *Marine Chemistry*, **57**, 61–84.

Newton, A. (1995) The water quality of the Ria Formosa Lagoon, Portugal. *Ph. D. Thesis*, University of Wales, Bangor. pp. 271.

Nichols, P. D. and Espey, Q. I. (1991) Characterisation of organic matter at the air-sea interface, in subsurface water and in bottom sediments near the Malabar sewage outfall in Sydney's coastal region. *Aust. J. Mar. Freshwater Res.*, **42**, 327–348.

Skerratt, J. H., Nichols, P. D., McMeekin, T. A. and Burton, H. (1995) Seasonal and interannual changes in planktonic biomass and community structure in Eastern Antarctica using signature lipids. *Mar. Chem.*, **51**, 93–113.