1 **Determination of refractory organic matter in marine sediments by chemical**

2 **oxidation, analytical pyrolysis and solid-state ^{13}C nuclear magnetic resonance**

3 **spectroscopy**

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6 J.M. DE LA ROSA[a], J.A. GONZÁLEZ-PÉREZ[a], P.G. HATCHER[b], H. KNICKER[c] & F.J.

7 GONZÁLEZ-VILA[a*]

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9 [a]*IRNAS, C.S.I.C, Avda. Reina Mercedes, 10, P.O. Box 1052, 41080-Sevilla, Spain,*

10 [b]*Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, VA*

11 *23529 USA, and* [c]*Lehrstuhl fur Bodenkunde, Technische Universitat Munchen, D-85350*

12 *Freising-Weihenstephan, Germany*

13

14 [*]Correspondence: Dr. F.J. González-Vila. E-mail: fjgon@irnase.csic.es

16   **Summary**

17   Seeking to quantify the amount of refractory organic matter (ROM), which includes

18   Black Carbon-like material (BC), in marine sediments, we have applied a two-step

19   procedure that consists of a chemical oxidation with sodium chlorite of the

20   demineralized sediments followed by integration of the aromatic C region in the

21   remaining residues by solid-state ^{13}C Nuclear Magnetic Resonance (NMR)

22   spectroscopy. The efficacy for lignin removal was tested by analytical pyrolysis in the

23   presence of tetramethyl ammonium hydroxide (TMAH). Riverine, estuarine and

24   offshore marine sediment samples were collected from the Southwest Atlantic coast of

25   Spain, a site of geological and environmental interest. Measured contents of BC-like

26   material ranged between 3.0 and 45.7 % of the total organic carbon. Greater relative BC

27   contents were found in riverine sediments close to urban areas, which shows an elevated

28   input of anthropogenic organic material. The contents of BC-like material in offshore

29   marine sediments (5.5 to 6.1 %) were similar to those previously reported for these

30   kinds of samples. However, NMR and pyrolysis-GC/MS of the isolated ROM reveals

31   that abundant refractory aliphatic organic material remains in most of the marine

32   samples after chlorite oxidation. We suggest that this pool of aliphatic carbon may play

33   an important role as a stable carbon pool within the global C cycle.

Introduction

Black carbon (BC), soot, elemental carbon and charcoal are different terms used to describe a chemically heterogeneous, biologically refractory form of organic matter that remains as a residue from incomplete combustion processes. This particular organic material is ubiquitous in the atmosphere, ice, soils and sediments due to its widespread production, and its stability and inertness in the environment (Goldberg, 1985). Moreover, it has been used as an indicator for the occurrence of forest fires and fossil fuel emissions (Bird & Cali, 1998). Thus BC can represent a significant carbon sink, because it represents carbon that is transferred from the rapidly cycling biological-atmosphere carbon cycle to the long-term geological carbon cycle (Middelburg *et al.*, 1999; Gustafsson *et al.*, 2001). However, despite its importance to carbon-cycling dynamics, BC remains a component of that large fraction of OC that is classified as biochemically uncharacterized (Hedges *et al.*, 2000). The main reason for this is that BC is not a pure substance but rather a *continuum* of condensed aromatic structures with different physicochemical properties (Jones & Chaloner, 1991) and its isolation and measurement is subject to serious experimental constraints (Kuhlbusch, 1998; Derenne & Largeau, 2001; Nguyen *et al.*, 2004; Brodowski *et al.*, 2005).

Seeking an accurate analytical protocol for quantifying BC stored in different environmental matrices, Simpson & Hatcher (2004) developed a procedure that is based on the removal of mineral fractions by treatment with hydrofluoric acid, followed by chemical oxidation with sodium chlorite to remove non-BC aromatic compounds (mainly lignin material). Because quantification of the BC involves use of solid-state ^{13}C NMR and aromatic lignin signals overlap those of BC, removal of lignin is essential for estimating BC contents. Effective removal of non-BC components was tested by Simpson and Hatcher (2004) using thermochemolysis with tetramethylamonium

59 hydroxide (TMAH) (thermochemolysis GC-MS), a method found useful for the

60 detection of lignin biomarkers (Martín *et al*., 1998; Del Río *et al*., 1998; Deshmukh *et*

61 *al*., 2001).

62   In the current study, we have applied the above procedures to quantify BC in

63 sediments from the Southwest Atlantic coast of Spain (Gulf of Cadiz), a site of

64 geological and environmental interest. Considering the fact that several model

65 calculations suggest that more than 90 % of BC deposition occurs on continental

66 shelves, we pose the question "Is the magnitude of this transfer similar to estimates of

67 continental BC emissions made by Suman *et al*. (1997) ?"   Undeniably, BC can

68 contribute greatly to the organic matter being buried in marine sediments (Masiello &

69 Druffel, 1998), and we seek to test this concept in sediments of the Gulf of Cadiz.

70

71 **Experimental methods**

72 *Area of study and sediment sampling*

73 The study area is geologically complex with great environmental interest due to its

74 proximity to the Doñana National Park. Including the estuaries of the Guadiana, Tinto,

75 Odiel and Piedras rivers, the area encompasses approximately 90.000 km^2 of

76 considerable deposition. Deep Mediterranean currents affect sedimentation and are

77 partly responsible for the mobilization, transport and deposition of large amounts of

78 detritus on the continental shelf and rise (Berasategui *et al*., 1998).

79   Surface sediment samples (0 to 20 cm depth) were collected on the Atlantic side

80 of the Gulf of Cadiz under the auspices of the Spanish BACH-Project (BACH, 2004).

81 Figure 1 and Table 1 show the geographical location and depth of the samples studied,

82 which consist of four river sediments (R-25; R-35; R-39; R-40), five estuarine samples

83 (E-202; E-209; E-213; E-218; E-226), a sediment from the mouth of the Odiel River

4

84   (R/E-28), another sediment from the mouth of the Guadiana River (R/E-211), and two

85   marine sediment samples (M-131, M-155). All samples were stored frozen in glass

86   containers to avoid microbial growth. Before analysis, samples were freeze-dried,

87   thoroughly ground in a mortar mill and homogenized to reduce variability.

88

89   *Elemental analysis*

90   Carbon, nitrogen, and sulphur analyses were performed on a Carlo-Erba NA 1500

91   Series 2 Elemental Analyzer (CE Elantech, Inc., Lakewood, NJ, USA). Samples were

92   analysed in triplicate and the total organic carbon (TOC) was obtained by carbon

93   elemental analysis after removal of inorganic carbonates by mild acidification of the

94   sample in silver capsules.

95

96   *Chemical treatments*

97   Dried samples were pre-treated with hydrocloric and hydrofluoric acid to enrich them in

98   organic material by removal of the mineral matrix, including paramagnetic compounds.

99   We demineralised approximately 10 g of dried sample with a 6M HCl solution followed

100   by a treatment with 10 % HF (50 ml) 5 times, stirred (2 hours) and centrifuged (2000g

101   for 15 min). After the HCl/HF treatment, samples were washed thoroughly, centrifuged

102   repeatedly (2000g for 15 min) with deionised water to remove excess salts, and finally

103   freeze-dried. This approach significantly improved the signal-to-noise ratio of the ^{13}C

104   NMR spectra.

105   To remove lignin-components known amounts (1g) of freeze-dried HCl/HF-

106   treated samples were chemically oxidized with sodium chlorite (NaClO$_2$) as described

107   by Simpson and Hatcher (2004). The final residue was freeze-dried for elemental

108   analysis and solid state ^{13}C NMR. The BC content was determined from the relative

109   amount of aromatic carbon (aromatic signal at 110 to160 ppm in solid state ^{13}C NMR)

110   and the amount of organic carbon left in the sample after sodium chlorite oxidation

111   (Simpson & Hatcher, 2004). The fraction of total organic carbon (TOC) in the form of

112   BC is obtained by dividing the BC content by the TOC content.

113

114   *Solid-state ^{13}C NMR*

115   The solid-state cross polarization and magic angle spinning (CP MAS) ^{13}C NMR

116   spectra were obtained on a 300 MHz NMR spectrometer (Bruker Avance, Germany)

117   equipped with a 4mm H-X MAS probe, and using the standard ramp-CP pulse program

118   (Cook *et al*., 1996). Approximately 100 mg of dry sample was packed into a 4mm

119   zirconium rotor with a Kel-F cap. The rotor was spun at 12 kHz. The ^{13}C chemical shifts

120   were referenced to glycine, and the acquisition parameters were the following: [13]C

121   spectral frequency of 75 MHz, 1s recycle delay and 1ms contact time. Between 40×10^3

122   and 100×10^3 acquisitions of 512 data points were signal averaged for each spectrum.

123   The free induction decays were zero-filled to 4 K of data and Fourier transformed, a line

124   broadening of 50 Hz was applied when fitting the NMR spectra. For quantification of

125   the solid-state ^{13}C NMR spectra, the integration routine of the spectrometer was used to

126   quantify the relative intensity of the following chemical shift regions defined Knicker &

127   Lüdemann (1995): 0-45 ppm (alkyl-C); 45-60 ppm (N-alkyl C, methoxyl C); 60-110

128   ppm (O-alkyl-C); 110-160 ppm (aromatic C, olefinic C); 160-185 ppm (carboxyl C,

129   amide C) and 185-245 ppm (ketone C, aldehyde C).

130

131   *Tetramethyl ammonium hydroxide (TMAH) thermochemolysis GC-MS*

132   Thermochemolysis   with   TMAH   was   performed   by   adding   15 μl   of   the

133   tetramethyammonium hydroxide (250g kg^{-1} in water; Sigma-Aldrich; Steinheim,

134   Germany) to 1–2 mg of freeze-dried sediment samples before and after the chlorite

135   treatment. The mixture of sample and TMAH was dried overnight at room temperature

136   in a dessicator under a reduced pressure. The mixture (1 mg) was then pyrolyzed in a

137   "Double Shot" microfurnace pyrolysis unit (Py-2020 iD; Frontier Lab. Ltd. Fukushima,

138   Japan). An initial thermal desorption was carried out from 100 to 300 °C using a heating

139   rate of 30 °C minute^{-1}. The temperature was then held at 300 °C for 1 minute.

140   Subsequently, pyrolysis was performed at 500 °C for 1 minute. The released desorption

141   products and pyrolysates were analysed on a GC–MS system (Agilent 6890, Santa

142   Clara, California, USA) equipped with a 30 m, 0.25 mm i.d., 0.25 μm film thickness

143   DB5 capillary column (J&W Scientific, Agilent, Palo Alto, USA). The GC injector was

144   held at 280 °C, and the transfer line at 300 °C. The detector consisted of an Agilent

145   5973 mass selective detector (Electronic Ionization at 70 eV) in the scan mode. The

146   carrier gas was helium with a flow of 1.5 ml minute^{-1}. The GC–MS analysis was carried

147   out at the following temperature program: from 50 to 100 °C at 30 °C minute^{-1}, and

148   then from 100 to 300 °C at 6 °C minute^{-1}, with a 10 minutes hold time. Compounds

149   were identified on the basis of their fragmentation patterns and by comparison with

150   NIST library spectra and previously published mass spectra.

151

152   **Results and discussion**

153   *Bulk analyses of the sediment samples*

154   Total carbon (TC), total nitrogen (TN), total sulphur (TS), total inorganic carbon (TIC)

155   and total organic carbon (TOC), as well as the values for the TOC/N and TOC/S ratios

156   of the sediments studied are listed in Table 2. The TC values ranged from 12.8 to 31.1 g

157   kg^{-1}, which are typical values for coastal and marine sediments (Nieuwenhuize *et al.*,

158   1994). Greater TOC values were found in riverine and in the Guadiana estuarine

159   samples. The TN values were found to be very similar and ranged between 0.9 % and

160   2.0 g kg^{-1}.

161   The paleoenvironmental significance of the C/N ratio and its usefulness as a

162   source characterization tool for OM have been discussed by numerous authors (Meyers

163   et al., 1984;; Andrews *et al*., 1998; Graham *et al*., 2001). Thus, C/N ratios have been

164   used to distinguish between different OM inputs in estuaries. For example,

165   autochthonous marine organisms rich in protein material have C/N values that range

166   from 4 to10; terrestrial plants, however, tend to have C/N ratios of 20 and greater

167   (Hollugan *et al*., 1984; Hedges & Mann, 1979). The C/N ratios in the sediments studied

168   (Table 2) range between 7 and 13. Larger C/N ratios ($\geq$ 13) are observed for the riverine

169   samples, indicative of a large contribution of terrestrial detritus.

170   Sulphur (TS) contents in the samples ranged from 1.8 to 13.5 g kg^{-1}. Larger

171   values were observed in Tinto and Odiel River samples (R-35, R-39, R-40), and the

172   smallest value was in the marine sample M-131. In the riverine samples, S mainly

173   derives from detrital pyritic-rich sediments deposited in the Tinto and Odiel rivers,

174   which drain a catchment area that traverses the pyritic belt in the Southwest of the

175   Iberian Peninsula.

176   As pointed out by Raiswell & Berner (1985) the TOC/S ratio is correlated with

177   microbial sulphate reduction processes. The greater the amount of metabolizable

178   organic matter available to sulphate-reducing bacteria, the greater the intensity of

179   sulphate reduction, and the amount of accumulated sulfides and resistant organic matter.

180   In our samples, the TOC/S ratio ranged between 0.8 and 6.8 (with an average of 3.4),

181   and no relationship between S content and TOC was found. It is known that marine

182   sediments deposited in oxygenated bottom water are usually characterized by a positive

183   correlation between sulphur and organic carbon contents. A typical TOC/S ratio of 2.8±

184 0.8 is the generally accepted value for normal oxygenated sediments. This is because

185 pyrite formation is controlled by the quantity and reactivity of the organic matter,

186 indicating early diagenesis in marine sediments (Urban *et al.*, 1999; Lallier-Vergès *et*

187 *al.*, 1997).

188

189 *Effects of the chemical treatments on the NMR spectral features of the sediment samples*

190 The CP MAS ^{13}C NMR spectra of the sediment residues after HCl-HF treatment and

191 NaClO$_2$ oxidation are displayed in Figure 2. These spectra show prominent signals in

192 the alkyl-C region (0-45 ppm), dominated by the signal peaking at 31-32 ppm, which is

193 characteristic of cross-linked methylene C or methylene C removed by two or more

194 carbons from a carboxyl C. This signal can be, in part, assigned to polymethylenic

195 chains. Table 3 shows the integration values of different regions of the solid state [13]C

196 NMR spectra after the successive treatments. The greatest intensity in the alkyl-C

197 region is found in sample R-35 (34.7 %). Strong signals were also found in the aromatic

198 C region (110-160 ppm), with integration values between 19.09 % (E-218) and 48.69 %

199 (R-25). Signals typically assigned to carboxyl and amide C (185-160 ppm) also show a

200 significant presence in every sample, with integration values in the range 10 to 12 %.

201   From 70 to 86 % (w/w) of sedimentary material was removed after HCl-HF

202 treatments, with greater weight losses (about 85 %) from estuarine samples (E-202, E-

203 209), and some riverine samples (R-39, R-40) from the Guadiana's estuary (R/E-211

204 and E-213) showed greater resistance to demineralization than the rest of the estuarine

205 samples (Table 4). The least weight loss occurred in marine samples (M-131, M-155).

206 Weight losses during this treatment are usually related to the presence of carbonates, but

207 no relationship was found between TIC content and the amount of material recovered

208   (%), so major weight losses are most likely attributed. Some removal of the most labile

209   and hydrophilic OM is also expected.

210   Material remaining after the chlorite oxidation step ranged widely in relative

211   amount, between 55 to 93 % (w/w) (Table 4). The greatest removal of material was

212   from riverine samples (R-35, R-40, R-25 & R-39), every one of which lost more than 20

213   % (by weight). In contrast, marine sediments (M-155 & M-131) lost the least amount of

214   material(<8 %). As shown in Table 3 and Table 4, chlorite treatment caused: 1) increase

215   in the relative abundance of O-alkyl-C (110-60 ppm) in every sample, 2) increase in the

216   alkyl-C region (0-45 ppm) in mainly estuarine and riverine samples, 3) a small

217   reduction in the carboxyl-amide C (185-160 ppm) (1-3 %), 4) significant signal

218   diminution (1-4 %) for the C=O region (245-185 ppm) and 5) signal reduction in the N-

219   alkyl/methoxyl C region (60-45 ppm). Few changes were observed in the R/E-28

220   sample compared to the others, due probably to the specific location of this sample

221   (Figure 1). It is subject to continuous erosion, and substantial and prolonged oxic

222   conditions could have produced natural oxidation of the more labile OM in the sample.

223   The relative decrease in the contents of aromatic C, more pronounced in several

224   riverine samples, seems to be due to an effective removal of lignin-like compounds,

225   although an important presence of aromatic-C is still observed (>14 % in every sample).

226   This residual aromatic carbon indicates the presence of BC, assuming that all the lignin

227   is removed.

228   Pyrolysis in the presence of TMAH was used to evaluate the efficacy of lignin

229   removal by the chlorite oxidation procedure. This pyrolysis approach was applied by

230   Pulchan *et al*., (1997) to characterize marine sediments and found suitable for

231   monitoring the occurrence of lignin and lignin degradation products in organic matter

232   from soils and sediments (Simpson & Hatcher, 2004). We found minor changes in the

233　pyrolytic behaviour of the sediment samples after the chlorite treatment except for the

234　general absence of lignin compounds among the pyrolyzates. Figure 3 shows the

235　pyrograms obtained after Py (TMAH)-GC–MS analysis of sample R-25 before and after

236　sodium chlorite treatment. The identity of the pyrolysis compounds is listed in Table 5.

237　In the original sample (Figure 3a) pyrolysis products are dominated by two series of

238　dicarboxylic acid C_4-C_{16} methyl esters (DAMEs) and fatty acid C_6-C_{28} methyl esters

239　(FAMEs). In addition, aromatic compounds (peaks 4, 6, 7 and 9) of undetermined origin

240　and a number of guaicyl (G) structures of lignin origin are observed. The major change

241　observed in the pyrogram after chlorite treatment (Figure 3b) is the removal of most of

242　the lignin-derived compounds. Only a trace of two lignin-derived compounds is found

243　in riverine samples (R-25, R-39). Additional aromatic compounds are detected in

244　chlorite-oxidized sample R-25 (peaks 13, 15, 18, 20 and 21; Table 4), while the relative

245　presence of FAMEs and DAMEs remains practically unaltered compared to pyrograms

246　of untreated sample.

247　　In general, pyrolysis results showed that the presence and abundance of lignin

248　markers in unchlorinated samples decreases remarkably with increasing distance from

249　the coast and with depth, in accordance with a seaward-declining contribution of

250　terrestrial OM. Chlorite oxidation treatment effectively removed most of the lignin-

251　derived compounds without noteworthy variation in other components in the sediments

252　studied. The residual presence of lignin markers in the chlorite oxidation residues means

253　that caution should be taken when quantifying BC in marine sediments by this method,

254　since it is based on the integration of the aromatic C region with the assumption that

255　lignin is completely removed by the treatment.

256

257　*Quantification of ROM (BC-like material) in the sediments by CP MAS ^{13}C NMR*

258   Table 4 shows the relative amount of material remaining after the treatments with HCl-

259   HF and NaClO$_2$, as well as the contents of BC-like material (calculated from the ^{13}C

260   NMR spectra elemental C data as described by Simpson & Hatcher, 2004). The BC-like

261   material ranged from low values of 0.47 g kg^{-1} (dry weight) in samples E-202 and R/E-

262   209 to high values of 8.09 g kg^{-1} in sample R-25 (Figure 1; R-25 > 45 % BC TOC^{-1}).

263   The high BC value in the Odiel river sample (R-25) can be explained, because this

264   location is protected from marine currents or river flooding that provides ideal

265   conditions for the accumulation of riparian sediments. It is also probable that sediments

266   in this area accumulate remains of charred material from the several wildfires that occur

267   regularly in the forest within the drainage basin.

268   Apart from sample R-25, the rest of the BC contents estimated fall within the

269   range previously reported for BC-like materials in marine sediments (Gustafsson &

270   Gschwend, 1997; Middelburg *et al*., 1999), from the 3.0 % BC TOC^{-1} obtained in the E-

271   218 sample located at the mouth of the Guadiana estuary to the 16.5 % in the R/E-211

272   sample (located in the mouth of the Guadiana river). Strong marine currents occur in

273   this region, which influence the relative accumulation of terrestrial organic matter. In

274   particular, in the Piedras estuary, high-energy coastal streams (Morales, 1995) probably

275   quench sediment accumulation and are, thus, responsible for the low BC values. It is

276   likely that Piedras estuary sediments are subjected to substantial and prolonged oxic

277   degradation during transport to their ultimate burial site elsewhere. While residing in

278   surface marine sediment horizons, the OC in these sediments is likely subjected to

279   extensive oxidation, greatly altering the OC and any accumulated BC (Hedges *et al*.,

280   1999; Keil *et al*., 2004).

281   The R-35 sample obtained at a depth of 18 m shows a low C/N ratio of 7.9,

282   together with an elevated aliphatic C content (41 %; 0-45 ppm) and a considerable

283   amount of carboxyl C (10 %; 185-160 ppm). Such characteristics are typical of organic

284   matter in marine environments (Hatcher *et al*., 1986: Hedges *et al*., 2001). The presence

285   of peptide-like material in the samples (inferred from the high C/N) could reflect a

286   recent input of marine OM for this particular sample. The enrichment in alkyl carbon in

287   marine ROM for this sediment could be indicative of selective preservation of refractory

288   marine alkyl carbon (Hatcher *et al*., 1983) or preservation of aliphatic-rich OC imparted

289   by organo-mineral associations (Dickens *et al*., 2006). Further analyses and seasonal

290   sampling could be helpful for a better understanding of the flux of ROM in the area.

291

292   **Conclusions**

293   Our study shows that the following:

294   1) Black Carbon (BC) values in marine sediments from the Southwest Atlantic coast of

295   Spain (Gulf of Cadiz) are similar to those reported for other coastal areas. However,

296   considerable accumulation of BC like material is found close to the city of Huelva,

297   Spain, in the sediments of the Odiel River. This BC derives from the accumulation of

298   charcoal transported through the watershed that experiences frequent forest fires, or may

299   arise from high-volume port traffic in the city of Huelva.

300   2) Both NMR spectra and Py (TMAH)-GC/MS analysis reveal a strongly aliphatic

301   nature of the refractory organic matter (ROM) isolated with recalcitrant OM in marine

302   sediments. These harbour lipidic and peptidic characteristics that resist the oxidation

303   and therefore can be consider as main components of the ROM in marine environments.

304   We believe that resistance to harsh oxidative treatment is related with biochemical

305   recalcitrance and we suggest that this refractory aliphatic material may play an

306   important role as a stable carbon pool in the fluxes and biogeochemical C cycle in

307   marine environments.

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432   Figures and tables

433   **Table 1**. Sample locations and site descriptions.

Sample	Latitude N	Longitude W	Origin	Depth /m
R-25	37º14´51.07´´	6º57´35.06´´	Odiel River	7
R-35	37º12´20.74´´	6º56´31.45´´	Tinto River	18
R-39	37º12´48.49´´	6º55´49.55´´	Odiel River	4
R/E-28	37º7´51.89´´	6º49´55.63´´	Odiel River mouth	3
R-40	37º12´34.20´´	6º56´12.37´´	Tinto & Odiel River	4
E-202	37º12´43.27´´	7º9´27.86´´	Piedras Estuary	12
E-209	37º9´23.87´´	7º17´34.51´´	Guadiana Estuary	4
R/E-211	37º12´17.06´´	7º20´42.83´´	Guadiana River-Estuary	5
E-213	37º12´20.74´´	7º20´19.32´´	Guadiana Estuary	13
E-218	37º8´48.66´´	7º20´0.60´´	Guadiana Estuary	12
E-226	37º8´48.23´´	7º15´59.58´´	Guadiana Estuary	17
M-131	37º4´23.05´´	7º9´53.10´´	Offshore marine sediment	36
M-155	37º1´59.84´´	7º21´58.25´´	Offshore marine sediment	70

434

435   **Table 2.** C, N and S elemental analyses, C/N and C/S ratios measured in bulk

436   sediments.

SAMPLE	Elemental analysis / g kg^{-1}						
	TC	TN	TIC	TOC	C/N	S	C/S
R-25	18.4	1.3	0.7	17.7	13.62	4.1	4.32
R/E-28	29.7	1.3	11.7	18.0	13.85	4.5	4.00
R-35	15.3	1.4	4.3	11.0	7.86	11.3	0.97
R-39	12.8	0.8	2.4	10.4	13.00	09.7	1.07
R-40	12.6	1.0	1.6	11.0	10.68	13.5	0.81
E-202	15.2	1.4	3.6	11.7	8.33	4.4	2.65
E-209	16.9	0.9	9.2	7.7	8.58	2.7	2.86
R/E-211	22.1	1.0	11.4	10.7	10.68	2.2	4.85
E-213	30.6	1.3	18.4	12.2	9.40	4.4	2.78
E-218	20.9	2.0	3.4	17.6	8.79	2.6	6.84
E-226	17.4	1.3	5.8	11.6	8.93	4.4	2.64
M-131	31.1	1.6	19.4	11.7	7.31	1.8	6.46
M-155	31.1	1.5	20.2	10.9	7.29	3.0	3.64

437 **Table 3.** Solid state ^{13}C NMR integrations and percentage of the areas and amounts

438 assigned to the remaining material after HCl/HF and NaClO$_2$ treatments

SAMPLE	Ketone C, aldehyde C	carboxyl C, amide C	aromatic C	O-alkyl-C	N-alkyl C, methoxyl C	alkyl-C	Remaining material / %	
	245-185	185-160	160-110	110-60	60-45	45-0		
R-25	2.93	10.35	48.69	14.50	5.65	17.87	16.89	HF/HCl
	2.15	9.32	37.82	23.63	5.24	21.81	12.95	NaClO$_2$
	-0.78	-1.03	-10.87	9.13	-0.41	3.94	-3.94	Difference
R/E-28	3.97	12.95	24.10	19.24	9.48	30.26	26.87	HF/HCl
	2.02	13.37	24.39	24.46	7.18	28.55	24.65	NaClO$_2$
	-1.95	0.42	0.29	5.22	-2.30	-1.71	-2.22	Difference
R-35	3.15	12.69	20.72	19.08	9.67	34.70	20.52	HF/HCl
	3.18	10.31	15.45	23.14	7.36	40.56	11.31	NaClO$_2$
	0.03	-2.38	-5.28	4.06	-2.31	5.86	-9.21	Difference
R-39	3.34	12.33	25.63	18.77	9.33	30.59	14.49	HF/HCl
	1.29	8.89	22.38	27.67	8.11	31.71	11.56	NaClO$_2$
	-2.05	-3.44	-3.25	8.91	-1.22	1.12	-2.93	difference
R-40	3.92	12.10	23.36	18.94	9.31	32.37	14.60	HF/HCl
	3.03	9.28	18.98	25.00	7.45	36.24	11.00	NaClO$_2$
	-0.89	-2.82	-4.38	6.06	-1.86	3.87	-3.60	difference
E-202	3.78	11.94	20.90	23.07	9.41	30.89	13.60	HF/HCl
	1.04	9.48	16.91	31.56	8.21	32.80	12.05	NaClO$_2$
	-2.74	-2.46	-3.99	8.49	-1.20	1.91	-1.55	difference
E-209	5.39	11.60	24.57	22.77	9.11	26.55	16.64	HF/HCl
	0.79	9.14	20.93	30.02	7.32	31.81	14.85	NaClO$_2$
	-4.60	-2.46	-3.64	7.25	-1.79	5.26	-1.79	difference
R/E-211	4.88	12.61	24.25	21.20	9.40	27.65	25.65	HF/HCl
	0.81	10.89	22.29	28.12	7.01	30.86	23.52	NaClO$_2$
	-4.07	-1.72	-1.97	6.92	-2.39	3.21	-2.13	difference
E-213	2.39	10.09	25.73	23.25	10.53	28.02	27.64	HF/HCl
	2.79	10.28	21.17	29.81	6.21	29.72	25.50	NaClO$_2$
	0.40	0.19	-4.56	6.56	-4.32	1.70	-2.14	difference
E-218	3.36	11.56	19.09	26.60	10.45	28.93	18.83	HF/HCl
	2.01	9.85	14.09	31.46	8.46	34.14	16.50	NaClO$_2$
	-1.35	-1.71	-5.00	4.86	-2.00	5.21	-2.33	difference
E-226	3.75	11.46	21.70	25.81	9.77	27.52	18.63	HF/HCl
	0.87	10.07	16.69	28.60	7.70	36.05	16.63	NaClO$_2$
	-2.88	-1.39	-5.01	2.79	-2.08	8.54	-2.00	difference
M-131	3.30	11.62	22.22	22.53	9.53	30.78	30.33	HF/HCl
	0.45	10.26	23.26	26.72	7.14	32.18	28.46	NaClO$_2$
	-2.85	-1.36	1.04	4.19	-2.39	1.40	-1.87	difference
M-155	4.73	12.56	22.62	21.76	9.03	29.29	29.14	HF/HCl
	2.47	11.55	22.68	27.02	7.82	28.45	27.02	NaClO$_2$
	-2.26	-1.01	0.06	5.26	-1.21	-0.84	-2.12	difference

Results are given in %. Differences were calculated by subtraction of the integration values of the HCl/HF treated samples from the values of the final residue (HCl/HF + NaClO$_2$ treated samples).

439

440 **Table 4.** Chemical oxidation treatments and BC results

441

SAMPLE	HCl/HF treatment		Sodium chlorite treatment		BC results		
	Remaining Material / %	^{13}C NMR Signal Aromatic C[†] / %	Remaining Material / %	^{13}C NMR Signal Aromatic C[†] / %	Final material remaining / %	gBC kg^{-1}	BC TOC^{-1}[‡] / %
R-25	16.89	48.69	76.70	37.82	12.95	8.09	45.7
R/E-28	26.87	24.10	91.75	24.39	24.65	0.78	4.3
R-35	20.52	20.72	55.10	15.45	11.31	0.90	8.2
R-39	14.49	25.63	79.78	22.38	11.56	0.81	7.8
R-40	14.60	23.36	75.40	18.98	11.00	0.87	7.9
E-202	13.60	20.90	88.57	16.91	12.05	0.47	4.1
E-209	16.64	24.57	89.23	20.93	14.85	0.47	6.1
R/E-211	25.65	24.25	91.71	22.29	23.52	1.76	16.5
E-213	27.64	25.73	92.27	21.17	25.50	1.87	15.3
E-218	18.83	19.09	87.65	14.09	16.50	0.52	3.0
E-226	18.63	21.70	89.25	16.69	16.63	0.59	5.0
M-131	30.33	22.22	93.83	23.26	28.46	0.64	5.5
M-155	29.14	22.62	92.71	22.68	27.02	0.67	6.1

[†] Values corresponding to the integrated signal in 110-160 ppm region in ^{13}C NMR spectra
[‡] Black carbon measured in relation to the TOC of each sample

442   **Table 5.** Peak identifications for the TMAH GC-MS results (non-lignin-derived

443   compounds).

Label	Non-Lignin Compounds released by Py-GC/MS
1	Dimethyl sulfone
2	N,N-dimethyl-t-butylamine
3	Glycine-N-acetyl methyl ester
4	Benzoic acid, methyl ester
5	Dimethyl, methyl fumarate
6	Benzoic acid, 3-methyl-methyl ester
7	Naphthalene, 2-methyl
8	1,3,5-Triazine-2,4,6 (1H,3H,5H)-trione-trimethyl
9	1,2-Benzenedicarboxylic acid dimethyl ester
10	1,2,4-Benzenetricarboxylic acid, trimethyl ester
11	N,N-Dimethyl-propanamide
12	Phenol
13	1,2,3-Trimethylbenzene
14	*m*-Cresol
15	Glycine, N(2-methoxy-2-oxoethyl)-methyl ester
16	Benzoic acid, 3-methyl-methyl ester
17	N-methylpyroglutamic acid, methyl ester
18	2-Naphthalenecarboxylic acid, methyl ester
19	2,4,6-Trimetoxybenzonitrile
20	2,6-Naphthalenedicarboxylic acid, dimethyl ester
21	1,2,4,5-Bencenetetracarboxylic acid, tetramethyl ester

444

Table 6. Existence of lignin-derived compounds released by TMAH Py-GC/MS before and after sodium chlorite treatment

Label	Lignin Compound	R-25		R/E-28		R-35		R-39		R-40		E-202		E-209		R/E-211		E-213		E-218		E-226		M-131		M-155	
	Guaiacyl-derived compounds	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
G1	*o*-Dimethoxybenzene (Veratrol)	X		X		X	X	X		X		X		X		X		X		X		X		X			
G2	*p*-Dimethoxybenzene	X				X		X						X		X		X									
G3	Benzoic acid 3,4-dimethoxy, dimethyl ester	X				X		X		X		X		X						X							
G4	Cinnamic acid 3,4-dimethoxy, methyl ester	X	X	X	X	X		X	X	X		X		X	X	X	X	X	X	X	X	X	X	X		X	
	Syringyl-derived compounds																										
S1	Benzaldehyde 3,4,5-trimethoxy, methyl ester	X		X		X		X		X		X		X		X		X		X		X					
S2	Benzoic acid 3,4,5-trimethoxy, methyl ester	X						X		X		X		X										X		X	
	Hydroxyphenyl-derived compounds																										
LG1	*p*-Methoxy benzaldehide	X		X				X		X		X				X		X		X		X					
LG2	Benzoic acid, 4-methoxy-methyl ester	X	X					X	X	X		X	X	X		X	X	X	X	X		X	X				
LG-V	Methylvanillin	X		X	X			X	X	X	X	X		X		X		X		X		X					

Before: Compound released before NaClO$_2$ treatment

After: Compound released after NaClO$_2$ treatment

446   FIGURE CAPTIONS

447

448   **Figure 1** Map of the sampling sites

449

450   **Figure 2** CP MAS ^{13}C NMR spectra of sediment samples after treatment with HCl/HF

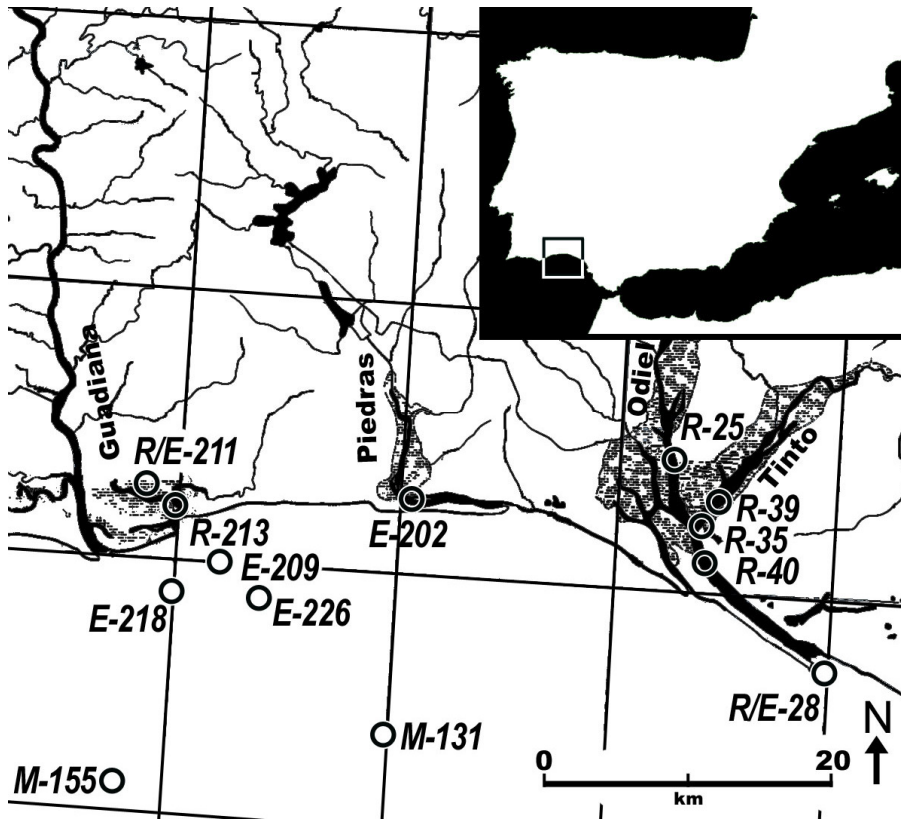451   and sodium chlorite solution

452

453   **Figure 3** Total Ion Current (TIC) of TMAH Py-GC/MS for riverine sediment before

454   and after chlorite treatment. The corresponding biomarker abbreviations are listed in

455   Table 1. Methyl esters of fatty acids are identified as FAME *n,* dimethyl diesters of fatty

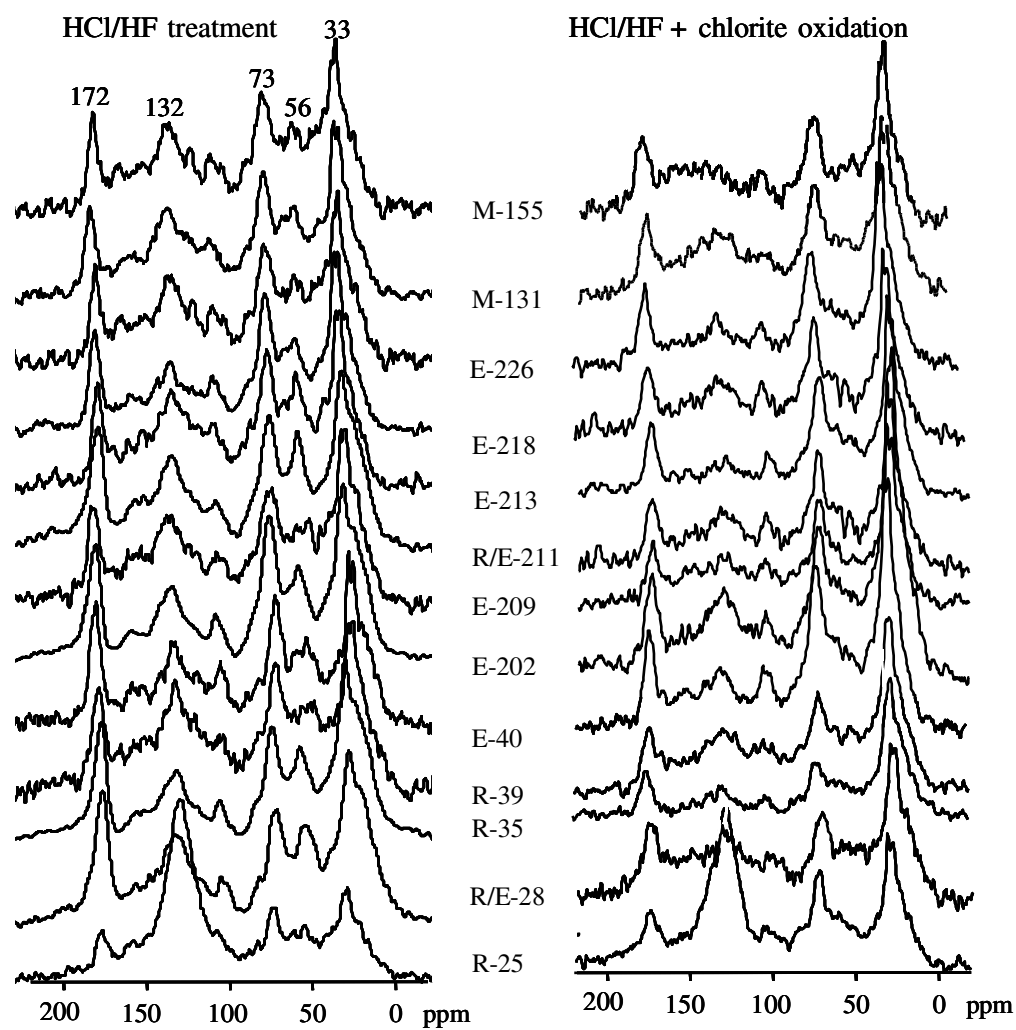456   acids are identified as DAME *n*, where *n* is the length of the carbon chain.
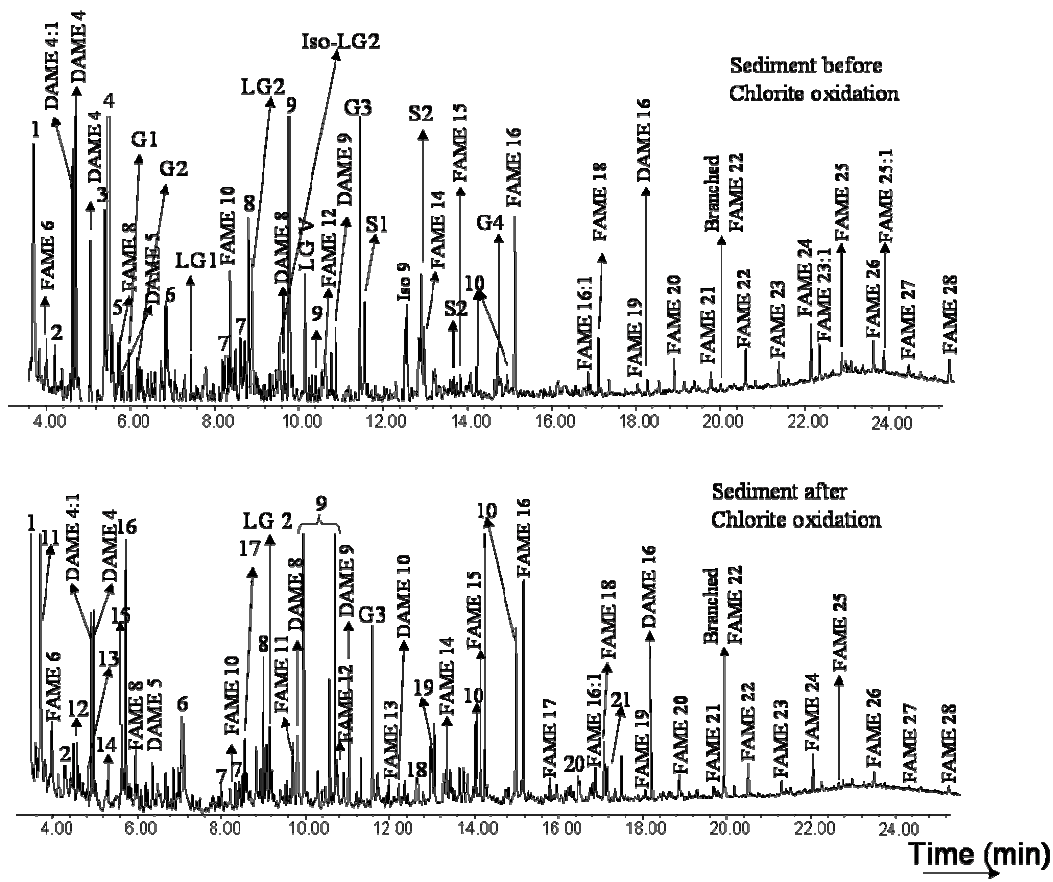
457

458   **Figure 1**

459

460

Figure 2

HCl/HF treatment

HCl/HF + chlorite oxidation

33

172   132   73   56

M-155
M-131
E-226
E-218
E-213
R/E-211
E-209
E-202
E-40
R-39
R-35
R/E-28
R-25

200   150   100   50   0   ppm

200   150   100   50   0   ppm

462   **Figure 3**.

463

464