Analytical pyrolysis in marine environments revisited

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This contribution outlines applications of analytical pyrolysis techniques (APTs) –in particular pyrolysis-gas chromatography-mass spectroscopy (Py-GC-MS) and thermally assisted hydrolysis and methylation (THM-GC-MS)– to marine geochemical research. It is subdivided into two main lines, i.e. the results obtained from (1) sedimentary, particulate and dissolved OM from open water environments (estuaries, seas, oceans), and (2) coastal vegetated habitats such as saltmarsh, mangrove and seagrass ecosystems (Blue Carbon). The main aim is to re-familiarize the marine research community with the possibilities of these infra-utilized techniques for the identification of sources and assessment of degradation/preservation state of macromolecular organic matter.

1. INTRODUCTION

This overview was motivated by the perceived unfamiliarity of scientists dedicated to different lines of marine geochemistry research with the potential of APTs for molecular characterization and thereby improved understanding of the biogeochemical cycle of organic matter (OM). Firstly, there is increased recognition of the importance of saltmarsh, mangrove, seagrass and other coastal vegetated habitats as hotspots of carbon sequestration (Geraldi et al., *in press*). This is best represented by the emerging Blue Carbon research and management initiatives. Even though Blue Carbon in coastal vegetated habitats is mainly composed of relatively well-preserved macromolecular detritus, the application of APTs over the last decade is almost nonexistent. Secondly, whereas the molecular composition of marine dissolved OM (DOM) is currently under scrutiny, few studies apply APTs to assist in the interpretation of common characterization methods such as fluorescence spectroscopy, FT-ICR-MS and stable isotope analysis. The same can be argued for the increasing effort of expeditions using advanced research vessels aiming to understand ocean circulations and materials fluxes therein.

Covering the last 40 yrs of APT applications in the marine environment, this work is dedicated to molecular probing by a combination of pyrolysis or thermochemolysis with GC-MS: studies using Py-GC, Py-MS, Py-FIMS, Py-GC-GC-(ToF)MS, Rock-Eval pyrolysis or hydropyrolysis are not discussed. Only publications in English are included. Research on geological sedimentary marine OM (Type I and II kerogen) (e.g. Larter et al., 1978; Sinninghe-Damsté et al., 1989) is discussed only if recent sediments are used for comparative purposes (and then only results from recent sediments are mentioned), and studies of shipwreck wood or other anthropic artefacts in the marine environment (Traoré et al., 2017) are also omitted. Furthermore, APT characterization of Py-GC-MS of marine biological materials without a clear connection to marine geochemistry are not discussed (e.g. biofuel potential assessment), even though such studies may be extremely helpful in source identification from environmental samples (Ross et al., 2008; Ní Fhlaithearta et al., 2013; Biller and Ross, 2014). Studies that characterized chemically treated fractions of algae biomass for isolation and characterisation of resistant biopolymers such as algaenan are not included (e.g. Gelin et al., 1996). For these topics and references to relevant Py-GC-MS studies, readers are referred to e.g. Versteegh and Blokker (2004), de Leeuw et al. (2005) or Derenne and Largeau (2011).

In order to discuss OM in the marine environment in a somewhat orderly manner, a subdivision is desirable, according to various criteria. Firstly, the marine environment includes coastal submerged semi-terrestrial systems (temporarily or spatially), estuaries and the "open water" environment with the important gradual trends from river plumers (continentally-affected) to oceanic, and from pelagic to benthic systems. Secondly, according to the operationally defined fractions that are concerned, i.e. DOM and particulate OM (POM), or the sample type (water column, suspended sediment, sinking sediment, sediment and coastal archives such as seagrass mats and saltmarsh/lagoon sediment). Here, the discussed literature is organized according to the environment (first open water systems, then coastal vegetated systems), then time of publication, then POM/DOM focus. This rule is sometimes broken to maintain the flow of discourse or to discuss various papers from the same line of research.

Another important aspect in marine OM geochemistry is the terminology employed. One of the main targets of analysis of OM in the marine environment is the identification of "allochthonous" and "autochthonous" sources. All too often these terms are used without describing what is meant, ignoring the fact that different disciplines have different interpretations of what is considered *in* and *ex situ*, especially in coastal environments. The most frequently targeted allochthonous (source of) OM type is lignin, as this phenylpropanoid polymer is often thought to be synthesized only by terrestrial vegetation. However, the "phanerograms that returned to the sea" (Klap et al., 2000), or seagrasses, and obviously mangrove and saltmarsh vegetation, produce lignin, which would be considered autochthonous by scholars of coastal vegetated marine habitats, but not by those that are studying the influence of terrestrial OM along a gradient of a river plume. Note that even some algae may contain some lignin or another substance that produces methoxyphenols upon Py-GC-MS (Ní Fhlaithearta et al., 2013), cupric oxide oxidation (CuO-GC-MS; Thomas et al., 2009) or derivatization followed by reductive cleavage (DFRC-GC-MS; Martone et al., 2009), albeit in negligible proportions when compared to land plants. Furthermore, the terms autochthonous and allochthonous are often invoked on the basis of results of analysis, for example, low C/N is marine and high C/N is terrestrial, but many marine sources of OM such as seagrasses or Black Carbon from hydrothermal vents, have high C/N, and many terrestrial sources that enter the marine system predominantly by rivers, may have low C/N (derivatives of fungi, bacteria, algae). Similar arguments can be made for d^{13}C and d^{15}N, fluorescence properties, or other frequently employed methods that essentially target elemental composition data such as the parameters obtained by FT-ICR-MS. In the light of the current efforts on quantifying the amount and cycling of coastal sources of OM and its inclusion in total (coastal and oceanic) Blue Carbon budgeting and modeling exercises, the imprecise dichotomization of autochthonous and allochthonous OM is of significant importance (Nelleman et al., 2009; Duarte et al., 2013; Serrano et al., 2014). Here we will avoid the use of these terms.

2. BACKGROUND ON ANALYTICAL PYROLYSIS

The APTs use rapid/instantaneous (“flash”) heating at 500-700 °C under inert conditions (usually helium flow) to cleave macromolecular organic matter into GC-amenable fragments, which may then be identified by means of MS, hence hyphenated Py-GC-MS (Moldoveanu, 1998; Voorhees, 2013). An alternative approach involves addition of a reagent prior to heating that stimulates hydrolysis and concomitant derivatization, called thermochemolysis, or thermally assisted hydrolysis and methylation (THM-GC-MS), and which can be but does not have to be performed at lower temperature (350-400 °C) (Hatcher and Clifford, 1994; Challinor et al., 2001; Shadkami and Helleur, 2010). APTs provide a fingerprint comprised of numerous pyrolysis products that can be translated to obtain detailed information on the molecular composition of a sample. Because of the invasive nature of the reactions involved APTs are quantitatively weak (they cannot provide absolute concentrations of any macromolecular OM constituent) and other pitfalls such as the fact that many of the common pyrolysis products can be sourced to more than one precursor (this is why we speak of fingerprinting, not biomarker analysis etc.), and sample matrix effects can severely compromise data quality, in particular when OM content is low and reactive mineral content is high (Sáiz-Jiménez, 1994). APTs have a long tradition in characterization of macromolecular constituents of natural OM, especially in mineral soils and peat deposits (see reviews by Buurman et al., 2006; Shadkami and Helleur, 2010; Derenne and Quénéa, 2015).

Theoretically, all the major biopolymers that can be found in marine environments, and their degradation products, can be pyrolytically cleaved into identifiable products. For example, using Py-GC-MS, (1) polysaccharides are identified from cyclopentenones, furans, furaldehydes, pyrans and anhydrosugars (Pouwels et al., 1989), (2) proteinaceous material forms pyrroles, pyridines, cyanobenzenes, indoles and diketopiperazines (Tsuge and Matsubara, 1985), (3) lignin produces phenols, guaiacols and syringols (Sáiz-Jiménez and de Leeuw, 1986; Ralph and Hatfield, 1991), (4) tannin generates phenols, catechols and pyrogallols (Galletti and Reeves, 1992), (5) chlorophyll forms phytadienes and pristenes (Ishiwatari et al., 1991), (6) chitin and peptidoglycan form acetamidosugars (Stankiewicz et al., 1998) and (7) various types of aliphatic biopolymers such as cutin, suberin, cutan, suberan and algaenan produce mainly alkanes and alkenes (Nip et al., 1986; Tegelaar et al., 1989; Gelin et al., 1996) (Table 1, see end of document). For THM-GC-MS, the pathways are less understood (and the chemical nomenclature less suitable for a brief summary). As different organisms consist of different relative proportions of these and other OM types, APTs allow for identification of source materials. Furthermore, biocomponent-specific proxies of degradation state allow for assessment of the preservation state of the different forms of OM. As such, APTs have a significant potential for OM provenancing and assessment of carbon sinks (recalcitrance/labilility), simultaneously.

As said, the quality of Py-GC-MS and THM-GC-MS data is negatively affected by the presence of reactive minerals, such as Fe- and Al-(hydr)oxides, and also carbonates. For sample matrices with significant amounts of such materials a purification/concentration of the OM is required before analysis. Elimination of carbonates by aliquots of HCl followed by repeated treatment with mild (2 %) HF solution has shown to induce only small organic C losses and significantly improve the quality of the data produced (pyrolysis chromatograms), for both Py-GC-MS (Zegouagh et al., 2004) and THM-GC-MS (Spacchini et al., 2013). Conveniently, such treatment is also adequate for elimination of paramagnetic minerals that interfere in NMR spectroscopy. Most of the studies reported here applied HF-HCl treatment to eliminate carbonates and reactive inorganic phases.

3. OVERVIEW OF APT APPLICATIONS IN MARINE SYSTEMS

A. Estuaries, seas, oceans, and their sediments

In two papers, van de Meent et al. (1980a/b) were early to target POM in marine suspended sediment, in particular of the Rhine delta (North Sea area and Lake Ijssel). The first paper involved analysis of 43 samples from 21 locations analysed by Py-MS but only “a selection” was analysed by Py-GC-MS. The authors identified van marine primary production from N-compounds (toluene, phenols, cyanobenzenes, pyrroles, indoles), carbohydrates (furans) and lipids (alkanes/alkenes, pristine, phytadiene), and terrestrial plant signals from lignin fingerprints. Many other aromatic products including PAHs were considered undiagnostic. The authors concluded that “the pyrolysis techniques used are very suitable for the analysis of non-volatile organic matter in a large number of samples”. In the second paper, the balance of prist-1-ene to phytadiene was used to demonstrate the rapid alteration of phytyl chains of chlorophyll.

Sigleo et al. (1982) analyzed “colloidal OM <0.4 µm” (hence DOM) from Chesapeake Bay (USA), which is known for high primary production, using Py-GC-MS. The samples produced large amounts of carbohydrate products (acetic acid, furaldehydes and lactones) and N-containing compounds (pyrroles, indole, pyridine and amides), whereas fatty acids and *n*-alkanes/*n*-alkenes were scarce and lignin markers were completely absent. They concluded that the DOM was largely derived from aquatic microorganisms (they also analysed cultured phytoplankton for comparison) and not terrestrial plant detritus. It is worth mentioning that different pyrolysis temperatures were applied using a Pyroprobe system, and notion was given of the large temperature gap between setpoint and actual pyrolysis temperature (e.g 750 °C setpoint corresponds to 610 °C for a Curie-point system). Interestingly, the authors detected acetamide (a chitin/peptidoglycan product) and not cyanobenzenes (protein) which may be an indication of a large zooplankton (e.g. copepods) or bacterial source, rather than phytoplankton. They also showed that the carbohydrate products were relatively abundant at 400 °C, the N-containing products at 600 °C and PAHs and other compounds of which the source was not discussed at 750 °C. Finally, DOM from the Patuxent River, which discharges into Chesapeake Bay, and is also known for high primary production, had negligible influence of lignin to the pyrolysis fingerprints as well, which implies that riverine DOM is sometimes (e.g. due to naturally high productivity or presence of upstream reservoirs) not easily distinguished from marine sources if both are dominated by algae-derived DOM. These results were in contrast with the common belief that marine DOM was largely derived from recalcitrant phenolic plant material (not to speak of riverine DOM). Research on the molecular composition of the global marine DOM pool has now debunked that idea.

Wilson et al. (1983) analyzed humic substances from terrestrial, freshwater and marine sources. The pyrolyzates of the (solvent-extracted) marine samples (“coastal seawater humic acid” and “aquatic extracellular material of *Phaeodactylum tricornutum* diatoms”, both from Vancouver area) were dominated by phenols, pyrroles and cyanobenzenes, with some carbohydrate products (furans and cyclopentenones) and fatty acids. Acetamide, (chloro)benzoic acid and PAH were also detected. Gilliam and Wilson (1985) used Py-GC-MS for the characterization of dissolved seawater humic substances from the same area and again an algal culture of *P. tricornutum*. The pyrolyzates were dominated by furans (carbohydrates), pyrroles and nitriles (from proteins, nucleic acids and/or tetrapyrroles) and phenols and methylphenols (non-lignin derived). The authors observed “the similarities in chemical structure of *P. tricornutum* exudate and dissolved marine humic material”. Both papers highlighted the complementarity of ^{13}C NMR and Py-GC-MS in source elucidation.

Saliot et al. (1984) analyzed suspended sediment, surface sediment and plankton from various sites in the Mediterranean Sea (five samples). Plankton was prolific of N-containing products, a sediment surface microlayer produced mainly fatty acids, whereas a samples from the Gulf of Marseille produced both set of compounds. Pollution was recognized from polystyrene products. The authors were surprised by the lack of chitin markers among the N-containing products of the plankton. This study included more samples, but those were analyzed by Py-MS only (the m/z fragment allocations were aided by Py-GC-MS).

Gadel and Bruchet (1987) analysed humic substances extracted from decaying algae (*Ulva sp.*, *Enteromorpha*) and phanerograms (*Phragmites*) from a Mediterranean lagoon, coastal sediment with *Posidonia* fragments and a fjord deposit and algae (*Macrocystis*) from the Kerguelen Islands. The *Phragmites* sample was prolific of lignin (G and S moieties) and very few N-containing products were detected, whereas the samples extracted from algae produce an abundant series of N-compounds (pyrroles, cyanobenzenes, pyridines, etc.). The N-compounds include acetamide ascribed to *N*-acetylaminosugars (bacterial/fungal cell walls growing on the decaying detritus). *Enteromorpha* algae produced some methoxyphenols upon pyrolysis. The sediment of the Kerguelen islands had a pyrolysis signature that was similar to that of the extracted algae, whereas the Quaternary sediment with *Posidonia* detritus was dominated by aromatic products toluene and phenol, and very low levels of lignin derivatives (methoxyphenols), which were attributed to proteinaceous sources, even though *Posidonia* itself has the particular feature of giving rise to pyrolysates that are very prolific of phenol (hence, scarcity of cinnamyl, guaiacyl and syringyl moieties does not imply scarcity of plant-derived lignin). The Sigean lagoon sample was prolific of N-compounds including acetamide, but also a smaller peak of propionamide, probably associated with bacterial peptidoglycan. This conclusion is based on the presence of the lactyl-peptide bridge in peptidoglycan, which transfer the necessary additional C atom to generate priopionamide upon pyrolysis and not acetamide which is also produced from chitin.

The study of Vandenbroucke and Behar (1988) is a good example of the pursuit of information on OM dynamics in recent sediments from diverse environments (lakes, estuaries and the Arabian and Mauritanian upwelling regions, a total of nine samples) to better understand the processes that drive (geological) kerogen formation (i.e. relevant to petroleum science). They compared humic acid, fulvic acid and kerogens from recent sediments, focussing on hydrocarbon chemistry (GC-MS, Py-GC-MS). Microscropic surveys showed the dominance of algae remains (diatoms, coccolithophores). It was shown that both lacustrine and marine recent kerogens contained/produced large amounts of isoprenoid hydrocarbons. The terrestrial signal (lignin) was recognized only in the sediments from deltaic areas. The *n*-alkane patterns of the stable OM in marine samples had maximum peak intensities around C_{16}. Fulvic acids have completely different aliphatic OM signals and are discarded as a major source of kerogen.

Gadel et al. (1993) applied Py-GC-MS to suspended POM from the Rhône delta (0.5, 1 and 2 m above the seafloor, i.e. bottom of the nepheloid layer). Signals of lignin were more pronounced near the river mouth and decreased in relative proportions in a seaward direction. However, depending on river discharge and currents, signals from the Liguro-provençal current were identified, which was recognized from molecular signals of phenols by Py-GC-MS and corroborated by HPLC. These waters originate from the east, with extensive *Posidonia* meadows, which is consistent with e.g. Klap et al. (2000) (see below). More seaward stations (and samples from pelagic layers) had stronger signals of N-compounds from marine primary production.

Ishiwatari et al. (1993) analysed 17 (sub) samples of sinking particles in the Japan Trench (9000 m water depth). The authors aimed to elucidate the source and degradation state of the OM. They identified (in decreasing order of average relative proportions based on peak areas): nitrogen compounds (pyrroles, indoles, cyanobenzenes and pyridines, assigned to proteins and derivatives), aromatic hydrocarbons (monoaromatic and polyaromatic), phenols (ascribed to protein due to absence of methoxyphenols), furans (from polysaccharides and related materials, i.e. melanoidins), aliphatic hydrocarbons (C_{10}-C_{27} linear alkanes/alkenes, isoprenoid compounds such as pristenes, pristane and phytane ascribed to chlorophyll) and aliphatic cyanides (C_{12}-C_{18} alkylnitriles). The authors described (time-dependent) variations in the relative proportions of the compounds, which makes this study the first attempt to numerically relate a parameter to analytical pyrolysis fingerprints in deep-sea marine environments. In 1995 (Ishiwatari et al., 1995), this line of research was expanded by including sinking particles from "only" 4500 m depth and samples from coastal Japan. The authors identified chemical changes during transport from surface waters to the seafloor on the basis of 16 samples analysed by Py-GC-MS. The same set of products were identified and a proxy of chemical alteration was based on the relative proportion of a product (normalized to the sum of alkanes/alkenes) in relation to its proportion in relatively unscathed reference material (also normalized to alkanes/alkenes in the corresponding sample). It was argued that the markers of relatively intact protein and polysaccharide (pyrroles, furfural) provided indicators of the degree of alteration. Highest proportions of fresh protein and carbohydrate were identified in samples during high foraminifera fluxes (during blooms).

Sicre et al. (1994) analysed suspended POM in surface and bottom waters of the Rhône delta, at various sites (representing a salinity gradient). Suspended particles of the Rhône river itself were prolific of polysaccharide markers (furans and furaldehydes) that were (due to absence of anhydrosugars such as levoglucosan) interpreted as possible products of transformed (dissociated) lignocellulose. The C_{10}-C_{24} alkanes/alkenes, fatty acids and phytadienes were detected as well. Lignin markers were scarce. In comparison with river POM, the surface waters were enriched in proteins (indoles and pyrroles). Bacterial protein was identified on the basis of similarity in mass spectral patterns of dipeptides reflected by m/z 195 and 209 in partial ion chromatograms, reported by Boon and de Leeuw (1987). Alkylnitriles (C_{14}-C_{18}) were also abundant. Further offshore the proportion of carbohydrates declined and fresh plankton markers increased. The bottom waters (nepheloid layer) were enriched in polysaccharides whereas many products of primary producers found abundantly in the surface waters were detected. It was concluded that the nepheloid POM was largely of terrestrial origin, which was in agreement with previous studies of the Rhône delta that showed that riverine suspended material enters into the marine environment through benthic currents. Finally, the authors found markers of anthropogenic inputs (polystyrene and a source of 1,1,3,3,5,5, hexamethylcyclotrioxane) and filter contaminant (C_8-alkenol).

Peulvé et al. (1996a) analysed POM from sediment traps deployed at 100, 200, 1000 and 2000 m along a mooring line in the NW Mediterranean Sea. Diatoms of *Biddulphia chinensis* were used as reference material of photosynthetic producers. The samples were prolific of alkanes/alkenes (C_{10}-C_{27}) as well as alkylnitriles throughout the water column whereas fatty acids (and phytadienes) were only detected at the surface, and the authors argued that the alkanes and alkenes must originate from resistant aliphatic macromolecules such as algaenan. They used the balance between alkanes and alkenes to distinguish fatty acid sources from algaenan. The *B. chinensis* samples only produced alkanes/alkenes in the C_{10}-C_{18} range, which is a particular feature of (some) diatoms. The alkylnitriles (C_{14}-C_{18}) were present in the pyrolyzates of deeper samples which did not produce fatty acids, and it was concluded that they cannot originate from the reaction between NH_4 and fatty acids during pyrolysis (Evans et al., 1985), but more likely from algaenen which was shown previously to produce alkylnitriles, probably due to dehydration of alkylamides (Derenne et al., 1991). Isoprenoid hydrocarbons, i.e. pristenes and phytadienes, were attributed to chlorophyll and the more resistant tocopherol, respectively, on the basis of their depth profiles. Nitrogen-containing products (pyrroles, indoles, cyanobenzenes) were also detected, and were enriched in deeper layers, which raised the question whether the N-compounds correspond to protein or another, unknown, non-proteinaceous, algae-derived, and more resistant precursor. In fact, the authors described the protein signal on the basis of the aforementioned ion fragments related to several dipeptide combinations, and all the other N-compounds (pyrroles, indoles, cyanobenzenes) were considered of non-proteinaceous sources (contrary to e.g. Ishiwatari et al., 1993). The authors considered that the strong contrast in depth distributions of the aforementioned amino acid dimers and the more common N-containing products implies that they cannot originate from the same source, disregarding the possibility of profound alteration of the protein. They did recognize, however, that they may correspond to melanoidin, a complex polymeric material which is now considered mainly as protein degradation product and not a different family of constituents. Interestingly, the degree of alkylation of the indoles increased with depth. The Py-GC-MS data was supported by some essential observations from SEM of the trap material, in particular the abundance of zooplankton detritus in all samples (foraminifera, radiolarians, dinoflaggelates, tintinnids), and arthropod exoskeletal, which pointed towards abundant chitinous materials, but the authors do not describe chitin pyrolyzates as possible dominant source of the N-compounds, instead of melanoidin or melanoidin-like POM. Carbohydrates (furans and benzofurans, but not levoglucosan) were argued to originate from polysaccharides with a rather stable character, and not storage polysaccharides (e.g. cellulose) (or chitin). Phenols and alkylbenzenes were also detected but considered of little diagnostic value (phlorotannins were inferred as a potential source of phenols, among other sources). Nonetheless, the more strongly alkylated phenols were argued as unlikely protein products and attributed to "from an autochthonous, unidentified, resistant macromolecule in algae" instead. The PAHs (naphthalenes) were ascribed to unknown resistant precursors. Lignin products were not detected. The authors concluded that "the preservation of known and some presently unknown biomacromolecules biosynthesized by algae, either by selective preservation or by rapid sinking, could be a major process determining the quality and quantity of sinking and depositing organic matter in the ocean". These materials were argued to originate from the surface productive layer (100 m).

Earlier that year, Peulvé et al. (1996b) reported the composition of suspended OM in the Laptev Sea (different depths: surface, intermediate, sediment surface) in the Arctic region. By comparison of POM signals from the Lena river that discharges into it, it was argued that the surface samples of the Laptev Sea contain POM of mixed terrestrial (furans and furaldehydes from degraded lignocellulose; pyrroles and cyanobenzenes from protein or chlorophylls; phenols from spores; alkanes and alkenes from higher plant lipids such as cutan and suberan)-marine (e.g. fatty acids, alkylnitriles, phytadienes) mixed origin. Deeper layers did not produce fatty acids or nitriles upon Py-GC-MS, but a series of C_{14}, C_{16} and C_{18} alkanes/alkenes. The alkenes contain large peaks of various isomers (alk-1-ene, (E)-alk-2-ene, (Z)-alk-2-ene), which were argued to originate from wax esters (C_{16}-C_{18} fatty acids esterified to C_{14}-C_{16} alkanols) on the basis of solubility in organic solvent and GC-MS data of the derivatized extract. They were tentatively attributed to non-chitinous microzooplankton or bacteria (Armada et al., 2017) identified the same set of compounds as the prevailaing pyrolysis products of a sample from archaeological jewellery that also contained large amounts of SEM-visible diatom skeletal.) The signal of alkylnitriles was relatively strong in the trap material from the water column-sediment interface, where wax ester products were scarce, possibly due to higher proportions of the more recalcitrant POM such as algaenan (and phenols allegedly from resistant terrestrial sources). Hence, the POM was largely ascribed to terrestrial sources, albeit that marine-derived macromolecules (algae) signal was also recognized. The authors describe the Laptev Sea as a good example of a shallow marine system with low primary production and high terrestrial influx.

Ergin et al. (1996) analyzed surface sediments (top 5 cm) from the coastal shelf of the southern Black Sea (9 samples) by Py-GC-MS (and ^{13}C NMR). The identified compound groups were lignin derivatives, carbohydrates (furan, cyclopentenes, pyrans), N-heterocyclic aromatics (pyrroles, pyridines, indole), aliphatics and alkylbenzenes. Most samples were prolific of phenol, guaiacol and 4-vinylguaiacol, indicative of a major terrestrial plant source. One sample contained pyrite and produced mainly elemental sulphur upon pyrolysis (samples were not pretreated and organic C content was below 3 %). The large proportion of lignin (allegedly "nondegradable by marine biota") and the complementarity between Py-GC-MS and solid-state ^{13}C NMR were highlighted.

Pulchan et al. (1997) used TMAH for THM-GC-MS of OM in marine sediments, also focusing on the identification of terrestrial sources in three cores (12 samples) of sediments in Trinity Bay (Newfoundland). The identified products included trimethoxybenzenes (1,2,3,-, 1,2,4-, 1,3,5-), P6, G4, G6, P18, G18 (lignin products nomenclature) and a series of fatty acid methyl esters (FAMEs, C_{14}-C_{26}) including mid-chain hydroxylated FAMEs. They linked 1,3,5-trimethoxybenzene to condensed tannin from terrestrial plants. They also applied THM-GC-MS using TEAH to distinguish native methoxylic groups in the OM from derivatized hydroxylic groups, confirming that the relevant dimethoxybenzene compounds (G4, G6, etc.) originated from guaiacol structures (and not catechols) and that the methoxylic group of P6 and P18 indeed originated from derivatized p-substituted phenols (not methoxybenzenes). The authors concluded that "the technique of batch-wise TMAH thermochemolysis is a quick and easy method of identifying chemical markers of terrestrial origin". In Pulchan et al. (2003), a modified THM-GC-MS method was applied to the fjord associated with Trinity Bay (Northwest Arm). Unsurprisingly, the sediments from this fjord were more prolific of terrestrial sources (again, lignin) than the further offshore samples of Trinity Bay itself.

Fabbri et al. (1998) applied Py-GC-MS to humins and humic acids from a surface sediment sample from the Adriatic Sea. They observed that, in comparison with samples from terrestrial or lacustrine systems, large contributions of phenols (non-lignin) and phytadienes were observed. They further developed the study of marine OM in the Adriatic Sea by Py-GC-MS in Fabbri et al. (2005), aiming to identify terrestrial precursors in marine sediments (five samples), after HCl and HF treatment. They identified (and performed a semi-quantitative exercise) lignin as diagnostic element of terrestrial OM (represented by guaiacols and syringols), and described the fingerprints of proteins (pyrrole, benzeneacetonitrile, indoles), carbohydrates (levoglucosan, furaldehydes) and chlorophyll (phytadienes) in detail. The alkane/alkene patterns were used to calculate proxies of terrestrial inputs which proved to be in accordance with the same proxies (TAR, terrestrial to aquatic ratio) from GC-MS data. It is worth mentioning that a series of PAHs were identified, but a possible pyrogenic component to the OM was not discussed. They concluded that "the pyrolytic marker approach by Py–GC–MS is valuable for sourcing marine OM on a semi-quantitative base".

Zegouagh et al. (1999) analyzed refractory (insoluble and non-hydrolyzable) OM in sediment cores from the NW African upwelling region, targeting a surface sample and two deep samples (70-90 kyr age). The identified refractory OM was argued to be primarily protein-derived/melanoidin-related. The pyrolyzates were dominated by alkylbenzenes, in particular toluene, C_2-benzenes and tetramethylbenzene. Phenols and N-compounds (ascribed to melanoidin-type OM) and PAHs (unassigned) were also abundant. Alkane/alkene doublets were surprisingly scarce in these samples. The authors conclude that selective preservation of algaenan was not relevant probably due to the prevailing diatoms with mineral skeletal instead of algaenan-rich organic-walled plankton groups, and that the main pathway of recalcitrant OM formation was the formation of melanoidins.

Ismaïli et al. (1999) examined pyrolyzates of suspended POM (surface and deep waters, but only bottom samples were analysed by Py-GC-MS) obtained during a series of N-S cruises in the English Channel. Samples from March '95 had lower C/N (8-11) than those of November '94 (10-15), whereas samples from July '95 had a wider range (8-20), and this was reflected by several molecular proxies. The dominant pyrolysis products of the bottom sediments (nine chromatograms from 3 sites x 3 expeditions) were benzene, styrene, furfural, pyrrole, methylpyrroles, indole and phenol/methylphenols. The sum of N-containing products ranged between 28 and 43 %. Acetamide was not including in this group, but with the aminosugars. The C/N ratios and sum of N-compounds clearly show that the POM is dominated by N-rich sources, allegedly of marine sources. The ratio of (3/2-furaldehyde + acetic acid)/pyrrole was used as a proxy of OM freshness. Other proxies used were benzene/toluene (degradation proxy) and acetonitrile/pyrrole (preservation proxy). All these proxies have serious limitations in mixtures of terrestrial and marine sources. Lignin signals, which were only picked up by CuO-HPLC but this may be ascribed to the fact that the authors only examined 23 major Py-GC-MS products, were stronger on the north side due to the fluxes of the Solent river, whereas the POM in the samples from the French side were almost completely of marine precursors. Decay intensity was strongest in the central waters (due to longer residence time of waters from the "Atlantic water vein"), reflected by high proportions of aromatic hydrocarbons. The authors highlighted the importance of phenolic POM from marine precursors (phytoplankton and coastal macroalgae). The authors discussed the phenolic proxies from CuO-HPLC in terms of likely origin (cinnamyl, guaiacyl and syringyl of continental origin, and p-hydroxyphenyl of multiple sources but argued to be mainly of algae/phytoplanktonic and bacterial sources).

Van Heemst et al. (1999) applied Py-GC-MS to a wide range of OM samples (DOM, POM, sediments, algae, tannins, lignin, coal, cuticles etc.) aiming to identify the source of alkylphenols in environmental samples. Their conclusion was that the alkylphenols are pyrolysis products of proteinaceous material that was subjected to oxidative cross-linking with protein and polysaccharides. Van Heemst et al. (2000a) applied Py-GC-MS to DOM samples from a salinity gradient in the North Sea (Ems-Dollard estuary). The carbohydrate fingerprint showed that all polysaccharides had been subjected to major alterations. Also, the aforementioned dipeptide products were not detected, pointing towards alteration of proteinaceous precursors. Alkylphenols were ascribed to heavily degraded proteins and/or lignins. In general, the results were considered evidence of the strong alteration of the prevailing polysaccharides and N-containing moieties. Van Heemst et al. (2000b) applied THM-GC-MS on DOM from fluvial and estuarine waters. Lignin markers were ascribed to terrestrial sources and abundant aliphatic compounds (FAMEs) were related to cutan. These results show that THM-GC-MS is biased towards aliphatic and phenolic, easily hydrolyzed/derivatized OM, whereas (structural plant) and microbial polysaccharides and protein are underrepresented. The authors used proxies that were frequently used as parameters of lignin source and oxidation degree (G6/G4, P/G, S/G) for lignin interpretation.

Çoban-Yıldız et al. (2000a) studied suspended POM from open (ten samples) and coastal waters (four samples) of the southern Black Sea, by Py-GC-MS and THM-GC-MS (using TMAH), focusing on the effects of vertical stratification. They identified markers of chlorophyll, lipids, carbohydrates (furans by Py-GC-MS, 1,2,4 trimethoxybenzenes and methylated deoxyaldonic acids by THM-GC-MS) and proteins/melanoidins (pyrroles, indoles and aromatic nitriles; Py-GC-MS only). Products of lignin or plant waxes, or any other signal of terrestrial OM (hence, including seagrasses) were not observed. Furthermore, the absence of levoglucosan and detection of the furans was interpreted as a negligible cellulose content. For Py-GC-MS, the alkane/alkene patterns (up to C19) maximized at C_{10}-C_{15}, of which the alkenes were dominant. These compounds were assigned to phytoplankton (fatty acids, waxes, algaenan, etc.). From THM-GC-MS, the polymethylene structures were mostly FAMEs (but not cutin-derived FAMEs). Markers of bacterial lipids were not found. The authors observed a clear correlation between the abundance of phytadienes and chlorophyll a concentration, confirming that phytadienes originate from chlorophylls (in this case). Interestingly, the depth trend showed two different protein sources, one dominated by pyrroles and cyanobenzenes (increase with depth) and another by indoles and phenols (decrease with depth) in the open water environment. This was explained in terms of relative proportions of phytoplankton in the euphotic zone and in deeper layers a mix of "aggregated bacteria, small fecal pellets and material adsorbed on inorganic particles". Çoban-Yıldız et al. (2000b) analyzed suspended POM from the (oligotrophic) Mediterranean Sea (two sites, water depth 1-110 m, a total of 16 samples) and compared the results from the 2000a paper from the (more productive) Black Sea. The authors described the products of chlorophyll, lipids, carbohydrates and proteins. Again, lignin was not detectable and the authors described the trends in molecular chemistry on the basis of lipid and protein markers. The level of maximum chlorophyll productivity marked a change in POM composition, albeit to a smaller extent as what had been observed for in the open sea environment of the Black Sea. THM-GC-MS was not performed here.

Aiming to increase the understanding of the formation of kerogen, Garcette-Lepecq et al. (2000) studied OM in surface sediments (three samples from several cores), in the Black Sea. Even though the authors applied a rather harsh HCl-HF attack, as they intended to isolate the refractory OM, the pyrolysis products include most of the products that are frequently observed in mixed marine/terrestrial materials. The observed Py-GC-MS products included PAHs, indoles, pyrroles, pyridines, cyanobenzenes, alkanes and alkenes (C_9-C_{33}, maximum at C_{13}) and fatty acids. The aliphatic products (alkanes/alkenes) were ascribed in part to recalcitrant biomacromolecules from higher plants (cutan, suberan) (the samples were obtained in the proximity of Danube delta). Algaenan was argued to be a minor source of alkane/alkene products. Prist-1-ene was ascribed to tocopherol, as the other major theoretical source of prist-1-ene, i.e. chlorophyll, was considered to be negligible on the basis of absence of phytadienes signal. Most phenols, catechols and methoxyphenols were ascribed to lignin and modified lignin. Melanoidins and proteins were considered a potential source of alkylphenols as well. Long-chain alkylbenzenes were ascribed to cyclization of polymetheylene chains during pyrolysis, whereas toluene and other short-chain alkylbenzenes were allocated to protein (phenylalanine). A C_4-alkylbenzene with 1,2,3,4-tetramethyl configuration was argued to originate from algae (*cf.* Hoefs et al., 1995; alternative source suggested by Hartgers et al. 1994: green sulphur bacteria). Pyrroles and indoles were ascribed to proteins and/or melanoidins (degraded proteins), whereas furans and furaldehydes were attributed to polysaccharides. In terms of rationale for source allocation of common Py-GC-MS products, this study represents one of the more detailed accounts. It is noted that the large Danube-fluxes of terrestrial OM imply that the OM is more terrestrial than may be expected in marine systems in general. In 2001, the same authors reported THM-GC-MS (using TMAH) data of one of the samples from the Danube delta/Black Sea interface (Garcette-Lepecq et al., 2001). The main products identified were lignin markers (including P18 and G18, indicative of well-preserved lignin and/or cinnamic acids), benzene carboxylic acids (detected as methyl esters), FAMEs (C_5-C_{30}, including *iso*- and *anteiso*-C_{15} and C_{17} aliphatic acids, associated with a bacterial contribution, and mid-chain hydroxylated C_{16} FAMEs from cutin), fatty diacid dimethyl esters, several N-compounds (including triazines, probably directly from TMAH reactant, and *N*,*N*-dimethyl-amino acid methyl esters of glycine, alanine, etc.), and 1,2,4-trimethoxybenzene ascribed to polysaccharides. All due to the predominantly terrestrial signal.

Mannino and Harvey (2000) evaluated the contribution of terrestrial OM in different fractions of POM and DOM obtained from Delaware Bay (estuary of the Delaware River) on the basis of the phenolic and aliphatic THM-GC-MS (using TMAH) products. The results showed a notable decline in the proportion of terrestrial-derived OM from the turbid estuary (C/N 25-30) to the coastal ocean (C/N 17-20) (seven samples were analyzed, salinity gradient). There was a clear decrease in the proportion of terrestrial DOM from the river to the coastal ocean. Reference samples for proteinaceous sources were also analyzed, and quite a few compounds could indeed be attributed to mainly tyrosine, phenylalanine, tryptophane and proline. The lignin products included many of the commonly detected H, G and S product from thermochemolyzates. Product P18 was attributed to protein rather than lignin or lignin-like sources. 1,2,4-Trimetoxybenzene, 1,3,5-trimethoxybenzene (ascribed to tannin and/or cutan) and a series of C_9-C_{26} FAMEs (the low molcular weight FAMEs were attributed to terrestrail but not necessarily plant-derived sources, and argued to be bound to the macromolecular matrix). Comparative analysis of POM showed that the proteinaceous OM was concentrated in POM fractions. For the POM fraction, lignin was concentrated in samples from the autumn period, when terrestrial debris is abundant and primary production is low.

Deshmukh et al. (2001) found a dominant signal of terrestrial OM (lignin products) in San Diego harbor area sediments but abundant alkane/alkene signal as well, which was assigned to marine primary production sources (algaenan). They applied Py-GC-MS and THM-GC-MS and detected products of protein, lignin, aliphatic OM and polysaccharides. THM-GC-MS was useful for identifying gymnosperm vegetation as the predominant source of the lignin products. In spite of clear PAH contamination at a polluted site in the region, a link between solvent-extricable PAH and PAH productivity upon Py-GC-MS and THM-GC-MS was not observed. Hence, the presence of solvent-extricable PAH and PAH in pyrolyzates are decoupled.

Guo et al. (2004) studied Siberian Arctic coastal sediments, focusing on the detection of fluxes from terrestrial environments, and thereby release of OM from thawing permafrost. They analyzed sediments collected from a wide range of estuarine regions of large arctic rivers (Ob, Yenisey, Khatanga, Lena, Indigirka) by Py-GC-MS. The study adopted a multi-proxy approach, including parameters from elemental and stable isotope analysis. Black Carbon analysis (chemothermal oxidation method) showed that on average approximately 10 % of the OM was pyrogenic, and detected an eastward increase in this proportions, which coincided with an increase in OM freshness as determined from the balance between furaldehydes to nitriles in the pyrolyzates. The authors found a startling correlation between the proportion of Black Carbon and 14C age, both of which increased eastward along the Siberian coast. The authors argued that this old pyrogenic OM (in terms of tens of Millennia) must originate from thawing permafrost regions. The evaluation of the Py-GC-MS data was based the selection of 21 products (from an original dataset based on identification of the 100 main peaks in each of the five chromatograms from five estuaries) which were grouped to create "index categories" (furfurals, alkylbenzenes, nitriles, and other). It seems that this sample set and pyrolysis dataset are suitable for a more detailed assessment of OM composition along the Siberian coast.

Hwang et al. (2006) aimed to improve the understanding of recalcitrant deep ocean POM by NMR and Py-GC-MS of non-hydrolyzable sinking POM. Both methods exhibited a prevailing aliphatic POM component (alkanes/alkenes). Benzene and alkylbenzenes, phenols, pyrroles, furans, pristenes, fatty acids and phytadienes were also detected. The alkanes/alkenes, alkylbenzenes and alkylphenols were more resistant against hydrolysis than the carbohydrate and protein/melanoidin products, but the latter series of products were also detected in the non-hydrolyzable deep ocean POM. The possible presence of pyrogenic POM (from wildfires, not anthropogenic) and their molecular products (aromatic compounds) were discussed.

Guo et al. (2009) studied high molecular weight DOM (>1000 D, <0.2 µm) in surface water samples from the Mississippi River plume, along a salinity gradient (12 samples). The desalted DOM samples had a high OM content. The C/N ratio decreased from 19-20 in the more terrestrial-influenced regions of the plume, to 12-15 offshore due to increased relative proportions of DOM from freshly photosynthesized structures. As in Guo et al. (2004), the pyrolysis fingerprints were summarized on the basis of an index set of only 8 products (C_0-C_1-furaldehydes, dimethylbenzene, phenol, C_1-cyclopentenone, indene, naphthalene and C_1-pyridine. There is a clear trend in the relative proportions of furaldehydes (increase with salinity) and, on the other hand, phenol and C_2-benzene, which was interpreted as an effect of the relative proportions of marine and terrestrial DOM.

De la Rosa et al. (2011) studied humic substances isolates from sedimentary OM in the SW Iberian continental shelf including the estuary of the Guadiana river, by means of Py-GC-MS. They reported the presence of vascular plant remains, represented predominantly by methoxyphenols (lignin) and long-chain linear alkane/alkene patterns, even far offshore. Nevertheless, there was a clear seaward increase of the relative proportion of marine sources recognized from alkanes/alkenes, alkylnitriles and alkylamides. The nitriles were thought to originate from algaenan. Interestingly, the samples produced large amount of PAHs (naphthalenes, fluorenes, phenanthrenes, etc.) which were ascribed to pyrogenic OM (the study area is fire-prone). The pyrolysis products included a wide range of benzonitriles and quinolones, presumably from bacterial input (a possible pyrogenic source of these products was not mentioned). In another work of the same group, González-Vila et al. (2009) had described the THM-GC-MS chromatograms of some of these samples but after degradation of labile OM (mostly lignin) by chlorite oxidation. Besides aliphatic (FAMEs) products, the authors reported the presence of "benzene and naphthalene derivatives". The compound list table includes methylated benzenepolycarboxylic acids (up to four methylated carboxylic groups), methylated naphthalenecarboxylic acids and methoxybenzonitriles, which are probably products of oxidized pyrogenic OM by this method (Kaal et al., 2008; Kaal and Filley, 2016). Polvillo et al. (2009) further elaborated on the THM-GC-MS profiles of various chemically isolated OM subfractions from the same set of samples, focusing on the abundance of lignin, benzenecarboxylic acids and aliphatic biopolymers, but other sources such as protein and polysaccharides were also detected. The benzenepolycarboxylic acids were conspicuous in the residue after the harshest treatment (RuO_4), supporting a source in pyrogenic OM.

Rocker et al. (2012) studied the salinity-dependent decomposition of humic acids by marine and estuarine bacterial communities, in an attempt to explain why humic substances are abundant in freshwater DOM but not in the DOM in the open ocean environment, by THM-GC-MS. Peat-derived humic acids were largely depleted of their aliphatic pyrolysis fingerprint (mostly derivatized carboxylated aliphatic compounds, including FAMEs) due to both estuarine and marine bacteria, whereas the lignin (benzoic acid derivatives such as benzenetricarboxylic acid trimethyl ester, cinnamic acid and G18) was largely unaffected. Tannin markers and other products were also detected.

Zhu et al. (2016) developed a Py-GC-MS protocol for the rapid (in comparison with procedures that involve PCR) screening of microbial biomass in sediment samples. The pyrolysis products benzyl nitrile (benzeneacetonitrile, not to be confused with benzonitrile or methylbenzonitrile), 2-furanmethanol, indole, phenol and pyrrole were evaluated. These compounds originate from DNA, protein and peptidoglycan, in different relative proportions. The approach and analytical conditions (e.g. polar column) are not comparable to most other studies mentioned in this review, but this study is a good example of exploring new potentials of Py-GC-MS to reduce time and expenses of more established methods. A table is provided in which the potential precursors of different N-containing pyrolysis products are reported, which may be helpful in the distinction of e.g. chitin and peptidoglycan in complex mixtures.

Zhang et al. (2016) applied Py-GC-MS to dissolved humic substances (humic acid, fulvic acid and the fraction obtained XAD resin) from coastal marine environments (estuary, continental shelf, and continental slope) in the Changjiang estuary. It was confirmed that the suspended sediment (all fractions) of the saltmarsh site had a relatively high proportion of lignin-derived pyrolysis products (phenols and alkylphenols, some methoxyphenols). Samples from the continental slope site in the adjacent South China Sea exhibited OM fingerprints typical of marine algae (aliphatic compounds, carbohydrates, and N-compounds), whereas the continental shelf site in the East China Sea contained signals of terrestrial and marine sources. Zhang et al. (2017) studied dissolved humic substances from the Southern Yellow Sea by Py-GC-MS, NMR and FTIR. The dominant pyrolysis products were aliphatic compounds (C_7–C_{17} alkanes/alkenes), other aromatic components (benzene, toluene, C_2–C_4-alkylbenzenes) and polysaccharides (furans, acetic acid, cyclopentenones), with smaller proportions of N-compounds, including alkylnitriles and -amides, phenols, fatty acids and PAH. Lignin products were not detected. Humic acid was enriched in aliphatic and proteinaceous OM which was attributed to a larger impact of degradation. Fulvic acids, which were more abundant, exhibited a relatively intense signal of aromatic OM. The most labile OM was in the XAD fraction, in particular polysaccharides. The authors mention the potential presence of pyrogenic OM in some of the samples.

Building further upon Guo et al. (2004), Sparkes et al. (2016) studied sediments from the East Siberian Arctic Shelf, aiming to identify the imprint of terrestrial OM from thawing permafrost deposits or coastal slumping in Siberia, and to demonstrate that Py-GC-MS is a rapid and robust procedure for macromolecular OM characterization. The sediments (the study is based on 42 samples) were subjected to solvent extraction to isolate the macromolecular OM, prior to analysis by Py-GC-MS. The semi-quantititave exercise was based on nine compounds, representative of main biomolecular sources (phenol from lignin; pyridines from marine N-rich OM; dimethylbenzene from anaerobic soils; furaldehydes from relatively intact polysaccharides; a cyclopentenone from soil polysaccharides; indene and naphthalene ("aromatics") from mature OM). The results showed an offshore decrease of the terrestrial signal (phenol, aromatics, cyclopentenone) with respect to the marine signal (represented by pyridines). The aromatics were ascribed to relatively old (degraded) terrestrial sources (thawing permafrost such as Yedoma deposits). The balance between phenols and pyridines (phenol/(phenol+pyridines)) is proposed as a proxy of terrestrial *vs.* marine macromolecular OM. The use of phenol as a proxy of terrestrial OM in marine environments is dangerous, in the light of the ample evidence of phenol production upon Py-GC-MS of algae and other marine sources, but an analysis of a riverine-distal shelf transect from the Kolyma river (this same study) showed that phenol correlates (very) strongly with lignin phenols from CuO-GC-MS data. It is not discussed why the five guaiacols and a syringyl (Supplementary Material), which are obviously useful as support of the phenol-based proxies, were not included in the argument. Other peaks detected but not used in the numerical evaluation of the data include benzonitrile, benzyl nitrile, an interesting furan carbonitrile, biphenyl and doublets of alkanes/alkenes, implying that the distribution of other OM constituents can be evaluated from the pyrolyzates. The absence of a trend in furaldehydes proportions was interpreted as a mixed marine/terrestrial origin, an interpretation that was supported by their behavior in PC1-PC2 space from PCA analysis.

Nierop et al. (2017) studied OM (after solvent extraction and HF/HCl treatment) from surface sediments (upper 18 cm) in the Murray Ridge of the Arabian Sea, and linked their pyrolyzates to oxygen exposure time via oceanographic parameters of the oxygen minimum zone (the ridge provides a transect from fully oxic to suboxic to dysoxic conditions with increasing altitude of the submarine mountain). The OM was predominantly marine, as shown by high proportions of chlorophylls, polysaccharides, proteins and phlorotannins. More specifically, Py-GC-MS produced furans, pyrans, anhydrosugars, pyrroles, pyridines, indoles, diketopiperazines (including diketodipyrrole; these are ubiquitous N-containing products that have been given surprisingly little attention), phenols, alkylbenzenes (dominated by 1,2,3,4-tetramethylbenzene), tocopherol, alkenes/alkanes and isoprenoid hydrocarbons (prist-1-ene and phytadienes). Oxygen exposure caused enrichment in linear alkanes/alkenes and alkylbenzenes, and depletion of polysaccharide products, protein products (drastically) and isoprenoid hydrocarbons (including prist-1-ene). THM chromatograms contained 1,3,5-trimethoxybenzenes but only in the samples that had not been subjected to prolonged O_2 exposure, indicative of selective decay of phlorotannins. Useful discussions on the likely sources of alkanes/alkenes (selective preservation of algaenan-like biopolymers vs. oxidatively polymerized fatty acids; *cf.* Versteegh et al., 2004), tetramethylbenzene (algae, not bacteria) and alkylpyrroles (pigments), pyridines (chitin, peptidoglycan) and indoles (protein) were provided. Differences between sediment cores were much larger than differences within cores, implying that O_2 exposure has a larger control on OM composition than subsequent (i.e. after burial) anaerobic decay.

Kaal et al. (2018a) assessed the molecular composition of POM in sediment traps underneath a series of mussel farms (rafts) in Galician Rías (NW Spain), and identified the fluxes of mussel debris (mostly faeces) on the basis of mucus/chitin after Py-GC-MS. The raft that was located more closely to the coastline (NW Spain) had a larger proportion of pyrogenic POM (concluded from the levels of PAHs). Organobromine and pollution products were also detected.

Zúñiga et al. (2019) applied Py-GC-MS to POM samples obtained from sediment traps deployed on a mooring line in the NW Iberian upwelling system. A total of 27 samples from 2002 and 2006 were analysed to witness temporal changes in POM composition. The molecular fingerprints, dominated by

N-containing products, confirmed what stable isotope data suggested in that most of the POM in all the samples originated from primary production (mainly phytoplankton). However, samples from upwelling seasons (the productive seasons) had a stronger expression of algae-derived OM (N-compounds, isoprenoid hydrocarbons, fatty acids) whereas the samples from downwelling seasons, which coincide with higher river discharge, had stronger signals of terrestrial signals of lignin and pyrogenic POM. These results were in good (statistical) agreement with d^{13}C and C/N data, and it was concluded that Py-GC-MS is a useful tool to understand the fluctuations in these commonly used parameters. Selected samples analysed by THM-GC-MS indicated the presence of cutin-derived aliphatic POM, which was more abundant in samples from high river discharge periods.

Stevenson and Abbott (2019) analyzed the composition of macromolecular OM (solvent-extracted before analysis) in sediments along a transect (5 stations, triplicate analysis) near Svalbard in the Arctic Ocean using Py-GC-MS. The balance between pelagic and benthic OM sources was determined using two different methods, both based on peak integration data of pyrolyzates but on the basis of different sets of pyrolysis products and one normalized to total peak area and the other not. Samples were treated with HCl to remove carbonates but not HF, even though total organic matter content was not high (1.6-2.5 %). Alkylbenzenes (especially toluene) were the dominant group of pyrolysis products. Among the N-containing products, pyridines were the most abundant. Indoles, pyrroles, phenols and furaldehydes, naphthalene and *n*-alkanes/*n*-alkenes were also detected. Terrigenous OM sources were argued to be negligible. High average annual ice cover (northern sediments) were enriched in alkanes/alkenes and N-compounds. They discuss most likely origins of several products (pyridines to chitin; tetramethylbenzenes to carotenoids; furaldehydes to labile algae-derived polysaccharides; alkanes/alkenes from algaenan and/or geopolymerization, etc.) on the basis of knowledge on organisms' distributions and foodweb structure in the study area, focusing on marine plankton (copepods, phytoplankton). On the basis of that information, the authors argue that poleward movement of ice cover/open ocean boundaries might enhance OM deposition/burial.

B. Coastal vegetated habitats (Blue Carbon systems)

In "Diagenesis of organic matter in Georgia salt marshes", Fogel et al. (1989) compared stable C and N isotopes with Py-GC-MS fingerprints of OM in saltmarsh sediments, suspended POM, humic acids and otherwise chemically-obtained fractions (35 samples, which was a lot for that time). The authors described molecular mechanisms behind decay processes and source variations that were identified by d^{13}C, focusing on the relative proportions of lignocellulose products (from Spartina cordgrass and other higher plants), short-chain alkanes (mainly from algae) and long-chain alkanes. For example, selective decay of polysaccharides and enrichment of lignin caused a shift in 13C of cordgrass detritus.

Klap et al. (1996) analysed seston samples from a creak in a large saltmarsh system from The Netherlands (Saeftinghe saltmarsh), and studied tidal effects on molecular composition of POM using Py-GC-MS and THM-GC-MS using TMAH (and Py-MS and Py-GC). The protein component of the POM was identified by indoles and the aforementioned dipeptides first reported by Boon and de Leeuw (1987). The phenols were also assumed to be partially protein-derived. The methoxyphenols with *p*-vinyl substitution were ascribed to lignin-hemicellulose complexes in non-woody (herbaceous) higher plant sources, and confirmed by peaks of P18 and G18 after THM-GC-MS. The tidal and seasonal fluxes indicated that the saltmarsh was not actively delivering lignin from halophytic vegetation to the aquatic system. Instead, lignin concentrations were higher in flood than in ebb samples. Using Py-MS, Hemminga et al. (1992) had come to a similar conclusion: negligible contribution of cordgrass (*Spartina* sp.)-derived POM to the tidal efflux from a creek in the 't Zwin slufter-saltmarsh (Dutch-Belgian border), and Saeftinghe (Hemminga et al., 1993). These studies were performed mainly to better understand the foodweb structure (source of C for microbial consumption) in the aquatic environment associated with the saltmarshes.

Klap et al. (2000) performed Py-GC-MS on tissues of two important seagrasses, i.e. *Posidonia oceanica* and *Zostera marina*, focusing on lignin content and composition. They found that *P. oceanica* contains more lignin than *Z. marina* and that leaves contain less lignin than roots and rhizomes. They argue that lignin content influences the recalcitrant of seagrass tissues (lignification for protection against microbial attack). The main finding of this study was that these phanerograms maintain the capacity to produce lignin, hence confirming the ambiguity and need for caution in provenancing lignin in marine systems (Opsahl and Benner, 1993). The analysis of MWL (milled wood lignin) from the studied species showed presence of phenols in all pyrolyzates, but not all samples produced G and S compounds. Phenol was the main product in all preparations of *Z. marina* and *Fucus vesiculosus*, and *P. oceanica* (except for rhizome, which had more 4-vinylsyringol), whereas the main peak of *Spartina anglica* was 4-vinylguaiacol. The authors did not consider the non-methoxyphenolic part of lignin, which in case of *S. anglica* or the leaves of *Zostera* may be reasonable, but for Posidonia it would later appear that the phenols are predominantly decarboxylation products of *p*-HBA units esterified to G moieties of the lignin backbone (see below).

Zang and Hatcher (2002) applied solid-state ^{13}C NMR and Py-GC-MS to sediments from Mangrove Lake (Bermuda). This 15 m thick sequence is an example of a system that shifted from predominantly terrestrial (peat) to freshwater aquatic to marine aquatic conditions (upper 8 meters), and currently the system is brackish/saline. Furans, several furancarboxaldehydes, phenols, catechol, guaiacols, N-compounds, pristenes, alkanes/alkenes and fatty acids were detected, indicative of a complex OM composition from mixed sources (chitin, protein polysaccharides, lignin, aliphatic biopolymers, chlorophyll/tocopherol, etc.). The signal of lignin was negligible in the aquatic section of the deposit, referred to as algal sapropel (1-14 m, spanning approximately 9000 yrs). The authors focused on organic forms of N and their depth trends. They showed that even though the majority of proteinaceous OM was lost during early decay processes, a minor fraction was preserved and obtained a recalcitrant character, arguably due to encapsulation in aliphatic OM. Protein was enriched in the marine section of the deposit, and the molecular fingerprinting of N sources focused on that. The N-compounds detected included pyrroles, acetamide, imidazoles, indoles, long-chain alkylamides and diketopiperazines. The latter products were considered the most useful markers of proteinaceous precursors of the OM (as they are dimerization products). Due to the fact that the peak intensities of the different diketopiperazines showed little change with depth, they were supposed to originate from a specific recalcitrant source that was barely affected by long-term decay. These compounds were also identified after acid hydrolysis of selected samples, which supported their argument. The co-abundance of alkane/alkene compounds in the sample after acid hydrolysis was interpreted as the main cause of protein preservation through encapsulation.

Maie et al. (2006) studied leachates (DOM) of various plant species from the Florida Coastal Everglades, including *Thalassia testudinum* (turtlegrass, a marine seagrass), periphyton (mixtures of algae, cyanobacteria, and detritus, attached to submerged surfaces / corresponds roughly to epilithic biofilms) and several wetland and coastal forest species including *Rhizophora* and *Typha*. The authors employed THM-GC-MS using TMAH for this purpose. The different sources of DOM produced markedly different phenolic fingerprints (only phenolic THM products were evaluated) but the authors mention that such compounds are unlikely to survive photo-oxidation and therefore possibly of little diagnostic value for marine DOM provenancing. The dominant phenolic products of mangrove were 1,3,5-trimethoxybenzene and 2,4,6-trimethoxytoluene, indicative of condensed tannin, and common G products (e.g. G6). Cattail was prolific *p*-methoxyphenol configuration (ascribed to propelargonidin in condensed tannin); spikerush was characterized trimethoxybenzenes such as product S6, from syringyl lignin or perhaps hydrolysable tannins; periphyton produced mainly 1,4-di- and 1,2,4-trimethoxybenzene, indicative probably of polysaccharides. Using PCA, differences/similarities between the samples were explored, which allowed to clarify the major sources of the products. The authors concluded that the results "will prove useful in estimating the contribution of DOM derived from the early stage of decomposition of various ecosystem components in spatially articulate models" but warned for their potential lability in the marine photo-environment.

Ferreira et al. (2009) studied soil OM from Spanish salt marshes (Atlantic and Mediterranean, dominant vegetation *Phragmites*, *Spartina*, *Scirpus*) and Brazilian mangrove forests (e.g. *Avicennia*, *Rhizophora*). It was noted that in spite of the importance of OM dynamics in these high-C coastal ecosystems, few studied aimed to identify mechanisms that control degradation/preservation on the molecular level. Multivariate statistical analyses were employed to cope with the diversity of the ecosystems (25 samples from 7 sites) and the resultant very complicated set of pyrolysis products and product distributions. The most important source in most samples was plant-derived material, of which the degree of lignification was a major source of statistical variability, accompanied by varying proportions of microbial and recalcitrant aliphatic OM. On the basis of Py-GC-MS fingerprinting and multivariate statistical tools, it was shown that OM preservation was better (less impact of selective decay of lignin and accumulation of aliphatic OM) under permanently anoxic conditions and allowed to distinguish woody and non-woody higher plant sources. Hence, plant-derived OM was better preserved in saltmarsh than in mangrove systems, probably because of the more continuous anoxic conditions in the former. They found that syringyl lignin in woody angiosperms (such as Rhizophora sp.) was particularly poorly preserved in the mangrove sediment, whereas guaiacyl lignin had a slightly stronger preservation potential. The authors concluded that Py-GC-MS "is a useful tool to study the behavior and composition of SOM in wetland environments such as mangroves and salt marshes. Additional profiles need to be studied for each vegetation type, however, to improve the interpretability of the chemical data".

Carr et al. (2010) performed a detailed palaeoenvironmental reconstruction spanning 32 thousand years using Py-GC-MS of samples from a coastal wetland deposit from South Africa (Rietvlei). Here, traditional palaeoecological evidence (e.g. palynological studies) is barely available (rapid decomposition and few wetlands or lakes) and molecular proxies from Py-GC-MS (21 samples) and THM-GC-MS (selected samples) were used to contribute to filling this gap. The C_7-C_{26} alkanes/alkenes were the most abundant class for most of the record, followed by aromatic (non-phenolic) products, especially in the deeper layers of the deposit. due to profound alteration of the OM. The surface was enriched in lignin products. The authors observed that the sequence was much less prolific of carbohydrate and protein products than previously analysed sequences, which was attributed primarily to the larger impact of selective preservation/degradation dynamics of pre-Holocene sections (most analogous archives from Northern Hemisphere peat deposits recorded the Holocene only). Correspondence analysis showed that OM "freshness" controlled the variability in the OM composition in the Rietvlei core (proteins, cellulose and lignin). Benzonitrile behaved like aromatic compounds and not the other N-containing compounds, suggesting it corresponds to a signal from fire residues. The record is more closely related to a terrestrial wetland than a saltmarsh system, but remains of semi-aquatic *Phragmites* (typical saltmarsh species) and molecular and physical (fragments of mollusc shells and marine micro-fauna) evidence temporary influx of marine detritus (here considered allochthonous). This illustrates the need for flexibility in the description of depositional environment (marine *vs.* terrestrial), especially in long coastal records of palaeoenvironment.

Kaal et al. (2016) published data of Py-GC-MS and THM-GC-MS of fresh plant materials from *Posidonia oceanica* seagrass organs (roots, rhizomes, sheaths, leaves). Results showed that P. oceanica sheaths and roots contain an extraordinarily large amount of phenolic constituents with a *para*-hydroxybenzoic acid skeleton (*p*-HBA), and that these are the dominant products of up to 750 yr old mat deposit from Port Lligat (Mediterranean), targeting the plant detritus (not the sediment). Changes in molecular composition with depth were discussed and interpreted in terms of relative abundance of the different organs and stability of the OM in general. In Kaal et al. (2018b), *P. oceanica* and *P. australis* were compared by Py-GC-MS, THM-GC-MS and 2D-NMR and DFRC approaches, demonstrating that the lignin of *P. australis* is not charged with *p*-HBA, a remarkable difference considering that they belong to the same genus. In Kaal et al. (2018c), *P. sinuosa* was studied and shown to have an intermediate lignin fingerprint, more similar to *P. australis* than to *P. oceanica* in terms of lignin composition. The authors hypothesized on the role that the *p*-HBA may have in transferring the exceptional recalcitrance of *P. oceanica* debris. The idiosyncratic nature of the important Posidonia seagrass environments demonstrates that at present our molecular-scale knowledge on OM dynamics is too limited to sustain tacit extrapolations of biomolecular lability/stability from non-Blue Carbon systems.

4. FINAL REMARKS

It appears that the application of APTs to samples from natural marine organic matter, and in particular by Py-GC-MS, started in the early 1980s, even though several years earlier experiments with Py-GC and/or Py-MS had been performed. The first studies were concerned mainly with humic substances characterization, which can be explained by the deep roots of the earth sciences field that applied Py-GC-MS in the terrestrial (soil) system (in this period, the composition of soil organic matter was being evaluated with massive interest due to the advances in molecular probing techniques such as Py-GC-MS but also solid-state 13C NMR that became suitable for analysis of complex and "dirty" mixes such as humic substances). The early studies established the set of dominant compounds that would be confirmed over the next decades, being mainly N-compounds (pyrroles, indoles, cyanobenzenes and sometimes pyridines, acetamidofurans and long-chain alkylnitriles and -amides), cyclopentenones, furans and furaldehydes from polysaccharides, phenols from several precursors (depending on the system parameters and anyway still under debate), linear alkanes/allkenes from both marine and terrestrial sources, and isoprenoid hydrocarbons mainly from chlorophyll and tocopherol from marine sources.

Process-oriented Py-GC-MS applications become more frequent in the 1990s, when controls such as salinity gradients in estuarine areas and seasonal changes in marine POM composition, were evaluated on the molecular level, and also water column depth trend examinations became more frequent. Sediment traps and water collection bottles (Niskin bottles) were usually used for these purposes. Original early applications included numerical studies on the differences in POM composition in tidal cycles on the basis of POM pyrolyzates. Several other studies targeted proxies of diagenesis, mainly aiming to link the knowledge on marine POM to molecular properties of kerogen, which was a major line of research in the 1980s and 1990s that also stimulated the analysis of numerous species of biological materials from cultures (e.g. phytoplankton, zooplankton and cyanobacteria), and the analysis of decaying POM under anaerobic surface sediment conditions. During this period many studies performed chemical degradation methods to isolate specific recalcitrant macromolecular materials such as algaenan, cutan, suberan and later (early 2000s) also pyrogenic OM (black carbon). These studies then allowed to address in more detail the most likely sources of important and ambiguous pyrolysis products, such as alkanes/alkenes (algaenan, polymerization, or other), alkylnitriles (artificial or algaenan or other), phenols (protein, melanoidin, degraded lignin, other sources in algae and degraded algal material), pristene (chlorophyll *vs.* tocopherols) and tetramethylbenzene (algae *vs.* bacteria), whereas few authors burned their fingers on the meaning of PAH patterns.

Molecular characterization of DOM followed initial attempts of analysis of dissolved humic substances, and became more frequent as the methods for isolation and purification improved (exchange resins) and allowed for the collection of sufficient solid material from oligotrophic waters, even though this process is still very time-consuming and requires processing of sometimes enormous amounts of seawater. There is still no consensus on the meaning of the Py-GC-MS data, and in particular the presence of large amounts of PAH and other indicators of thermally altered precursors such as heavily carboxylated aromatic moieties, which may be ascribed to photo-oxidized lignin, to Black Carbon from charcoal/soot from wildfires and anthropogenic combustion, or hydrothermal vents. In general terms, DOM samples appeared to have a much smaller aliphatic imprint than POM, and larger aromatic (including heterocyclic) fraction.

It is surprising that very few studies, to date, have applied APTs as tools to understand deep and multi-sample sediment records from the marine environment (e.g. ocean drilling programs). In fact, the vast majority of Py-GC-MS studies in the marine environment have targeted areas under influence of terrestrial fluxes (continental shelf), and open ocean studies have barely been evaluated. It may be considered that Py-GC-MS is not likely to provide definite information on new sources of OM that had hitherto not been identified: the suite of compounds in the pyrolyzates may be largely anticipated at this point. But this does not imply that the changes in relative proportions of the pyrolysis products can provide crucial information on the underlying causes of variations in proxies form other, more frequently applied methods that target bulk OM chemical properties, such as C/N, d^{13}C, d^{15}N, fluorescence parameters, etc., and to aid in the interpretation of GC-MS and LC-MS data of soluble components such as linear alkane patterns and br-GDGT proxies. This also goes for detailed long-term palaeoenvironmental records from lacustrine deposits. The APTs, when used in combination with multivariate statistics, will always provide proxies of environmental changes that can be cross-compared to other data. The same can be argued for the surprisingly infrequent application of APTs to the materials sampled during modern oceanographic expeditions. Whereas expensive technology such as HRMS (e.g. FT-ICRMS) is massively used or even acquired for DOM fingerprinting, few studies target the HWM fraction of DOM which for now escapes the window of such methods (up to 1000 or 2000 D, typically).

In summary, APTs are useful to obtain a rapid screening of the different sources of marine OM, facilitating rather straightforward identification of different constituents (protein, polysaccharides, aliphatic biopolymers, lignin, etc.) of the major source types (bacteria, algae, vascular plants) and their degradation state (from abiotic, biotic or thermal decay). These parameters also give an idea of the mechanism of Blue Carbon preservation in coastal vegetated habitats.

5. REFERENCES

Armada, X.-L., García-Vuelta, O., Kaal, J., Martín-Seijo, M., Porto, Y., 2017. Characterization of cores and organic remains in Iron Age gold objects: The Recouso treasure. Materials and Manufacturing Processes 32, 740-748.

Biller, P., Ross, A.B., 2014. Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae. Algal Research 6. 91–97.

Boon, J.J., de Leeuw, J.W., 1987. Amino acid sequence information in proteins and complex proteinaceous material revealed by pyrolysis-capillary gas chromatography-low and high resolution mass spectrometry. Journal of Analytical and Applied Pyrolysis 11, 313-327.

Buurman, P., Nierop, K.G.J., Pontevedra Pombal, X., Martínez Cortizas, A., 2006. Molecular chemistry by pyrolysis–GC/MS of selected samples of the Penido Vello peat deposit, NW Spain. *In* I.P. Martini, A. Martínez Cortizas, W. Chesworth (Eds.), Peatlands. Evolution and Records of Environmental and Climate Changes, Elsevier, Amsterdam, pp. 217-240.

Carr, A.S., Boom, A., Chase, B.M., Roberts, D.L., Roberts, Z.E., 2010. Molecular fingerprinting of wetland organic matter using pyrolysis-GC/MS: an example from the southern Cape coastline of South Africa. Journal of Paleolimnology 44, 947–961.

Challinor, J.M., 2001. Review: the development and applications of thermally assisted hydrolysis and methylation reactions. Journal of Analytical and Applied Pyrolysis 61, 3-34.

Çoban-Yıldız, Y., Chiavari, G., Fabbri, D., Gaines, A.F., Galletti, G., Tugrul, S., 2000a. The chemical composition of Black Sea suspended particulate organic matter: pyrolysis-GC-MS as a complementary tool to traditional oceanographic analyses. Marine Chemistry 69, 55–67.

Çoban-Yıldız, Y., Fabbri, D., Tartaro, D., Tugrul, S., Gaines, A.F., 2000b. Application of pyrolysis–GC/MS for the characterisation of suspended particulate organic matter in the Mediterranean Sea: a comparison with the Black Sea. Organic Geochemistry 31, 1627-1639.

de la Rosa, J.M., González-Pérez, J.A., González-Vila, F.J., Knicker, H., Araújo, M.F., 2011. Molecular composition of sedimentary humic acids from South West Iberian Peninsula: A multi-proxy approach. Organic Geochemistry 42, 791–802.

de Leeuw, J.W., Versteegh, G.J.M., van Bergen, P.F., 2005. Biomacromolecules of algae and plants and their fossil analogues. Plant Ecology 182, 209-233.

Derenne, S., Largeau, C., 2011. A review of some important families of refractory macromolecules: composition, origin, and fate in soils and sediments. Soil Science 166, 833-847.

Derenne, S., Quénéa, K., 2015. Analytical pyrolysis as a tool to probe soil organic matter. Journal of Analytical and Applied Pyrolysis 111, 108–120.

Derenne, S., Largeau, C., Casadevall, E., Berkaloff, C., Rousseau, B., 1991. Chemical evidence of kerogen formation in source rocks and oil shales via selective preservation of thin resistant outer walls of microalgae: Origin of ultralaminae. Geochimica et Cosmochimica Acta 55, 1041-1050.

Deshmukh, A.P., Chefetz, B., Hatcher, P.G., 2001. Characterization of organic matter in pristine and contaminated coastal marine sediments using solid-state 13C NMR, pyrolytic and thermochemolytic methods: a case study in the San Diego harbor area. Chemosphere 45, 1007-1022.

Duarte, C.M., Losada, I.J., Hendriks, I.E., Mazarrasa, I., Marbà, N., 2013. The role of coastal plant communities for climate change mitigation and adaptation. Nature Climate Change 3, 961–968.

Ergin, M., Gaines, A., Galletti, G.C., Chiavari, G., Fabbri, D., Yücesoy-Erilmaz, F., 1996. Early diagenesis of organic matter in recent Black Sea sediments: characterization and source assessment. Applied Geochemistry 11, 711-720.

Evans, E.J., Batts, B.D., Cant, N.W., Smith, J.W., 1985. The origin of nitriles in shale oil. Organic Geochemistry 8, 367-374.

Fabbri, D., Mongardi, M., Montanari, L., Galletti, G.C., Chiavari, G., Scotti, R., 1998. Comparison between CP/MAS 13C-NMR and pyrolysis-GC/MS in the structural characterization of humins and humic acids of soil and sediments. Fresenius Journal of Analytical Chemistry 362, 299–306.

Fabbri, D., Sangiorgi, F., Vassura, I., 2005. Pyrolysis–GC–MS to trace terrigenous organic matter in marine sediments: a comparison between pyrolytic and lipid markers in the Adriatic Sea. Analytica Chimica Acta 530, 253-261.

Ferreira, F.P., Vidal-Torrado, P., Buurman, P., Macías, F., Otero, X.L., Boluda, R., 2009. Pyrolysis-gas chromatography/mass spectrometry of soil organic matter extracted from a Brazilian mangrove and Spanish salt marshes. Soil Science Society of America Journal 73, 841-851.

Fogel, M.L., E.K. Sprague, A.P. Gize, and R.W. Frey. 1989. Diagenesis of organic matter in Georgia salt marshes. Estuarine, Coastal and Shelf Science 28, 211–230.

Gadel, F., Bruchet, A., 1987. Application of pyrolysis-gas chromatography-mass spectrometry to the characterization of humic substances resulting from decay of aquatic plants in sediments and waters. Water Research 21, 1195-1206.

Gadel, F., Charrière, B., Serve, L., 1993. Chemical characterization of suspended particulate organic matter by pyrolysis-gas chromatography coupled with mass spectrometry and high performance liquid chromatography in the bottom nepheloid layer of the Rhône delta. Estuarine, Coastal and Shelf Science 37, 221-236.

Galletti, G.C., Reeves, J.B., 1992. Pyrolysis/gas chromatography/ion-trap detection of polyphenols (vegetable tannins): preliminary results. Journal of Mass Spectrometry 27, 226-230.

Garcette-Lepecq, A., Derenne, S., Largeau, C., Bouloubassi, I., Saliot, A., 2000. Origin and formation pathways of kerogen-like organic matter in recent sediments off the Danube delta (northwestern Black Sea). Organic Geochemistry 31, 1663-1683.

Garcette-Lepecq, A., Derenne, S., Largeau, C., Bouloubassi, I., Saliot, A., 2001. Thermally assisted hydrolysis and methylation of kerogen-like organic matter in a recent sediment off the Danube delta (northwestern Black Sea). Journal of Analytical and Applied Pyrolysis 61, 147–164.

Gelin, F., Boogers, I., Noordeloos, A.A.M., Sinninghe Damsté, J.S., Hatcher, P., de Leeuw, J.W., 1996. Novel resistant microalgal polyethers: an important sink of organic carbon in the marine environment Geochimica et Cosmochimica Acta 60, 1275-1280.

Geraldi, N.R., Ortega, A., Serrano, O., Macreadie, P.I., Lovelock, C.E., Krause-Jensen, D., Kennedy, H., Lavery, P.S., Pace, M.L., Kaal, J., Duarte, C.M. Fingerprinting blue carbon: Rationale and tools to determine the source of organic carbon in marine depositional environments. Frontiers in Marine Chemistry, under review.

Gilliam, A.H., Wilson, M.A., 1985. Pyrolysis-GC-MS and NMR studies of dissolved seawater humic substances and isolates of a marine diatom. Organic Geochemistry 8, 15-25.

González-Vila, F.J., De la Rosa, J.M., González-Pérez, J.A., 2009. Black carbon and other refractory forms in recent sediments from the Gulf of Cadiz, Spain. Earth and Environmental Science 5, p. 012009, 10.1088/1755-1307/5/1/012009.

Guo, L., Semiletov, I., Gustafsson, Ö., Ingri, J., Andersson, P., Dudarev, O., White, D., 2004. Characterization of Siberian Arctic coastal sediments: Implications for terrestrial organic carbon export. Global Biogeochemical Cycles 18, GB1036, doi:10.1029/2003GB002087.

Guo, L., White, D.M., Xu, C., Santschi, P.H., 2004. Chemical and isotopic composition of high-molecular-weight dissolved organic matter from the Mississippi River plume. Marine Chemistry 114, 63–71.

Hartgers, W.A., Sinninghe Damsté, J.S., Requejo, A.G., Allan, J., Hayes, J.M., Ling, Y., Xie, T.-M., Primack, J., de Leeuw, J.W., 1994. A molecular and carbon isotopic study towards the origin and diagenetic fate of diaromatic carotenoids. Organic Geochemistry 22, 703-725.

Hatcher, P.G., Clifford, D.J., 1994. Flash pyrolysis and in situ methylation of humic acids from soil. Organic Geochemistry 21, 1081-1092.

Hemminga, M.A., Klap, V.A., van Soelen, J., de Leeuw, J.W., Boon, J.J., 1992. Shifts in seston characteristics after inundation of a European coastal salt marsh. Limnology and Oceanography 37, 1559–1564.

Hemminga, M.A., Klap, V.A., van Soelen, J., Boon, J.J., 1993. Effect of salt marsh inundation on estuarine particulate organic matter characteristics. Marine Ecology Progress Series 99, 153–161.

Hoefs, M.J.L., van Heemst, J.D.H., Gelin, F., Koopmans, M.P., van Kaam-Peters, H.M.E., Schouten, S. et al., 1995. Alternative biological sources for 1,2,3,4-tetramethylbenzene in flash pyrolysates of kerogen. Organic Geochemistry 23, 975-979.

Hwang, J., Druffel, E.R.M., Eglinton, T.I., Repeta, D.J., 2006. Source(s) and cycling of the nonhydrolyzable organic fraction of oceanic particles. Geochimica et Cosmochimica Acta 70, 5162-5168.

Ishiwatari M., Ishiwatari R., Sakashita H., Tatsumi T., Tominaga, H., 1991. Pyrolysis of chlorophyll a after preliminary heating at a moderate temperature: implications for the origin of prist-1-ene on kerogen pyrolysis. Journal of Analytical and Applied Pyrolysis 18, 207-218.

Ishiwatari, R., Yamamoto, S., Handa, N., Nozaki, Y., 1993. Organic composition of sinking particles (JT-01) in Japan Trench as revealed by pyrolysis gas chromatography/mass spectrometry. *In:* T. Teramoto (Ed.), Deep Ocean Circulation, Physical and Chemical Aspects, Elsevier, Amsterdam, pp. 235-252.

Ishiwatari, R., Yamamoto, S., Handa, N., 1995. Characterization of sinking particles in the ocean by pyrolysis-gas chromatography/mass spectrometry. Journal of Analytical and Applied Pyrolysis 32, 75-89.

Ismaïli, M.M., Serve, L., Gadel, F., Lafite, R., Texier, H., 1999. Biogeochemical markers of organic matter along the Wight-Cherbourg transect (central English Channel). Oceanologica Acta 22, 397-412.

Kaal, J., Filley, T., 2016. Novel molecular proxies for inferring pyrogenic black carbon oxidation state using thermally assisted hydrolysis and methylation (THM-GC-MS) with ^{13}C-labeled tetramethylammonium hydroxide (TMAH). Journal of Analytical and Applied Pyrolysis 121, 146-154.

Kaal, J., Brodowski, S., Baldock, J.A., Nierop, K.G.J., Cortizas, A.M., 2008. Characterisation of aged black carbon using pyrolysis-GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarisation ^{13}C nuclear magnetic resonance (DP/CP NMR) and the benzenepolycarboxylic acid (BPCA) method. Organic Geochemistry 39, 1415-1426.

Kaal, J., Serrano, O., Nierop, K.G.J., Schellekens, J., Martínez Cortizas, A., Mateo, M.A. 2016. Molecular composition of plant parts and sediment organic matter in a Mediterranean seagrass (*Posidonia oceanica*) mat. Aquatic Botany, 133, 50-61.

Kaal, J., and the CSIC-PROINSA Mussel Team, 2018a. Sources of organic detritus under mussel farms in the Ría de Betanzos (NW Spain). Analytical Pyrolysis Letters APL003. www.pyrolyscience.com/apl003/.

Kaal, J., Serrano, O., Del Río, J.C., Rencoret, J., 2018b. Radically different lignin composition in *Posidonia* species may link to differences in organic carbon sequestration capacity. Organic Geochemistry 124, 247-256.

Kaal, J., Serrano, O., Del Río, J.C., Rencoret, J., 2018c. Chemotyping the lignin of *Posidonia* seagrasses. Analytical Pyrolysis Letters APL001. www.pyrolyscience.com/apl001/.

Klap, V.A., Boon, J.J., Hemminga, M.A., van Soelen, J., 1996. Assesssment of the molecular composition of particulated organic matter exchanged between Saeftinghe salt marsh (southwestern Netherlands) and the adjacent water system. Marine Chemistry 54, 221–243.

Klap, V.A., Hemminga, M.A., Boon, J.J., 2000. Retention of lignin in seagrasses: angiosperms that returned to the sea. Marine Ecology Progress Series 194, 1-11.

Larter, S.R., Solli, H., Douglas, A.G., 1978. Analysis of kerogens by pyrolysis gas chromatography-mass spectrometry using selective ion detection. Journal of Chromatography 167, 421–431.

Maie, N., Jaffe, R., Toshikazu, M., Childers, D.L., 2006. Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland. Biogeochemistry 78, 285-314.

Mannino, A., Rodger Harvey, H., 2000. Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. Organic Geochemistry 31, 1611-1625.

Martone, P.T., Estevez, J.M., Lu, F., Ruel, K., Denny, M.W., Somerville, C., Ralph, J., 2009. Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. Current Biology 19, 169-175.

Moldoveanu, S.C.,1998. Analytical Pyrolysis of Natural Organic Polymers. Elsevier, Amsterdam.

Nellemann, C., Corcoran, E., Duarte, C.M., Valdés, L., de Young, C., Fonseca, L., Grimsditch, G., 2009. Blue carbon - The role of healthy oceans in binding carbon. http://www.grida.no/publications/145.

Nierop, K.G.J., Reichart, G.-J., Veld, H., Sinninghe Damsté, J.S., 2017. The influence of oxygen exposure time on the composition of macromolecular organic matter as revealed by surface sediments on the Murray Ridge (Arabian Sea). Geochimica et Cosmochimica Acta 206, 40–56.

Ní Fhlaithearta, S., Ernst, S. R., Nierop, K. G., de Lange, G. J. Reichart, G.-J., 2013. Molecular and isotopic composition of foraminiferal organic linings. Marine Micropaleontology 102, 69–78.

Nip M., Tegelaar E. W.. Brinkbuis H., de Leeuw J. W., Schenck P. A., and Holloway P. J. (1986) Analysis of modern and fossil plant cuticles by Curie point Py-GC and Curie point Py-GC-MS: Recognition of a new, highly aliphatic and resistant biopolymer. Organic Geochemistry 10, 769-778.

Opsahl, S., Benner, R., 1993. Decomposition of senescent blades of the seagrass Halodule wrightii in a subtropical lagoon. Marine Ecology Progress Series 94, 191–205.

Peulvé, S., de Leeuw, J.W., Sicre, M.-A., Baas, M., Saliot, A., 1996a. Characterization of macromolecular organic matter in sediment traps from the northwestern Mediterranean Sea. Geochimica et Cosmochimica Acta 60, 1239-1259.

Peulvé, S., Sicre, M.-A., Saliot, A., de Leeuw, J.W., Baas, M., 1996a. Molecular characterization of suspended and sedimentary organic matter in an Arctic delta. Limnology and Oceanography 41, 488-497.

Polvillo, O., González-Pérez, J., Boski, T., González-Vila, F., 2009. Structural features of humic acids from a sedimentary sequence in the Guadiana estuary (Portugal–Spain border). Organic Geochemistry 40, 20-28.

Pouwels, A.D., Eijkel, G.B., Boon, J.J., 1989. Curie-point pyrolysis-capillary gas chromatography high- resolution mass spectrometry of microcrystalline cellulose. Journal of Analytical and Applied Pyrolysis 14, 237-280.

Pulchan, J., Abrajano, T.A., Helleur, R., 1997. Characterization of tetramethylammonium hydroxide thermochemolysis products of near-shore marine sediments using gas chromatography/mass spectrometry and gas chromatography/combustion/isotope ratio mass spectrometry. Journal of Analytical and Applied Pyrolysis 42, 135-150.

Pulchan, J., Helleur, R., Abrajano, T.A., 2003. TMAH thermochemolysis characterization of marine sedimentary organic matter in a Newfoundland fjord. Organic Geochemistry 34, 305–317.

Ralph, J., Hatfield, R.D., 1991. Pyrolysis-GC-MS characterization of forage materials. Journal of Agricultural and Food Chemistry 39, 1426-1437.

Rocker, D., Kisand, V., Scholz-Böttcher, B., Kneib, T., Lemke, A., Rullkötter, J., Simon, M., 2012. Differential decomposition of humic acids by marine and estuarine bacterial communities at varying salinities. Biogeochemistry 111, 331–346.

Ross, A.B., Jones, J.M., Kubacki, M.L., Bridgeman, T., 2008. Classification of macroalgae as fuel and its thermochemical behaviour. Bioresource Technology 99, 6494–6504.

Sáiz-Jiménez, C., 1994. Analytical pyrolysis of humic substances: pitfalls, limitations, and possible solutions. Environmental Science and Technology 28, 1773–1780.

Sáiz-Jiménez, C., de Leeuw, J.W., 1986. Lignin pyrolysis products: Their structures and their significance as biomarkers. Organic Geochemistry 10, 869-876.

Saliot, A., Ulloa-Guevara, A., Viets, T.C., de Leeuw, J.W., Schenck, P.A., Boon, J.J., 1984. The application of pyrolysis-mass spectrometry and pyrolysis-gas chromatography-mass spectrometry to the chemical characterization of suspended matter in the ocean. Organic Geochemistry 6, 295-304.

Serrano, O., Lavery, P., Rozaimi, M., Mateo, M.A., 2014. Influence of water depth on the carbon sequestration capacity of seagrasses. Global Biogeochemical Cycles 28,950–961.

Shadkami, F., Helleur, R., 2010. Recent applications in analytical thermochemolysis. Journal of Analytical and Applied Pyrolysis 89, 2-16.

Sicre, M.-A., Peulvé, S., Saliot, A>, De Leeuw, J.W., Baas, M., 1994. Molecular characterization of the organic fraction of suspended matter in the surface waters and bottom nepheloid layer of the Rhone delta using analytical pyrolysis. Organic Geochemistry 21, 11-26.

Sigleo, A. C., T. C. Hoering, and G. R. Helz, 1982. Cornposition of estuarine colloidal material: organic components. Geochimica et Cosmochimica Acta 46, 1619-1626.

Sinninghe-Damsté, J.S., Eglinton, T.I., De Leeuw, J.W., Schenck, P.A., 1989. Organic sulphur in macromolecular sedimentary organic matter: I. Structure and origin of sulphur-containing moieties in kerogen, asphaltenes and coal as revealed by flash pyrolysis. Geochimica et Cosmochimica Acta 53, 873-889 .

Spaccini, R., Song, X., Cozzolino, V., Piccolo, A., 2013. Molecular evaluation of soil organic matter characteristics in three agricultural soils by improved off-line thermochemolysis: The effect of hydrofluoric acid demineralization treatment. Analitica Chimica Acta 802, 46-55.

Sparkes, R.B., Dogrul Selver, A., Gustafsson, Ö., Semiletov, I.P., Haghipour, N., Wacker, L., Eglinton, T.I., Talbot, H.M., van Dongen, B.E., 2016. Macromolecular composition of terrestrial and marine organic matter in sediments across the East Siberian Arctic Shelf. Cryosphere 10, 2485–2500.

Stankiewicz, B.A., Mastalerz, M., Hof, C.H.J., Bierstedt, A. , Flannery, M.B., Briggs, D.E.G., Evershed, R.P., 1998. Biodegradation of the chitin-protein complex in crustacean cuticle, Organic Geochemistry 28, 67–76.

Stevenson, M.A., Abbott, G.D., 2019. Exploring the composition of macromolecular organic matter in Arctic Ocean sediments under a changing sea ice gradient. Journal of Analytical and Applied Pyrolysis, in press.

Tegelaar, E.W., de Leeuw J.W., Holloway P.J., 1989. Some mechanisms of flash pyrolysis of naturally occurring higher plant polyesters. Journal of Analytical and Applied Pyrolysis 15, 289-295.

Thomas, E.K., Gao, L., Huang, Y., 2009. A quantitative and qualitative comparison of aquatic and terrestrial plant lignin phenols: Critical information for paleoecological reconstructions. American Geophysical Union, Fall Meeting 2009, abstract id. B33C-0404.

Traoré, M., Kaal, J., Martínez Cortizas, A., 2017. Potential of pyrolysis-GC-MS molecular fingerprint as a proxy of Modern Age Iberian shipwreck wood preservation. Journal of Analytical and Applied Pyrolysis 126, 1-13.

Tsuge, S., Matsubara, H., 1985. High-resolution pyrolysis-gas chromatography of proteins and related materials. Journal of Analytical and Applied Pyrolysis 8, 49-64.

van de Meent, D., de Leeuw, J.W., Schenck, P.A., 1980a. Chemical characterization of non-volatile organics in suspended matter and sediments of the river Rhine Delta. Journal of Analytical and Applied Pyrolysis 2, 249-263.

van de Meent, D., de Leeuw, J.W., Schenck, P.A., 1980b. Origin of unsaturated isoprenoid hydrocarbons in pyrolysates of suspended matter and surface sediments. In: Douglas, A.G., Maxwell, J.R. (Eds.), Advances in Organic Geochemistry, 1979. Pergamon Press, Oxford, pp. 469-474.

Vandenbroucke, M., Behar, F., 1988. Geochemical characterization of the organic matter from some recent sediments by a pyrolysis technique. Geological Society, London, Special Publications, 40, 91-101, doi: 10.1144/GSL.SP.1988.040.01.09.

van Heemst, J.D.H., van Bergen, P.F., Stankiewicz, B.A., de Leeuw, J.W., 1999. Multiple sources of alkylphenols produced upon pyrolysis of DOM, POM and recent sediments. Journal of Analytical and Applied Pyrolysis 52, 239-256.

van Heemst, J.D.H., del Río, J.C., Hatcher, P.G., de Leeuw, J.W., 2000a. Nature, origin and average age of estuarine ultrafiltered dissolved organic matter as determined by molecular and carbon isotope characterization. Organic Geochemistry 31, 847-857.

van Heemst, J.D.H., del Río, J.C., Hatcher, P.G., de Leeuw, J.W., 2000b. Characterization of estuarine and fluvial dissolved organic matter by thermochemolysis using tetramethylammonium hydroxide. Acta Hydrochimica et Hydrobiologica 28, 69–76.

Versteegh, G.J.M., Blokker, P., 2004. Resistant macromolecules of extant and fossil microalgae. Phycological Research 52, 325–339.

Versteegh, G.J.M., Blokker, P., Wood, G.D., Collinson, M.E., Sinninghe Damsté, J.S., de Leeuw, J.W., 2004. An example of oxidative polymerization of unsaturated fatty acids as a preservation pathway for dinoflagellate organic matter. Organic Geochemistry 35, 1129-1139.

Voorhees, K.J., 2013. Analytical Pyrolysis: Techniques and Applications. Elsevier, Amsterdam.

Wilson, M.A., Philp, R.P., Gilliam, A.H., Gilbert, T.D., Tate, K.R., 1983. Comparison of the structures of humic substances from aquatic and terrestrial sources by pyrolysis gas chromatography-mass spectrometry. Geochimica et Cosmochimica Acta 47, 497-502.

Zang, X., Hatcher, P.G., 2002. A Py–GC–MS and NMR spectroscopy study of organic nitrogen in Mangrove Lake sediments. Organic Geochemistry 33, 201–211.

Zegouagh, Y., Derenne, S., Largeau, C., Bertrand, P., Sicre, M.-A., Saliot et al., 1999. Refractory organic matter in sediments from the North-West African upwelling system: abundance, chemical structure and origin. Organic Geochemistry 30, 101-117.

Zegouagh, Y., Derenne, S., Dignac, M-F., Baruiso, E., Mariotti, A., Largeau, C., 2004. Demineralisation of a crop soil by mild hydrofluoric acid treatment: influence on organic matter composition and pyrolysis. Journal of Analytical and Applied Pyrolysis 71, 119–135.

Zhang,Y., Du, J., Ding, X., Zhang, F., 2016. Comparison study of sedimentary humic substances isolated from contrasting coastal marine environments by chemical and spectroscopic analysis. Environmental Earth Sciences 75, 378.

Zhang, Y., Yang, K., Du, J., Zhang, F., Dong, Y., Li, W., 2017. Chemical characterization of fractions of dissolved humic substances from a marginal sea—a case from the Southern Yellow Sea. Chinese Journal of Oceanology and Limnology, doi:10.1007/s00343-017-6202-6.

Zhu, R., Versteegh, G.J.M., Hinrichs, K.-U., 2016. Detection of microbial biomass in subseafloor sediment bypyrolysis–GC/MS. Journal of Analytical and Applied Pyrolysis 118, 175–180.

Zúñiga, D., Kaal, J.,Villacieros-Robineau, N., Froján, M., Alonso-Pérez, F., de la Granda, F., Castro, C.G., in press. Tracing sinking organic matter sources in the NW Iberian upwelling system (NE Atlantic Ocean): Comparison between elemental, isotopic and molecular indicators. Journal of Analytical and Applied Pyrolysis, https://doi.org/10.1016/j.jaap.2019.01.016.

Table 1. **Some major sources of organic matter that can be encountered in OM from marine environments, and main Py-GC-MS and THM-GC-MS products. This list is incomplete and simplified: it is an example of main sources and potential products.**

Components	Sources	Py-GC-MS	THM-GC-MS
Polysaccharides	Multiple	acetic acid, cyclopentenones, furans furaldehydes, pyrans, anhydrosugars	C_5/C_6 saccharinic acid MEs 1,2,4-trimethoxybenzene
Proteins	Multiple	pyrroles, pyridines, imidazoles indoles, cyanobenzenes, diketopiperazines, phenols, toluene	N,N-dimethylated peptides (MEs) of any amino acid C_2-C_5-pyrroles, C_1-C_3-indoles
Chitin	Fungi, zooplankton, etc.	acetamide, acetamidofurans, other (common) N-products.	?
Peptidoglycan	Bacterial cell walls	Acetamide, acetamidofurans, propionamide other (common) N-compounds	?
Lignin	Vascular plants	phenols, guaiacols, syringols	mono-, di- and trimethoxybenzenes, benzene carboxylic acid methyl esters
Cinnamic acids	Grasses, aquatic plants	4-vinylphenol, 4-vinylguaiacol, 4-vinylsyringol	P18, G18, S18
Cutin	Plant cuticles: leaves of plants	alkanes/alkenes, fatty acids	short-chain FAMEs, mid-chain methoxylated FAMEs
Suberin	Bark/root of higher plants	alkanes/alkenes, fatty acids	long-chain FAMEs, DAMES, $\omega-$methoxy FAMEs
Algaenan	Algae	alkanes/alkenes, alkylnitriles	FAMEs, aliphatic hydrocarbons
Fatty acids	Multiple	fatty acids	FAMEs
Phlorotannins	Brown algae	phenols, dihydroxybenzenes	1,3,5-trimethoxybenzenes
Hydrolyzable tannins	Gymnosp/Angiosp.	phenols, catechols, pyrogallols	1,2-di-, 1,2,3-trimethoxybenzenes
Condensed tannins	Angiosp.	phenols, catechols, pyrogallols	1,2-di-, 1,2,3-trimethoxybenzenes
Chlorophyll	Photosynthetic precursors	pyrroles, phytadienes, pristenes	phytadienes, etc.
Pyrogenic OM	Charred material	benzene, toluene, benzonitrile, PAHs	PAHs, benzenepoly-carboxylic acid (methyl esters)