Assessment of distribution and sources of pyrogenic carbon in the lower course of the Guadiana River (SW Iberian Peninsula)

José M. De la Rosa 1,2,*
Email: jmrosa@irnase.csic.es

José M. Martins 3
António Monge Soares 2
M. Fátima Araújo 2

1 Instituto de Recursos Naturales y Agrobiología de Sevilla, Consejo Superior de Investigaciones Científicas (IRNAS-CSIC), Avenida Reina Mercedes 10, 41012 Sevilla, Spain
2 Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Estrada Nacional 10 (km 139,7), Bobadela LRS, 2695-066 Lisbon, Portugal
3 CIMA, Faculdade de Ciências e Tecnologia, Campus de Gambelas, Universidade do Algarve, 8005-139 Faro, Portugal

Abstract

Purpose

The main goal of this work was to determine the pyrogenic carbon (PyC) distribution in surficial sediments for the last 40 km of the Guadiana River (SW Iberian Peninsula). In addition, the changes in the contribution of terrestrial and marine organic matter (OM) sources and their relationship with the PyC contents were assessed.

Materials and methods

Sediments were collected from 22 stations within the lower course of the Guadiana River. All the samples were analyzed for total organic carbon (TOC), total nitrogen (TN), organic carbon to nitrogen atomic ratios (C/N), and stable carbon isotope ratio (δ13C) as indicators of changes in the sources of OM. PyC was determined according to the chemo-thermal oxidation method (CTO-375).

Results and discussion

TOC ranged from 0.25 to 2.37 %dw. The greatest TOC contents were located in sediments collected at the middle estuary. The C/N values ranged from 7.7 to 12.7, with slight relative increase in the marine input compared to pre-Alqueva times. Similar C/N ranges are representative of native soil OM, lacustrine OM, and local sedimentary OM. The δ13C values of the sediment samples ranged from −25.0 to −27.6 ‰ illustrating a signature of C3 higher plants, marsh-plants, lacustrine plants, and freshwater algae present in the area of study and in the forests located upstream. PyC ranged between 0.17 and 0.74 gkg⁻¹, with the highest contents measured in samples located close to urbanized areas. Sediments collected at the middle estuary, within a rural area, showed the lowest proportions of PyC relative to the percent of TOC (≤4 % TOC). The negative relationship between PyC to TOC ratio and TOC contents confirmed the selective preservation of PyC to degradation in the sedimentary OM of the Guadiana estuary. The carbon isotope composition of the final material after applying the CTO-375 method (hereafter called δ13C_PyC) was <−30 ‰, suggesting that the PyC fraction was mainly composed of a mixture of fossil fuels and C3-charred biomass. Nevertheless, selective loss of the less recalcitrant OM fractions due to degradation and/or chemical alteration might contribute to lighter carbon isotope values of PyC.
Conclusions

Geochemical data showed that sedimentary OM was composed of a mix of different OM sources, with a slight increase in the marine input after the Alqueva dam went into operation. The PyC content was higher close to urbanized areas of the Guadiana River estuary. The selective preservation of PyC in the sedimentary OM of the Guadiana estuary was observed.

Keywords

- Black carbon
- CTO-375 method
- Estuaries
- Recent sediments
- Refractory organic matter
- Soot

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1. Introduction

Pyrogenic carbon (PyC), also called black carbon, is a relatively inert form of condensed carbon products originated from incomplete combustion processes of biomass or fossil fuels, which are heated to relatively high temperatures (<700 °C) under low or no oxygen. It comprises a variety of materials, from lightly charred biomass to soot (Lehmann 2007). The PyC can be found in soils, sediments, ice, atmospheric aerosols, rainwater, and river and ocean water (Masiello and Druffel 1998; Dittmar et al. 2008). The traditional view of PyC, being an inert C pool, was based on radiocarbon measurements supporting its persistence in some soils and sediments for millennia (Thevenon et al. 2010; de Lafontaine and Asselin 2011). However, recent experiments suggested rather rapid degradation of some PyC forms (De la Rosa and Knicker 2011; Zimmermann et al. 2012), with mean residence times considerably lower than those estimated by other authors (Hockaday et al. 2006; Norwood et al. 2013). In fact, this is consistent with the heterogeneity of PyC formed under different conditions. For instance, every wildfire provides unique combinations of fuels and temperatures, which in turn will produce a range of different forms of PyC. Low-temperature PyC will undergo biologically driven mineralization faster than high-temperature PyC (Ballock and Smernik 2002). Yet, the pyrogenic process confers larger chemical and microbial recalcitrance to these materials, and consequently, longer mean residence times in the environment in comparison to the original biomass from which they are produced. Therefore, PyC can be considered a genuine C sink and a significant component of the global C cycle (Kuhlbusch 1998; Gustafsson et al. 2001). There are contrasting estimations of the PyC discharged annually into the ocean, ranging from 1 Tg yr⁻¹ up to 16–275 years⁻¹ (Dickens et al. 2004; Flores-Cervantes et al. 2009). Nevertheless, Suman et al. (1997) estimated that about 10 Tg of PyC are discharged annually into coastal areas via aeolian and fluvial contributions. Similarly, Ducret (1994) calculated that the deposition of atmospheric PyC into oceans is approximately 7 ± 3 Tg C yr⁻¹. Jaffé et al. (2013) estimated the global riverine flux of dissolved PyC from soils to oceans is 26.5 ± 1.8 Tg C yr⁻¹. This value is in the same order of magnitude as previous estimations of annual PyC production from vegetation fires (Kuhlbusch and Crutzen 1995). Fossil fuel combustion-derived particles, charcoal produced by biomass burning, or petrogenic C forms are transported through aeolian, surface runoff, and fluvial transport, being coastal sediments and marine waters the ultimate receiver of these forms of PyC (Lim and Cachier 1996). All estimation models indicated that riverine fluxes and surface runoff are significant PyC contributors to sediments at the continental shelf and coastal areas, whereas only half of the total atmospheric deposition of PyC to the ocean surface occurs over the continental margins. Moreover, almost all PyC deposition (>90 %) to ocean sediments occurs in nearshore sediments rather than in open ocean sediments (Suman et al. 1997). In summary, less than 10 % of the area under the world oceans receives the major contribution of sedimentary PyC. Thus, even small changes in the relative abundance of PyC in these environments may be significant at a global scale. In addition, it is well known that storage conditions in coastal and estuarine environments, particularly in anoxic sediments, are ideal for PyC preservation (De la Rosa et al. 2008). Consequently, alterations of environmental conditions and fluxes could affect PyC preservation and dynamics.
This study focuses on the assessment of PyC stored in recent sediments from the lower course and estuary of the Guadiana River (SW Iberian Peninsula). This region is of environmental importance because it comprises several protected areas included in Spanish and Portuguese National-Preserve Networks and the European Natura 2000 Network, such as the Natural reserve “Sapal de Castro Marim e Vila real de Sto Antonio” and The Natural Area “Marismas de Isla Canela e Isla Cristina.”

The increase of anthropogenic activities carried out during the last decades at the Guadiana estuary, including urbanization, port, and recreational development, have caused significant environmental impacts (Erzini 2005). Also noticeable is the construction of several dams in the middle and lower course of the Guadiana River. For instance, the Alqueva dam, the biggest dam in Europe with a storage capacity of ca. 4150 hm³, became operational in 2001. Several recent studies have reported changes in the sediment load discharges and estuarine dynamics after this dam went into operation (Caetano et al. 2006; Chicharo et al. 2006; Oliveira et al. 2006; Delgado et al. 2010; González et al. 2007).

The southwestern (SW) area of the Iberian Peninsula has a Mediterranean climate with very high forest fire incidence due to the combination of high temperatures and dry periods (González-Pérez et al. 2004). During the twentieth century, fire was used as a management practice by grassland farmers of SW of the Iberian Peninsula. An estimation of PyC production in the form of particulate residues or aerosols (soot) emitted by forest fires was conducted for Andalusia. It was estimated that up to 31,222 Mg of PyC can be produced by forest fires every year, at a rate of 1.8 Mg burnt ha⁻¹. Part of this PyC, between 767 and 920 Mg year⁻¹, can be emitted in the form of aerosols (soot) to the atmosphere and after some time (40 h to 1 month) deposited and incorporated into sediments worldwide. Part of this considerable amount of charred material is transported by wind or by the Guadiana River to the estuary.

A couple of recent studies assessed the abundance of black carbon (PyC-like material) in a few sediments taken from the Guadiana estuary and the adjacent continental shelf before Alqueva dam became operational (De la Rosa et al. 2011; Sánchez-García et al. 2013). In this study, the state of the OC and PyC pools were analyzed for the first time, as well as the sedimentary OM sources in Guadiana riverine sediments collected after the dam became operational.

Despite the important roles and significance played by PyC, ambiguity persists when complex matrices, such as aquatic sediments, are considered. One of the major constraints to establish confident PyC estimations at a regional and global scale is the lack of a standardized method capable of detecting all types of PyC. Consequently, there is a diversity of analytical techniques currently in use (Kuhlbusch 1998; Middelburg et al. 1999; Druffel 2004; Hammes et al. 2007; De la Rosa et al. 2011). Most studies performed thermal and chemical oxidations of OM, being the C residue after oxidation determined as the PyC. However, other highly aromatic materials from sedimentary metamorphism (petrogenic) are accounted by using these PyC assessment methods. Thus, coal, graphitized carbon, and pure graphite are included in the oxidized material (Veillieux et al. 2009). In this study, the chemo-thermal oxidation method (CTO-375; Gustafsson et al. 2001) was applied. This method is able to isolate the most recalcitrant fraction of PyC (i.e., soot, graphitic black carbon) from complex sedimentary matrices containing abundant carbonates. Therefore, it has been widely applied to quantify PyC in sediment matrices.

The sedimentary OM was characterized by means of elemental analysis: TOC, TN, C/N, and stable carbon isotope analysis (δ¹³C). Such parameters have been widely used to determine changes in OM sources in lacustrine sediments, tidal marsh sediments, coastal areas, or continental shelves (e.g., Meyers 1994; Hedges and Oades 1997; Lamb et al. 2006; Perdue and Kopriwnjak 2007; Burdloff et al. 2008). The δ¹³C of the PyC-enriched material was also measured. The degree to which the δ¹³C value of pyrogenic material recovered from sediments and soils can currently be interpreted as an environmental indicator is hampered by a poor understanding of the processes leading to physical fractionation of C₂⁻ and C₄⁻derived pyrogenic OM during both production (Das et al. 2010) and transport to a site of deposition. This study brings further data in this field, which are strictly needed for a development of its potential as a natural tracer for the study of pyrogenic OM degradation and its interaction with the environment (Bird and Ascough 2012).

2. Study area

The Guadiana River basin has an area of ca. 67,000 km² and a length of 810 km. It is the fourth largest river in the
Iberian Peninsula; the last 60 km form the natural border between Portugal and Spain (Fig. 1).

**Fig. 1**

Map of the lower course of the Guadiana River and location of the 22 sampling stations

Before reaching the estuarine zone, the Guadiana River crosses approximately 130 km of rural area without point sources of anthropogenic pollutants (Ferreira et al. 2003). The flow of the Guadiana estuary is highly variable, at a seasonal and inter-annual scale (González et al. 2007). This pattern produces severe droughts and episodic floods in the river basin. For example, the monthly river discharge ranged from <10 to 4660 m$^3$ s$^{-1}$ for the period 1947–2001, with an average flow of 156 m$^3$ s$^{-1}$, decreasing to 83 m$^3$ s$^{-1}$ during the last decades. The flow of the Guadiana River has been extensively modified during the decades due to a range of anthropogenic activities, including damming, mining, urbanization, deforestation, and dredging (Delgado et al. 2010). Besides this, due to the Mediterranean climate of the area, characterized by hot and dry summers followed by cool and relatively wet winters, which alternate with frequent annual droughts, forest fires are a recurrent phenomenon. An average of 205 forest fires per year is reported by the Huelva fire department statistics, burning approximately 4985 ha yr$^{-1}$ of forest area (Zamora et al. 2010). In addition, grassland farmers of the SW Iberian Peninsula commonly used the fire-after-crop practice to facilitate tillage and to sterilize the soil (as disease control) during the twentieth century. Thus, biomass-derived PyC, dominantly composed of char and charcoal particles more prone to sedimentation and transport by means of rivers or water runoff, is likely to occur in the study area. Additional sources of PyC are port traffic in the Ayamonte (Spain) and Vila Real de Sto. Antonio (Portugal) harbors, vehicular traffic over the Guadiana international bridge as well as other anthropogenic activities in the towns located upstream.

The Guadiana estuary is divided into three sub-areas: upper (fluvial), middle, and lower (marine) estuary (Fig. 1). The fluvial area comprises mainly the freshwater section, still with a tidal influence but with a salinity close to zero (fluvial freshwater <0.5 g kg$^{-1}$). The middle section is the salinity-mixing zone (0.5–25 g kg$^{-1}$), whereas in the lower estuary, salinity is usually very close to seawater (>25 g kg$^{-1}$) (Chicharo et al. 2006).

The upper estuary consists mainly of gravel and sand from the drainage basin. The middle estuary is dominated by poorly sorted sediment, with grain size ranging from gravels to clay and silt. The fraction of mud decreases downstream and well-sorted medium sand (quartz, feldspar, bioclasts, plus lithic components of diverse origin) lies
at the lower estuary. In addition, some gravel, either mixed with sand or in small isolated pockets, are observed in few (and generally deep) locations of the lower estuary (Dias et al. 2004). These (lower, middle, upper) sectors are concordant with distinct ecohydrological characteristics that were described by Chicharo et al. (2001).

The estuary is influenced by semi-diurnal mesotidal tides and is usually partially stratified in its lower and middle sections, depending on river flow and tidal stage, being well mixed in the upper section. The estuary is located in a region highly vulnerable to climate change, due to a predicted decrease in rainfall (IPCC 2001), and increasingly subjected to urban development.

For this study, a total of 22 surficial (0–15 cm) sediment samples were collected using a Smith–McIntyre grab, in June 2008, along the lower course of the Guadiana River, covering a section of ca. 40 km between the river mouth and the Alcoutim town (Fig. 1). Sampling sites were selected along the riverbed of the main stream channel and at the shores of the Spanish and Portuguese margins. For each sampling site, a combination of three subsamples were taken, immediately stored in sealed plastic bags, and kept at −20 °C. Finally, samples were freeze dried and homogenized prior to analysis.

3. Analytical work

3.1. Elemental analysis

Freeze-dried samples were used for geochemical analysis. Total C (TC) and TN contents were determined in triplicate on ground and homogenized aliquots (10–20 mg) using an elemental analyzer (Carlo–Erba EA-1110 microanalyzer). Total OC content determination was performed on decarbonated samples. Finely ground and homogenized sediment (1 g) was treated with 10 ml of 2 M HCl (3×) in 50 ml Teflon centrifuge tubes (NALGENE Labware), washed (30 ml distilled water) and centrifuged (4000 rpm, 10 min). This step was repeated (3×) before the samples were freeze dried and reweighed. The instrumental readings were checked against aliquots of 2 mg of sulfanilamide standard (C₉H₇N₄O₂S from Sigma-Aldrich Co, USA). The analytical error based on triplicate analyses was within 4 to 6 % for both TOC and TN.

3.2. Stable carbon isotope analysis (δ¹³C)

Carbon isotope analysis (δ¹³C) was carried out on decarbonated sediments and PyC-enriched sediments with an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (Sercon 20-20 EA-IRMS; Sercon Ltd). Aliquots (5–10 mg) of each sample were packed into Sn capsules and combusted in excess O₂ at 1000 °C in a reactor packed with chromium oxide, copper oxide, and silver wool. Values are expressed in the δ notation as per mil deviation (%) from corresponding international standards and referred to Pee Dee Belemnite (¹³C/¹₂C, PDB). External working standard materials were inserted every five samples to monitor the working conditions of the analyzer. The analytical precision (±1σ) was in the range ±0.1–0.2 %. Data reproducibility was checked by replicate analysis of samples.

3.3. Pyrogenic C determination; chemo-thermal oxidation method (CTO-375)

We applied the method developed by Gustafsson et al. (2001). Briefly, small aliquots (ca. 40 mg, to prevent charring) of dry and finely ground decarbonated sediment were weighted (ρ ± 0.01 mg) and heated (375 ± 1 °C; 24 h) in duplicate to remove non-PyC material. A constant air flux of 250 mL min⁻¹ was maintained through the furnace. After the thermal treatment, the residual C (i.e., the PyC content) was determined as above for elemental analysis. The analytical error was within 3 to 7 % for all the samples analyzed.

4. Results and discussion

4.1. Elemental and isotopic analysis of sediments

Table 1 shows the TOC, TN, C/N, and δ¹³C values of the bulk sediments and of the remaining material after the CTO-375 treatment. The TOC content of sediments ranged from 0.25 (G22) to 2.37 (G9) wt% (mean 1.25 ± 0.50 wt%) and TN ranged from 0.03 to 0.28 wt% (mean 0.16 ± 0.06 wt%), which are typical values for estuarine sediments (Nieuwenhuize et al. 1994). The TOC concentration highly correlated with TN concentration (r = 0.959, p < 0.001), indicating that N is predominantly fixed in the OM (Sabel et al. 2005). In general, TOC and TN
contents were greater in samples from the middle estuary (G9 to G18). Lower values of TOC (0.50 to 1.40 wt%; mean 0.88 wt%) and TN (0.07 to 0.16 wt%; mean 0.10 wt%) were reported recently by Sánchez-García et al. (2009, 2013)) for sediments taken in the continental-marine transition area of the Gulf of Cádiz before the Alqueva dam was operational. However, we cannot establish a direct comparison to the data presented here because those samples belong to an area located from 5 to 48 km of distance to the Guadiana River mouth.

Concerning the atomic TOC/TN ratio (C/N index), the presence or absence of cellulose in the plant sources of OM to estuaries and lakes influences the C/N ratios of sediments. Thus, they can be used as an indicator of the predominant OM sources in aquatic ecosystems (e.g., Thornton and McManus 1994; Meyers 1997).

Phytoplankton C/N ratio varies from 6 to 9 in natural systems (Hollugan et al. 1984). Bacterioplankton is N-rich, with C/N 2.6–4.3 (Lee and Fuhrman 1987). In contrast, terrestrial vegetation normally has relatively high C/N ratios of >12 (Prabh et al. 1980) as it is composed predominantly of lignin and cellulose, which are nitrogen poor. C3 vascular plant material has C/N ratios of around 12 and over (Tyson 1995), while C4 grasses typically have C/N ratios of above 30 (Meyers, 1994). C/N values for the sedimentary OM (Table 1) ranged from 7.7 (G13) to 12.7 (G17), being ≤10 in most cases (avg. of 9.5 ± 1.3). Similar C/N values were reported for salt marsh sediments (Alberts and Filip 1989) and for surface sediments from lacustrine samples (Meyers and Ishiwatari. 1993). In general, C/N values obtained are slightly lower than those reported for surficial sediments from the marine estuary taken in 2001 before the Alqueva dam was operational (avg. 10.9 ± 1.1; De la Rosa et al. 2008) and similar to those obtained from the Iberian continental shelf, which ranged from 7.0 to 9.8 by Sánchez-García et al. (2009; 2013). Coastal sediments receive OM from autochthonous sources (derived from in situ sources, such as plants and algae growing on the sediment surface) and allochthonous sources (organic material transported to the sediment from elsewhere, such as with the tide or a river). The slight reduction on the C/N values compared to pre-Alqueva data may indicate a dilution of terrestrial and freshwater sources of OM by a relative increase in marine (tidal) sedimentary OM, which could be due to a smaller reach of freshwater phytoplankton and terrigenous OM. Nevertheless, early diagenesis can modify elemental composition and hence may be also responsible for a relative diminution of C/N values (Meyers and Ishiwatari 1993), due to microbial immobilization of nitrogenous material accompanied by the remineralization of carbon. Nevertheless, this would indicate an alteration on the conditions of the ecosystem.

Table 1
Location details, analytical data, and pyrogenic carbon (PyC) concentrations of sediment samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Latitude N</th>
<th>Longitude W</th>
<th>Depth (m)</th>
<th>Location detail</th>
<th>TOC (%)</th>
<th>TN (%)</th>
<th>δ13C Sed (‰)</th>
<th>OC/N atomic ratio</th>
<th>PyC (g kg⁻¹ sed.)</th>
<th>PyC (% TOC)</th>
<th>δ13C PyC (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G22</td>
<td>37° 27’ 51.42”</td>
<td>7° 27’ 58.20”</td>
<td>3.1</td>
<td>Close to Alcoutim town</td>
<td>0.25</td>
<td>0.03</td>
<td>−25.9</td>
<td>9.7</td>
<td>0.29</td>
<td>11.7</td>
<td>−31.4</td>
</tr>
<tr>
<td>G21</td>
<td>37° 27’ 15.48”</td>
<td>7° 27’ 23.76”</td>
<td>2.8</td>
<td>“Barranco de los mosqueteros’ stream input”</td>
<td>0.88</td>
<td>0.11</td>
<td>−27.6</td>
<td>9.3</td>
<td>0.55</td>
<td>6.2</td>
<td>−32.1</td>
</tr>
<tr>
<td>G20</td>
<td>37° 26’ 11.88”</td>
<td>7° 27’ 29.70”</td>
<td>2.0</td>
<td>“Torneiro” stream input</td>
<td>0.38</td>
<td>0.04</td>
<td>−26.9</td>
<td>11.1</td>
<td>0.45</td>
<td>11.8</td>
<td>−30.4</td>
</tr>
<tr>
<td>G19</td>
<td>37° 25’ 12.78”</td>
<td>7° 26’ 49.86”</td>
<td>3.9</td>
<td>“El Romerano” meander</td>
<td>1.81</td>
<td>0.24</td>
<td>−27.1</td>
<td>8.8</td>
<td>0.71</td>
<td>3.9</td>
<td>−30.3</td>
</tr>
<tr>
<td>G18</td>
<td>37° 24’ 16.56”</td>
<td>7° 27’ 15.18”</td>
<td>3.6</td>
<td></td>
<td>2.01</td>
<td>0.24</td>
<td>−26.7</td>
<td>9.8</td>
<td>0.62</td>
<td>3.1</td>
<td>−30.4</td>
</tr>
<tr>
<td>G17</td>
<td>37° 23’ 53.52”</td>
<td>7° 26’ 50.52”</td>
<td>2.3</td>
<td>Alamo town</td>
<td>1.31</td>
<td>0.12</td>
<td>−27.3</td>
<td>12.7</td>
<td>0.74</td>
<td>5.6</td>
<td>−32.5</td>
</tr>
<tr>
<td>G16</td>
<td>37° 23’ 45.06”</td>
<td>7° 26’ 40.74”</td>
<td>1.6</td>
<td></td>
<td>1.68</td>
<td>0.23</td>
<td>−27.2</td>
<td>8.5</td>
<td>0.54</td>
<td>3.2</td>
<td>−32.0</td>
</tr>
<tr>
<td>G15</td>
<td>37° 23’ 39.42”</td>
<td>7° 26’ 37.02”</td>
<td>2.3</td>
<td></td>
<td>1.71</td>
<td>0.22</td>
<td>−27.4</td>
<td>9.1</td>
<td>0.55</td>
<td>3.2</td>
<td>−30.2</td>
</tr>
<tr>
<td>G14</td>
<td>37° 20’ 57.60”</td>
<td>7° 26’ 42.90”</td>
<td>3.6</td>
<td>“Odeleite” stream input</td>
<td>1.65</td>
<td>0.22</td>
<td>−27.2</td>
<td>8.8</td>
<td>0.69</td>
<td>4.2</td>
<td>−30.4</td>
</tr>
<tr>
<td>G13</td>
<td>37° 18’ 55.98”</td>
<td>7° 26’ 36.30”</td>
<td>1.2</td>
<td></td>
<td>1.25</td>
<td>0.19</td>
<td>−27.2</td>
<td>7.7</td>
<td>0.54</td>
<td>4.3</td>
<td>−30.3</td>
</tr>
<tr>
<td>G12</td>
<td>37° 16’ 47.40”</td>
<td>7° 25’ 22.98”</td>
<td>1.8</td>
<td></td>
<td>1.47</td>
<td>0.19</td>
<td>−27.1</td>
<td>9.0</td>
<td>0.27</td>
<td>1.8</td>
<td>−30.1</td>
</tr>
<tr>
<td>G11</td>
<td>37° 15' 52.32&quot;</td>
<td>7° 25' 52.50&quot;</td>
<td>1.9</td>
<td>1.20</td>
<td>0.14</td>
<td>−25.3</td>
<td>10.0</td>
<td>0.17</td>
<td>1.4</td>
<td>−30.0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G10</td>
<td>37° 14' 59.04&quot;</td>
<td>7° 26' 07.14&quot;</td>
<td>3.0</td>
<td>1.51</td>
<td>0.16</td>
<td>−25.9</td>
<td>11.0</td>
<td>0.59</td>
<td>3.9</td>
<td>−31.3</td>
<td></td>
</tr>
<tr>
<td>G9</td>
<td>37° 14' 31.56&quot;</td>
<td>7° 25' 17.52&quot;</td>
<td>5.3</td>
<td>2.37</td>
<td>0.28</td>
<td>−26.1</td>
<td>9.9</td>
<td>0.67</td>
<td>2.8</td>
<td>−31.7</td>
<td></td>
</tr>
<tr>
<td>G8</td>
<td>37° 13' 39.78&quot;</td>
<td>7° 25' 02.10&quot;</td>
<td>3.7</td>
<td>1.36</td>
<td>0.18</td>
<td>−26.3</td>
<td>8.8</td>
<td>0.53</td>
<td>3.9</td>
<td>−31.8</td>
<td></td>
</tr>
<tr>
<td>G7</td>
<td>37° 13' 20.28&quot;</td>
<td>7° 24' 34.74&quot;</td>
<td>4.4</td>
<td>1.04</td>
<td>0.13</td>
<td>−26.1</td>
<td>9.3</td>
<td>0.62</td>
<td>5.9</td>
<td>−32.5</td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>37° 12' 52.50&quot;</td>
<td>7° 24' 29.76&quot;</td>
<td>1.1</td>
<td>0.79</td>
<td>0.08</td>
<td>−25.5</td>
<td>11.5</td>
<td>0.47</td>
<td>5.9</td>
<td>−32.4</td>
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<td>7° 25' 00.24&quot;</td>
<td>1.2</td>
<td>1.02</td>
<td>0.14</td>
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<tr>
<td>G4</td>
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<td>7° 24' 38.58&quot;</td>
<td>6.1</td>
<td>1.04</td>
<td>0.15</td>
<td>−26.5</td>
<td>8.1</td>
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<td>−31.6</td>
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<td>7° 24' 51.60&quot;</td>
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<td>1.01</td>
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<td>0.74</td>
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Values obtained for decarbonated sediments by the CTO-375 method

TOC total organic carbon, TN total nitrogen

The δ¹³C values ranged from −25.0‰ (G5) to −27.6‰ (G21) (avg., −26.5‰; Table 1), most of the samples located in the middle estuary being depleted in δ¹³C (≤−27‰). Stable isotopes (δ¹³C) are usual indicators for reconstructing OM sources and transformation of sedimentary OM (Meyers, 1994). The δ¹³C for C₃ biomass ranges from −23 to −34‰, with an average of ca. −27‰ (Meyers 1997). Lacustrine-derived OM is normally isotopically indistinguishable from OM from land plants (Meyers and Ishiwatari 1993), while typical marine end member values are −20 to −18‰ (Holtvoeth et al. 2005). Bulk δ¹³C and C/N should in theory reflect the relative amounts of the OM sources. The values in Table 1 illustrate mixed contributions of C₃ marsh-plants (Cistus grandiflorus, Cistus sp., and Halimium halimiforum) are abundant in the area; Ruiz de la Torre 1990), lacustrine plants (Meyers and Ishiwatari 1993), C₃ freshwater algae (−26 to −30‰; Meyers, 1994), and C₃ vascular plants (Castanea sativa, Pinus pinea, Pinus pinaster, or Quercus suber are abundant in the forests located upstream; González-Pérez et al. 2008). Nevertheless, the reliability of δ¹³C values as recorders of the source of OM is uncertain when decomposition affects the sediments (Thornton and McManus 1994). Some studies have suggested that microorganisms may also have an impact on the δ¹³C and C/N of recent sediments (e.g., Malamud-Roam and Ingram 2001). For instance, the δ¹³C values of living Spartina alterniflora, a C₄ marsh grass present in the area of study (typically δ¹³C = −11 to −13‰), changed in surface sediments from salt marshes of the eastern USA due to the presence of bacteria from −18 up to −24‰ (Peterson et al. 1980). Benner et al. (1987) demonstrated that laboratory decomposition of S. alterniflora produced a progressive shift in the δ¹³C of sedimentary OM from −13 to −17‰. Thus, the marine signature could be masked by microbial diagenesis of the sedimentary OM.

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Figure 2 plots C/N vs. δ¹³C data. Usually, a significant negative correlation would occur between δ¹³C and C/N because sedimentary OM with high C/N ratio has much lighter δ¹³C values (Wu et al. 1999). In addition, numerous studies across the world showed a gradient of δ¹³C and C/N in estuaries along their river axis, which
characterized the shift downstream from fluvial to marine OM inputs (e.g., Thornton and McManus 1994; Middelburg et al. 1999; Lamb et al. 2006). In our case, there is no correlation between $\delta^{13}C$ and C/N ($r^2 = 0.049$). This fact could be due to efficient sediment mixing and/or the presence of numerous OM sources with differing $\delta^{13}C$ values (Graham et al. 2001).

**Fig. 2**

Plot of the stable carbon isotopic composition ($\delta^{13}C$ %) versus the total organic carbon to total nitrogen atomic ratio (C/N). Values of source materials (boxes) are based on Meyers (1994)

[Graph showing C/N ratio vs. $\delta^{13}C$]

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### 4.2. Pyrogenic C distribution and sources

PyC was detected in all sediment samples with concentrations ranging from 0.17 (G11) to 0.74 (G17) g kg$^{-1}$ dry weight (average $0.54 \pm 0.14$ g kg$^{-1}$ dw). These values are of the same order of magnitude than those previously published for graphitic black carbon in sediments from the Gulf of Cádiz. They consisted of 0.47 to 1.63 g kg$^{-1}$ dw at the Atlantic Iberian margin (Middelburg et al. 1999), 0.30 to 1.10 g kg$^{-1}$ dw and 0.10 to 1.10 g kg$^{-1}$ dw (avg. 0.40 g kg$^{-1}$ dw) by De la Rosa et al. (2011) and Sánchez-García et al. (2013), respectively, for sediments taken in 2001 at the continental margin of the Gulf of Cádiz.

Samples G11 and G12 located in the central estuary, presented the lowest concentrations (0.17 and 0.27 g kg$^{-1}$ dw, respectively), in contrast with the samples taken close to residential areas that exhibited the highest PyC contents (G17 at Alamo town, 0.74 g kg$^{-1}$ dw; G1 at Ayamonte harbor, 0.71 g kg$^{-1}$ dw). Nevertheless, no significant trend was found related with the river axis. This result seems to confirm the efficient sediment mixing as well as the importance of anthropogenic sites as sources of PyC.

Concerning the method of PyC assessment, Gélinas et al. (2001) noted that previous measurements of PyC in sediments by the CTO-375 method might have been biased because of charring of the sedimentary OM. This problem was illustrated by increasing PyC concentrations with increasing OC. Figure 3 displays the determined PyC fraction versus TOC content. In this study, no significant correlation between PyC and TOC is evidenced ($r^2 = 0.1424, p < 0.001$), which confirms that measurements were not affected by in situ charring. The relative contribution of PyC to TOC (in wt%) ratio has been used in discussing pollution phenomena (Gustafsson and Gschwend 1998). Results obtained for the studied samples ranged from 1.4 wt% (G11) to 11.8 wt% (G20), which are comparable to the PyC/TOC ratios reported by Sánchez-García et al. (2013) by using the same method for sediments from the Gulf of Cadiz (2–15 wt%). In our case, sediments from the middle estuary, within a rural area, presented the lowest values of PyC/TOC (≤ 4 %). In contrast, enhanced ratios (≥10 %) were found in samples located close to Ayamonte harbor, Ayamonte town, Vila Real de Sto. Antonio town and Alcoutim town (G1, G20, G22; Table 1 and Fig. 1). The relationship between PyC and the distance from the Guadiana mouth found by Sánchez-García et al. (2009) for continental margin sediments of the Gulf of Cadiz was not observed. Taking into account the characteristics of the PyC assessed by the CTO-375 analysis within the combustion continuum model, the location of the samples enriched in PyC (close to sites with clear anthropogenic activities) may be interpreted by the contribution of soot-derived condensates.

**Fig. 3**

Relation between total organic carbon (TOC; wt%) and pyrogenic carbon content (PyC wt%)
The correlation between the PyC to TOC and the TOC values ($r = -0.8027; p<0.001$) are depicted in Fig. 4. A trend for TOC poor samples (≤1 % TOC) to contain a larger relative abundance of PyC is shown (see G1–G7; G20–G22). In general, the contribution of PyC to TOC is the result of two independent processes: the fluxes of PyC and OC to the sediments and the remineralization processes removing organic C from the sediments (Burdige 2005). In our case, this trend is not explained by lower sedimentation rates, as usually occurs in deep-sea sediments, but by the selective preservation of PyC, less sensitive to postdepositional degradation than bulk sedimentary OM. This means that biogeochemical processes may remove PyC less effectively than OC in this estuarine environment.

**Fig 4**
Relation between total organic carbon (TOC; wt%) and the contribution of pyrogenic carbon to organic carbon (PyC/TOC %)

The isolated PyC-enriched fractions were analyzed for δ¹³C ratios (δ¹³C_{PyC}). They ranged from –32.5 to –30.4 ‰ (avg. δ¹³C_{PyC} = –31.2 ‰). PyC in sediments is usually derived from the burning of fossil fuels, vegetation biomass, or weathering of rocks. The typical δ¹³C values of PyC from petrogenic nature range from –19.4 to –21.3 ‰ (Dickens et al. 2004); thus, the isotopic data suggest that the PyC present is a mixture of soot derived from fossil fuels and C₃ charred biomass (Friedli et al. 1986; Bird and Ascough 2012). In addition, depletion in the δ¹³C values of PyC-enriched material after the CTO-375 method (<–30 ‰) could be interpreted as indicative of a greater contribution of fossil fuels to the PyC instead of vegetation biomass burning. Other aspects such as the isotopic fractionation during the formation of soot PyC (charring) from starting biomass or the different fractionation processes occurring postdeposition for OC and soot PyC may alter the C isotope composition. Bird and Ascough (2012) reviewed the effects of charring on the stable isotope fractionation (Δ δ¹³C_{PyC}) during pyrolysis of C₃ and C₄ plant material. In the majority of instances, Δ δ¹³C_{PyC} consisted of a depletion of 1–2 ‰, but changes up to –11.0 ‰ were identified in some studies (Czimczik et al. 2002; Krull et al. 2003; Das et al. 2010). During charring, oxidative “weathering” has been reported (Ascough et al. 2011), which may be responsible for a loss of isotopically distinct indigenous carbon, with the addition of oxygen to the pyrogenic aromatic skeleton, and an increase in carboxylic groups.

Concerning microbial diagenesis, OM is a mixture of different types of compounds with different isotopic content. Amino acids from marine plankton, for instance, are enriched in ¹³C by –17 ‰ on average. Bacteria and algae, which are usually enriched in ¹³C compared with C₃ vascular plants, contain a high level of labile compounds and decompose chemically and biologically more rapidly than vascular plants (Valiela et al. 1985). The CTO-375 oxidation process of the sedimentary OM removes preferentially labile OM, which becomes less significant to bulk...
organic δ¹³C, producing a shift in the δ¹³C_{PyC} to lighter carbon isotope values. Taking into account the depletion in the δ¹³C_{PyC} (mean value -31.2 ‰) and the negative correlation between the PyC and the TOC contents (r = -0.8027; p < 0.001), we hypothesize that postdepositional degradation/alteration contributed to losses of isotopically heavy OM.

5. Conclusions

Elemental (TOC, TN, C/N) and isotopic (δ¹³C) data of sediments collected from the last 40 km of the Guadiana River revealed an efficient mixing of OM from terrestrial, estuarine salt marshes, lacustrine, and freshwater contributions. The C/N data suggested a slight increase in the marine contribution, or a smaller reach of freshwater phytoplankton and terrigenous OM compared to samples taken in the same area before the Alqueva dam became operational.

PyC accounted from 0.17 to 0.74 g kg⁻¹, constituting a significant portion of the sedimentary OM (ca. 2 to 12 % of TOC), especially in the vicinity of urbanized areas of the Guadiana River estuary. The relative enrichment in PyC measured in sediments with low OC content indicated a selective preservation of PyC to postdepositional degradation when compared to bulk sedimentary OM. According to the δ¹³C_{PyC} data, fossil fuels and C₃-charred biomass comprised the main sources of PyC, whereas the presence of PyC weathered from rocks was negligible. Nevertheless, δ¹³C_{PyC} was affected by postdepositional alteration, which may contribute to a shift of the δ¹³C values to lighter carbon isotope values.

Acknowledgments

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References


Flores-Cervantes DX, Reddy CM, Gschwend PM (2009) Inferring black carbon concentrations in particulate


Rapid degradation of pyrogenic carbon. Global Ch Biol 18:3306–3316