

## - **Purpose of the visit**

The main purpose of this short stay is the evaluation of changes in the labile (lipidic) and recalcitrant (humic acids) fractions of organic matter in surface sediments from the Guadiana Estuary (SW-Iberian Peninsula) by chromatographic techniques (GC/MS and Py-GC/MS) available at IRNAS-CSIC.

The IRNAS-CSIC group is integrated in the Geoecology and Biogeochemistry department of the Institute of Natural Resources and Agrobiology (IRNAS, Seville). This Department includes a laboratory of international reference. It is specialized in organic matter chemistry, environmental analyses and added considerably to progresses in the chemistry of lignin, research of humic substances in soils, waters and sediments, in the structure of fossil resources (peats, carbons and kerogens) and in the examination of Black Carbon in soils and sediments. This group is a referent laboratory in the analysis of complex organic matrices using Analytical Pyrolysis techniques. Relevant methodological advances in the chemolysis and sequential specific degradation techniques, actually in use worldwide for the structural elucidation of organic macromolecules, have been developed in this lab.

## - **Description of the work carried out during the visit**

### **ENVIRONMENTAL STUDY OF THE GUADIANA RIVER BASIN AND ADJACENT SHELF: BY USING LIPID BIOMARKERS AND HUMIC FRACTIONS**

#### **Introduction:**

Coastal/estuarine environments are sensitive areas of great environmental and socio-economic importance in terms of ecosystem preservation and sustainable management. Research on transport and storage of materials in these areas has been recognized as critical for the understanding of the global carbon cycle. The significant continental input occurring in these areas is the main source of the broad spectrum of organic materials, including pollutants, introduced in the marine environment. The understanding of biogeochemical processes and environmental conditions that lead to the preservation of sedimentary organic matter (SOM) in marine sediments has been the subject of many studies. It is estimated that more than 80% of the total organic carbon preserved in marine sediments is buried in deposits along the continental margins in "terrigenous-deltaic" regions near river mouths. (Hedges et al., 1999).

Due to the rapid accumulation and isolation of organic materials from biological degradations, estuarine zones are good environments for studying the origins, pathway and fates of SOM. In particular the study of the lipid fraction at a molecular level, can contribute in a significant manner to a better delineation of the multistage geochemical processes that occur in estuarine sediments. The biomarker assemblages found in the lipid fraction of SOM may also provide a valuable information about the structure of the trophic system involved in the formation of the original organic fraction and some are used to trace specific biogenic sources.

On the other hand, it is well known that humic substances (HS) play a key role in a range of environmental issues, such as soil and water acidification, nutrient control, weathering, mobility and distribution of heavy metals, ecosystem buffering, etc. These macromolecules are also

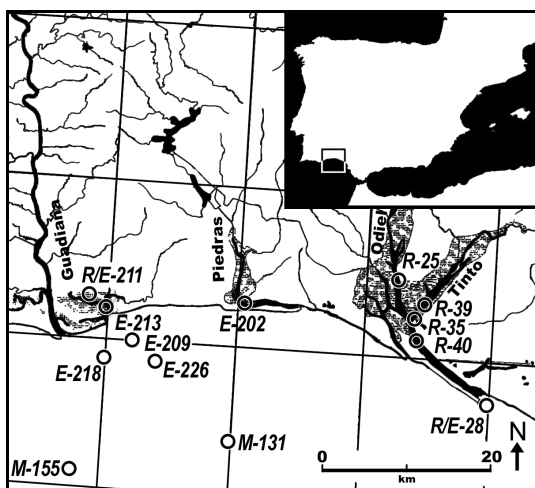
interesting because of their structural features, which includes binding sites with different complexing strength, able to form inert and labile complexes with inorganic cations (metals) and organic compounds. The information on the compositions and functional groups of HAs is critical for understanding their reactivity with organic and inorganic contaminants.

The proposed research is focused on the evaluation of changes in the labile (lipidic) and recalcitrant (humic acids) fractions of organic matter in surface sediments (0-30 cm) from the Guadiana Estuarine area (SW Iberian Peninsula).

Run-off and sediment load carried by Guadiana river to estuarine region and towards the continental shelf has been highly modified mainly during last century because of a range of anthropogenic activities including damming, mining, urbanisation, deforestation and dredging. River discharge modification is particularly evident in regions of Mediterranean climate where the precipitation is high in winter, often associated with frequent natural floods, and low in summer, associated with low river run-off.

### Methodology:

*-Sampling Site:* Surface sediment samples (0 to 20 cm depth) were collected on northern of the Gulf of Cadiz (Figure 1) during the 2001 year. It is a site of geological and environmental interest, highly influenced by water run-off and sediment load derived from the Guadiana River, which is a major river of the Iberian Peninsula (742 km of length). To avoid microbial growth, all the samples were stored frozen in glass containers and freeze dried before analysis. (Samples used for this study were collected during the year 2001).



**Figure 1.** Area of study and location of studied samples.

*-Lipid analysis:* Sediment samples (40 g) were Soxhlet-extracted with a solution containing dichloromethane-methanol (3:1) for 16 h. Total extracts were saponified with 0.5 mol/l KOH/methanol for 8 h under reflux. In order to remove elemental sulfur, copper curls were added. Neutral lipids were separated by extraction with hexane and acidic lipids were isolated after acidification to pH<1; both fractions were then methylated with trimethylsilyldiazomethane before analysis by gas chromatography and gas chromatography-mass spectrometry (GC-MS). Further experimental details can be found in González-Vila et al. (2003).

#### *-Humic acids isolation and analysis:*

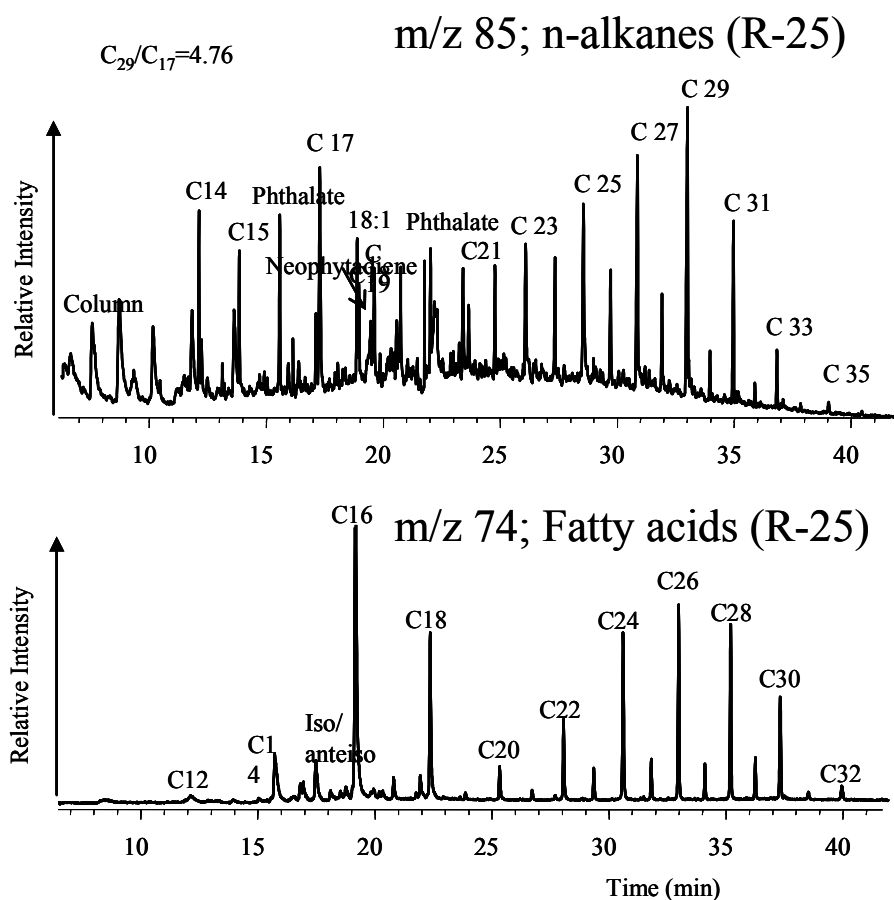
Humic acids were isolated from seven sediments representing the studied area, by following the procedure suggested by the International Humic Substances Society (IHSS). Analytical pyrolysis (Py-GC/MS) will be used to discern changes in the molecular composition of humic acids. Bearing in mind the fact that cation-humic interactions depend on the presence of reactive acidic functional groups, such as carboxylic and phenolic groups, their characterization can provide a guide to modeling metal-humate interactions. The analytical characterization of samples was carried out by using analytical pyrolysis (Py-GC/MS).

*-Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS):* The pyrolysis was performed in a double shot Frontier Laboratories pyrolyzer (model 2020) directly connected to a GC-MS system Agilent 6890 equipped with a fused silica capillary column HP 5MS (30m x 250  $\mu\text{m}$  x 0.25  $\mu\text{m}$  inner diameter). The detector consisted of an Agilent 5973 mass selective detector (EI at 70 eV). The GC-MS conditions were the same for both experiments. Oven temperature was held at 50°C for 1 min and then increased up to 100°C at 30°C  $\text{min}^{-1}$ , from 100 to 300°C at 10°C  $\text{min}^{-1}$  and isothermal at 300°C for 10  $\text{min}^{-1}$  using a heating rate of 20  $\text{min}^{-1}$  in the sacon modus. The carrier gas used was helium with a controlled flow rate of 1  $\text{ml min}^{-1}$ . The identification of individual compounds was achieved by single ion monitoring for different homologous series, low-resolution mass spectrometry and comparison with published and stored (NIST and Wiley libraries) data.

## **- Description of the main results obtained**

### **Results and discussion:**

*-Biomarker distribution:* TIC of samples were similar, mainly composed by the most abundant biomarker series, i.e. *n*-alkane/*n*-alkene pairs, *n*-fatty acid methyl esters (FAMES) and *n*-2-alkanones. However significant differences were found when monitoring selected ions. Fig. 2 corresponds to the selected ion chromatograms of *m/z* 85 and *m/z* 74 of sediment R-25. The homologous series of  $\text{C}_{12}$ - $\text{C}_{35}$  *n*-alkanes was found in most samples studied showing a bimodal distribution with maxima at  $\text{C}_{29}$  and  $\text{C}_{17}$ . A strong predominance in the  $\text{C}_{27}$ - $\text{C}_{31}$  range 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  $\text{C}_{15}$ ,  $\text{C}_{17}$  and  $\text{C}_{21}$  are thought to be synthesised by phytoplankton and benthic algae (Wakeham et al., 1991). They are less abundant than the higher homologues due, presumably, to their preferential degradation during early diagenesis. The high values of the  $\text{C}_{31}/\text{C}_{17}$  or  $\text{C}_{31}/\text{C}_{19}$  ratios ( $> 0.4$ ) found in this study, can indicate the relative proportions of allochthonous and autochthonous organic matter inputs, suggesting that the main sources of organic matter are non-marine in origin. The predominance of the  $\text{C}_{29}$  and  $\text{C}_{31}$  homologues found in the estuarine sediments may indicate typical input from a pine assemblage (Gonzalez-Vila et al., 2003). Specific bacterial biomarkers, branched chain iso- and anteiso- acids were also detected in several lipid extracts.

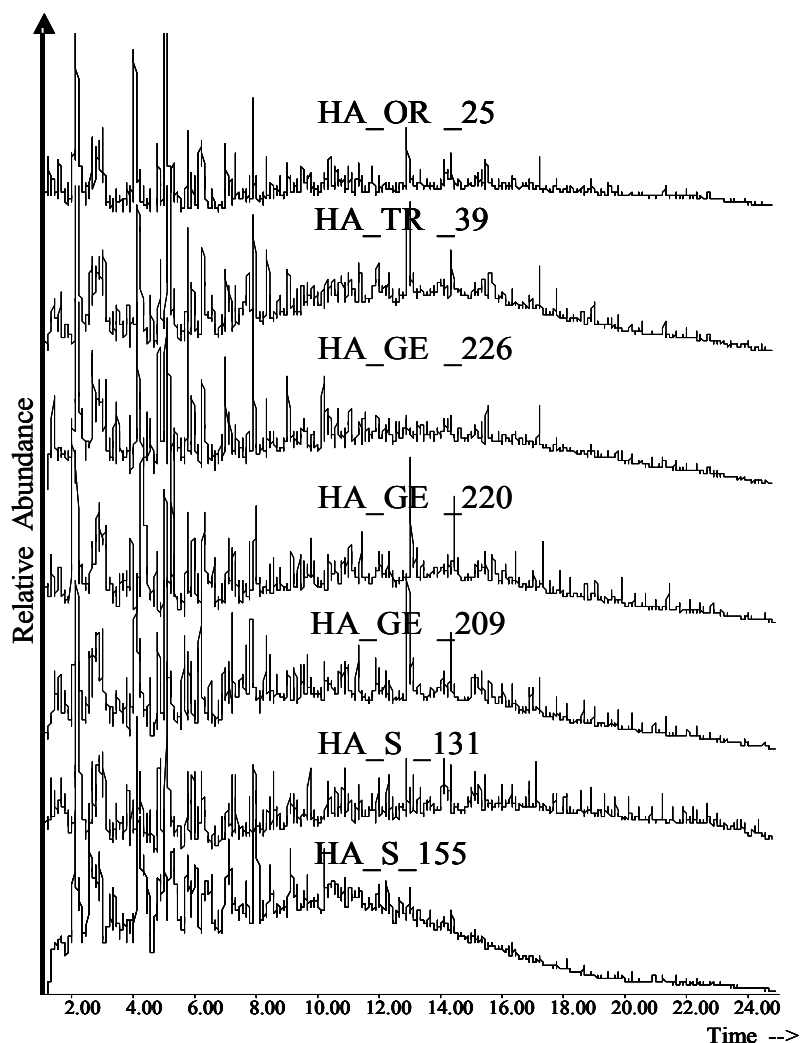


**Figure 2.** Selected ion chromatograms of m/z 85 and m/z 74 of sediment R-25.

*-Py-GC/MS:*

Pyrolysis-GC/MS experiments revealed similar compositions for flash pyrolysates generated from the seven samples.

Similar TICs were obtained from the studied Humic acids. In general, these pyrolysates were dominated by the presence of aromatic compounds (a complex mixture of phenols, benzenes, naphthalenes, etc) and alkane/ene pairs. A slight increase in the PAHs released during the pyrolysis of riverine and estuarine HAs were also observed, whereas alkane/ene pattern were more abundant in HAs from the shelf (S).



**Figure 3.** Py-GC/MS total Ion Chromatogram from Humic acids.

**Conclusions:**

Few variations were observed in the patterns of the most abundant alkyl series (*n*-alkanes, *n*-fatty acids, *n*-alkanols and *n*-2-alkanones). In general, our findings are indicative of the existence of a complex input of organic matter (OM) to the estuarine areas studied, mainly from terrestrial origin. Temporal variations could also be attributed to terrigenous contributions after flooding from rivers, although different degradation rate of individual biomarkers as well as that of OM from different origins should not be neglected when interpreting biomarker assemblages in these geodynamically active environments.

The variability observed in the biomarker distribution in sediments from estuary samples provides a good indicator of differences in the supply of terrigenous and marine OM. It is suggested that OM inputs fundamentally derived from soil erosion and the deposition of continental OM. The exposure of sediment layers to periods with oxic conditions may also exert a selective degradation of the more labile planktonic marine OM. TIC pyrolysates pointed to a relative increase in the terrestrial OM, enriched in aromatic compounds, in estuarine Humic acids. Further research is needed in order to improve those partial conclusions.

**References:**

González-Vila, F.J., Polvillo, O., Boski, T., Moura, D., de Andres, J.R., 2003. Biomarker in a time-resolved holocene/terminal Pleistocene sedimentary sequence from the Guadiana river estuarine area (SW Portugal/Spain border) *Organic Geochemistry* 34. 1601-1613.

Hedges, J.I., Hu, F.S., Devol, A.H., Hartnett, H.E., Tsamakis, E., Keil, R., 1999. Sedimentary organic matter preservation: a test for selective degradation under oxic conditions. *American Journal of Science* 299. 529-555.

Jaffé, R., Wolff, G.A., Cabrera, A.C., Carvajal-Chitty, H., 1995. The biogeochemistry of lipids in rivers from the Orinoco Basin. *Geochimica et Cosmochimica Acta* 59. 4507-4522

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**- Future collaboration with host institution (if applicable)**

It is the intention of PhD. J.A. Gonzalez Pérez and other members of the IRNAS-CSIC group to continue the scientific collaboration with PhD. J.M. de la Rosa and ITN institution. In addition it is my personal intention to continue the collaboration with IRNAS-CSIC group in order to apply in a future call for a wider joint project founded by National (Spanish and Portuguese governments) such as the bilateral research programme between Spain and Portugal.

**- Projected publications/articles resulting or to result from your grant**

It is expected submitting a manuscript before the end of this year to a peer-reviewed journal, included in the science citation index, based on the scientific work developed during this stay.

**- Other comments (if any)**

The travel was carried out with my private car because it was cheaper than a return flight ticket in economic class from Lisbon to Seville.