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1 **Occurrence of phthalate acid esters (PAEs) in the northwestern Mediterranean Sea and the**
2 **Rhone River**

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32

33 **Abstract:**

34 Phthalate acid esters (PAEs) which are mainly anthropogenic molecules with endocrine
35 disrupting effects in animals and humans, have been detected in terrestrial and aquatic environments.
36 However, little is known about their distribution in the Mediterranean Sea, mainly because of
37 analytical difficulties and the high possibility of ambient sample contamination. Here, we report the
38 optimization of an existing protocol for the determination of PAEs in seawater and freshwater
39 samples, as well as the first estimation of the source and distribution of phthalates acid esters (PAEs)
40 in coastal waters from the NW Mediterranean Sea. By passing 1 L of sample through glass cartridges
41 packed with 200 mg of Oasis HLB and eluted with 6 mL of ethyl acetate, the recoveries for DMP,
42 DEP, DPP, DiBP, DnBP, BzBP, DEHP and DnOP were 101, 98, 115, 110, 99, 98, 103 and 95 %,
43 respectively, with acceptable blank values (below 0.4-4.0 % of the masses measured in different
44 seawater samples). By using this method, we detected PAEs in the Marseilles coastal area, offshore
45 (2000 m depth) and in the Rhone River with total concentrations ranging from 75.3 ng/L offshore in
46 surface water to 1207.1 ng/L a few meters above the bottom of the Marseilles Bay. High
47 concentrations were also observed in deep waters offshore (310.2 ng/L) as well as in the Rhone River
48 (615.1 ng/L). These results suggest that Marseilles urban area, Rhone River and sediment are potential
49 sources of PAEs in the areas studied.

50 In the Rhone River, DEHP was the most abundant PAE (66.1 %) followed by DiBP (20.5 %)
51 and DnBP (6.6 %), whereas a gradual change was observed in the plume of the river with increasing
52 salinity. In the Marseilles Bay, DiBP was the most abundant PAE at the surface (47.3 % of total PAEs)
53 followed by DEHP (22.7 %) and DnBP (19.1 %), whereas DnBP was predominant (38.6 %) a few
54 meters above the bottom. By contrast, DEHP was the dominant species in the first 50 m (66.9-76.7 %)
55 offshore followed by DnBP (10.9-15.2 %), whereas DnBP was the most abundant (57.0-72.6 %)
56 followed by DEHP (20.1-33.1 %) in the deepest waters. This study suggests that in addition to direct
57 PAEs injection in marine waters, different processes regulate PAE distribution in Mediterranean Sea
58 including photochemical oxidation reactions, bacterial degradation and possible diffusion following
59 release from marine litter near the bottom.

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1. Introduction

63 Phthalates or phthalic acid ester (PAEs) with low volatility and low water solubility are
64 colorless and odorless. They are mainly produced by anthropogenic activity such as the manufacture
65 and processing of plastic products (Simoneit et al., 2005; Serôdio and Nogueira, 2006; Lyche et al.,
66 2009). Their annual production reached 8 million tons in 2011 (Net et al., 2015 and references therein)
67 and they represent approximately 70% of the world consumption of plasticizer in 2014 (CEH, 2015).
68 The structure of PAEs consists of a di-alkyl ester of *o*-benzenedicarboxylic acid. The most
69 commercially significant PAEs, sorted by increasing number of carbons in the ester side chains, are
70 dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), benzylbutyl
71 phthalate (BzBP), di-ethylhexyl phthalate (DEHP) and di-*n*-octyl phthalate (DnOP) the later being the
72 most abundant (Holahan and Smith, 2015). PAEs of lower molecular weight are used as essential
73 components of solvents, adhesives, waxes, pharmaceutical products, insecticide materials and
74 cosmetics, whereas PAEs of higher molecular weight are instead used to improve the flexibility and
75 handling of industrial materials (IARC, 2000; Halden, 2010). PAEs are mixed with the polymers and
76 are not covalently bound to them. During aging, they are likely to migrate out of the plastic and to be
77 released directly into the environment or inside an animal's stomach or tissue (Andrady, 2011).

78 As widely used compounds, PAEs are ubiquitous in terrestrial, marine and atmospheric
79 environments (Xie et al., 2007; Fu et al., 2013; Dargnat et al., 2009; Gao et al., 2014; Staples et al.,
80 1997; Net et al., 2014; 2015a-b; Wang et al., 2014). PAEs have been detected in surface seawater
81 (Turner and Rawling, 2000; Horn et al., 2004; Brossa et al., 2005), in indoor air (Becker et al., 2004),
82 in urban environments in soils (Ferreira and Morita, 2012), in urban lakes (Zeng et al., 2008), in
83 drinking water (Yang et al., 2014), in terrestrial and marine remote aerosols (Xie et al., 2006, 2007; Fu
84 et al., 2008, 2009, 2013), and in marine sediment, as well as in biota, including several aquatic species
85 (Wofford et al., 1981), algae (Gao and Chi, 2015), and the tissues of mammals (Staples et al., 1997)
86 and humans (Liou et al., 2014). PAEs can be degraded by some microalgal species (Gao and Chi,
87 2015), fish and shellfish (Munshi et al., 2013), bacteria (Chang et al., 2004) and intracellular and

88 extracellular algal enzymes (Gao and Chi, 2015) as well as by the combined action of UV and H₂O₂
89 (Xu et al., 2007). PAEs are of particular concern to humans since they are considered as emerging
90 environmental contaminants due to the endocrine disruption and carcinogenic effects detected in
91 laboratory animal experiments and in humans (Crisp et al., 1998; Latini, 2005; Kamrin, 2009; Meeker
92 et al., 2009).

93 The Mediterranean Sea is a semi-enclosed basin covering an area of 2.5 million km² with a
94 slow turnover time of ~ 80 years implying a sensitive response to anthropogenic impact (The Mermex
95 group, 2011). Urbanization has been growing particularly along the coastline, with the result of a
96 substantial modification of the coast and adverse effects on the quality of the environment (Pergent,
97 2012). There is a large range of industrial activities spread all along the Mediterranean basin, and a
98 number of highly industrialized spots that are concentrated mainly in the northwestern Mediterranean
99 Sea (Zorita et al., 2007). All these activities constitute a source of pollution through direct disposal,
100 continental runoff and atmospheric transport (UNEP Chemicals, 2002; The Mermex group, 2011 and
101 references therein). These industrial, agricultural and domestic activities generate a large volume of
102 wastewater, which provides nutrients, trace metals (Cossa et al., 2017, this issue) organic matter,
103 persistent organic pollutants (POPs) and related anthropogenic contaminants such as polycyclic
104 aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs)
105 (The Mermex group, 2011; Berrojalbiz et al., 2011; Guigue et al., 2011, 2014; Castro-Jiménez et al.,
106 2008, 2010, 2012) that impact the coastal marine ecosystems. The Mediterranean Sea is also impacted
107 by marine litter and microplastics (Deudero and Alomar, 2015; Cózar et al., 2015; Faure et al., 2015;
108 Pedrotti et al., 2016; Suaria et al., 2016 Schmidt et al., submitted), which can supply PAEs to
109 surrounding waters during their degradation (Andrady, 2011). Although PAEs have already been
110 detected in aquatic environments (Xie et al., 2005; Net et al., 2015), only a few studies have reported
111 the occurrence of individual phthalates in the Mediterranean Sea (Sanchez-Avila et al., 2012; Brossa et
112 al., 2005), and there is no study dealing with the distribution of a complete series of PAEs in the
113 Mediterranean basin, including the sea and rivers, mainly because of analytical difficulties.

114 Indeed, PAE analysis of environmental samples is a challenging task because of the low

115 concentrations, which is associated with the risk of contamination during sampling and processing in
116 relation with their ubiquity in the laboratory atmosphere, tools, glassware and various containers (Net
117 et al., 2015b and references therein). For environmental waters, several extraction procedures have
118 already been used, including liquid-liquid (EPA, 1996; Zeng et al., 2008a; Li et al., 2013; Gao et al.,
119 2014), solid-liquid (SLE), solid phase (SPE), magnetic (MSPE) dispersive (DSPE), solid phase micro
120 extraction (SPME) (Zhang et al., 2013; Liou et al., 2014; Yang et al., 2014) and high volume sample
121 techniques with PAD-2 resin columns and Soxhlet extractor (Xie et al., 2005). SPE appears to be an
122 appropriate technique for seawater because of its ease of implementation, its ability to save time and
123 solvent (compared to liquid-liquid extraction) and the decreased contamination (Zhang et al., 2013;
124 Liou et al., 2014; Yang et al., 2014; Net et al., 2015b). However, the SPE protocol uses a tube made of
125 polyethylene or polypropylene, which can release DiBP, DnBP and DEHP (Tienpont, 2004, Fasano et
126 al., 2012, Bonini et al., 2008; Paluselli unpublished results). This technique has been used by a great
127 number of reported protocols, concerning the type, amount and conditioning of the phase (Liou et al.,
128 2014; Sanchez-Avila et al., 2011-2012; Yang et al., 2014) and the sample volume as well as the
129 volume and type of solvent (He et al., 2013; Sanchez-Avila et al., 2011-2012; Yang et al., 2014; Liou
130 et al., 2014). Previous protocols have shown a recovery variability ranging from 50-60% to 120-150%
131 (Del Carlo et al., 2008; Wang et al., 2008; He et al., 2013, Sanchez-Avila et al., 2011), especially for
132 DEHP, which ranges from 60-80% (He et al., 2013) to 151% (Sanchez-Avila et al., 2011).

133 The objectives of this study are to examine, for the first time, the concentration levels and
134 distribution of a homologous series of PAEs in the coastal northwestern Mediterranean Sea. This
135 environmental study was associated with an optimization of a detection protocol for PAEs at trace
136 level in seawater. Based on previous studies (Liou et al., 2014; Gao and Chi, 2015; Yang et al., 2014),
137 conditions for the SPE extraction of PAEs have been modified and optimized for seawater. The
138 method we report here was tested in seawater spiked with authentic standards and then applied to
139 coastal and offshore Mediterranean seawater as well as to Rhone River freshwater in the framework of
140 the 'Particule –MERMEX' project.

141

142 **2. Experimental section**

143 **2.1 Materials and reagents**

144 The selected phthalates are dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl
145 phthalate (DPP), di-isobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), benzylbutyl phthalate
146 (BzBP), di-ethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP). The deuterated internal
147 standards were DEP-d4, DnBP-d4 and DEHP-d4. All native and labeled standards were of high purity
148 grade (> 98 %) and were purchased from Supelco (Bellefonte, USA). Stock solutions were prepared in
149 hexane and stored in the dark at 4 °C. Working solutions (unlabeled and labeled standards) were
150 prepared by dilution of these solutions to 20 mg/L. All solvents were glass-distilled grade and supplied
151 by Rathburn Chemicals Ltd. (Walkerburn, UK). Ultra-pure water was produced on-site by a Milli-Q
152 system, Millipore (Molsheim, France) with a specific resistivity of 18.2 MΩ.cm (25 °C) and a total
153 organic carbon content of < 2 ppb. The extractions were carried out in controlled air conditions in an
154 ISO class 6 cleanroom (temperature: 22 °C; SAS pressure: +15 Pa; SAS brewing rate: 30 vol/h; lab
155 pressure: +30 Pa; brewing rate: 50 vol/h). All material was produced in borosilicate glass and that was
156 previously kept in an acid bath overnight (10 % hydrochloric acid), rinsed with ultrapure water,
157 combusted at 450 °C for 6 hours and rinsed with methanol and dichloromethane just before use.

158 **2.2 Solid phase extraction (SPE)**

159 The protocol was adapted from that of Yang et al. (2014) and Liou et al. (2014) and optimized
160 for seawater in this study. The mentioned methods were modified in order to perform easy and rapid
161 analysis on a large-scale with low contamination and to improve the recovery. The amount of Oasis
162 HLB phase, the elution solvent type and volume, the sample volume, deuterated internal standards and
163 glass materials were considered to improve the recoveries. Extraction cartridges were prepared in our
164 laboratory with 200 mg of Oasis HLB sorbent (Waters Corporation, 30 µm, 100 g) in a precombusted
165 6 mL glass reaction tube equipped with methanol-precleaned Teflon frits (Supelco, Sigma-Aldrich).
166 SPE was conducted on a Visiprep vacuum manifold from Sigma-Aldrich (Saint Quentin Fallavier,
167 France). Before sample extraction, the SPE cartridges were sequentially cleaned three times with 5 mL
168 of acetone and 5 mL of dichloromethane. The cartridges were then conditioned with 5 mL of ethyl

169 acetate, 5 mL of acetone and 5 mL of ultrapure water prior to sample processing. Seawater samples
170 were spiked with DEP-d4, DnBP-d4 and DEHP-d4 at 50, 200 and 400 ng/L, respectively, and
171 percolated onto the cartridges under vacuum at a flow rate of 1-2 drops per second. After percolation,
172 the cartridge was washed with 5 mL of ultrapure water to remove the remaining salt and dried under
173 vacuum for 1 h. The retained phthalates were then eluted with 2 × 3 mL of ethyl acetate into
174 precombusted (450 °C, 6 hours) 10 mL vials. Ethyl acetate was then gently evaporated under a gentle
175 stream of nitrogen (purity > 99,995 %) at room temperature to a final volume of 200 µL. The final
176 samples were immediately closed with Teflon-lined screw caps, wrapped with Teflon ribbon and
177 stored in the dark at -20 °C until analysis.

178 **2.3 GC-MS analysis**

179 Analysis was performed using an Agilent Technologies 6850 GC system coupled to an Agilent
180 Technologies 5975C mass spectrometer (GC/MS) operated with electron impact ionization (70 eV).
181 Chromatographic separation was achieved on an Agilent HP-5MS capillary column (30 m × 0.25 mm,
182 0.25 µm film thickness) with the GC oven programmed at 70 °C for 1 minute and then up to 230 °C at
183 30 °C/min, to 250 °C at 8 °C/min and to 280 °C at 30 °C/min with a final isothermal hold for 7.50
184 min. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. The sample was manually
185 injected (2 µL) in splitless mode at an injector temperature of 250 °C. A Merlin Microseal injector
186 was used as a low carryover septum and a gas purifier (Charcoal, CP17972) was fitted on the carrier
187 gas line to prevent contamination during injection. Data were acquired in selected ion monitoring
188 (SIM) mode for increased sensitivity (SIM parameters given in Table 1) with a dwell time of 100 ms.
189 Two fragment ions were monitored per compound, with the most abundant ion used for quantification,
190 except for DMP, DPP, DiBP and DnBP, which were ionized in a unique fragment. Data were collected
191 and analyzed with the Agilent ChemStation software. The PAEs were quantified by isotopic dilution
192 of 3 deuterated PAEs. The use of internal standard homologues to the target molecules has been shown
193 to improve the recoveries by accurately compensating for loss of analyte during sample treatment
194 (Aminot et al., 2015). The response factors were assessed for each analytical sequence from freshly
195 prepared standards.

196

197 Table 1. Retention time and ions for selected ion monitoring (SIM) of PAEs by GC/MS.

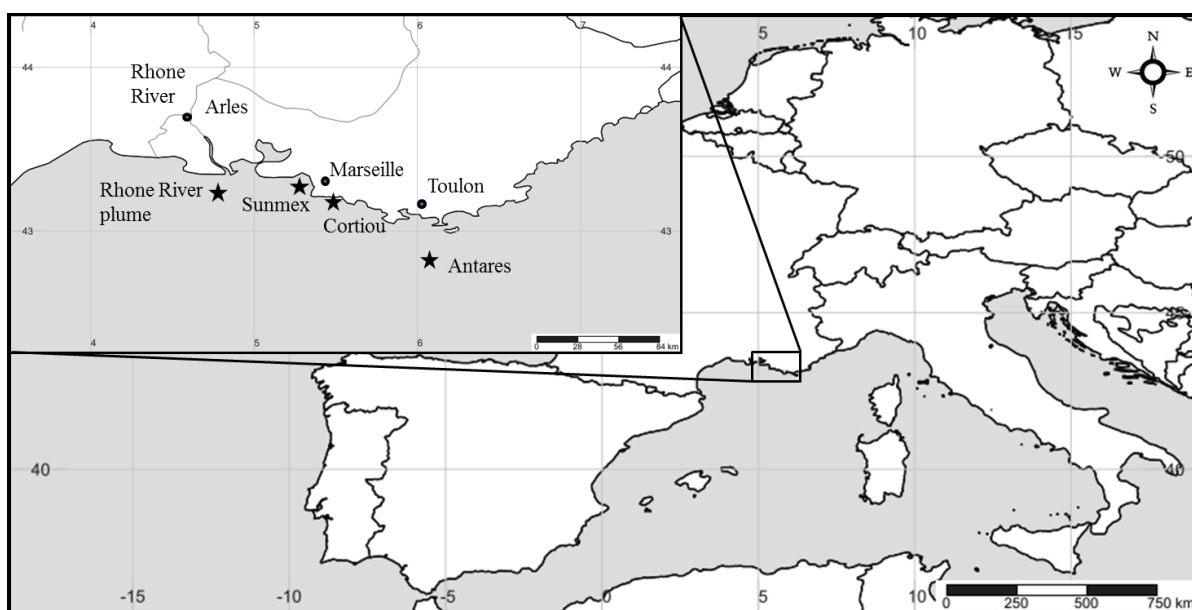
Compound	Molar Mass (g mol⁻¹)	Retention Time (min)	Quantifier ion (abundance)	Qualifier ion (abundance)
DMP	194.2	5.55	163 (100)	194, 135, 77
DEP	222.2	6.12	149 (100)	222, 121
DPP	250.0	6.84	149 (100)	209, 191
DiBP	278.4	7.22	149 (100)	223, 205
DnBP	278.4	7.65	149 (100)	223, 205
BzBP	312.4	9.72	149 (100)	206, 238
DEHP	390.6	10.62	149 (100)	279, 167
DnOP	390.6	11.76	149 (100)	279
DEP-d4	226.2	6.11	153 (100)	181
DnBP-d4	282.4	7.64	153 (100)	209
DnBP-d4	282.4	7.64	153 (100)	209
DEHP-d4	394.6	10.61	153 (100)	171, 283

198

199 **2.4 Application to seawater and freshwater samples**

200 Seawater samples were collected from SUNMEX station in the northwestern Mediterranean
201 Sea, Bay of Marseilles (43°18' N, 05°22' E) in October 2014 and offshore at 'Antares station' (42°
202 47'856 N; 6° 04'395 E) in March 2015 (Fig. 1), with a 12 L GO-Flo bottle previously rinsed with 1%
203 hydrochloric acid and ultrapure water to avoid contamination. The Go-Flo bottle tested with Milli-Q
204 water showed only trace levels of PAEs which were not subtracted from our dissolved PAEs
205 concentrations in this study. Immediately after collection, seawater samples were transferred into 5 L
206 precombusted glass bottles. Additional samples were directly collected in the Rhone River and Rhone
207 River plume in March 2014 using 5 L precombusted glass bottles. General information on the
208 Marseilles Bay, the Antares station and the Rhone River were given by Sempéré et al. (2015), Martini
209 et al. (2016) and Sempéré et al. (2000), respectively. Less than two hours after collection, the samples
210 were filtered through precombusted (450 °C for 6 h) GFF filters (47 mm filter diameter, rinsed with 2
211 L of Milli-Q water and 150 mL of sample prior to filtration) in a precombusted glass apparatus directly
212 in a filtered-air clean laboratory. Milli-Q water rinse limit additional contamination (Yoro et al., 1999).

213 After filtration, duplicate subsamples (10 mL) were poisoned with sulfuric acid to approximately pH 2
214 to avoid any biological activity and stored in the dark in precombusted Teflon-capped glass vials for
215 dissolved organic carbon (DOC) analysis. DOC concentrations were measured using a Shimadzu
216 TOC-5000 carbon analyzer (Sempéré et al., 2008). Some seawater samples (Bay of Marseilles) were
217 spiked with authentic PAEs (from 5 to 800 ng/L final concentration) to perform recovery experiments.
218 All the seawater analyses were conducted in duplicate in the laboratory in Marseilles.



219
220 Figure 1. Area of study. The map shows the sampling stations: SUNMEX in the Bay of Marseilles
221 (October 2014), offshore ‘Antares station’ (March 2015), Arles in Rhone River and the river plume
222 (March 2014).

223

224

225 3. Results and Discussion

226 3.1 Extraction of PAEs in seawater samples (optimization of the method)

227 Numerous factors are known to significantly influence the efficiency of SPE extraction of
228 phthalates from water. The most influential factors are elution the solvent, sample volume and sorbent
229 mass. To increase the recovery, the elution solvent, sample volume and sorbent mass were studied in
230 order to optimize efficient and reliable conditions for SPE extraction of seawater samples.

231

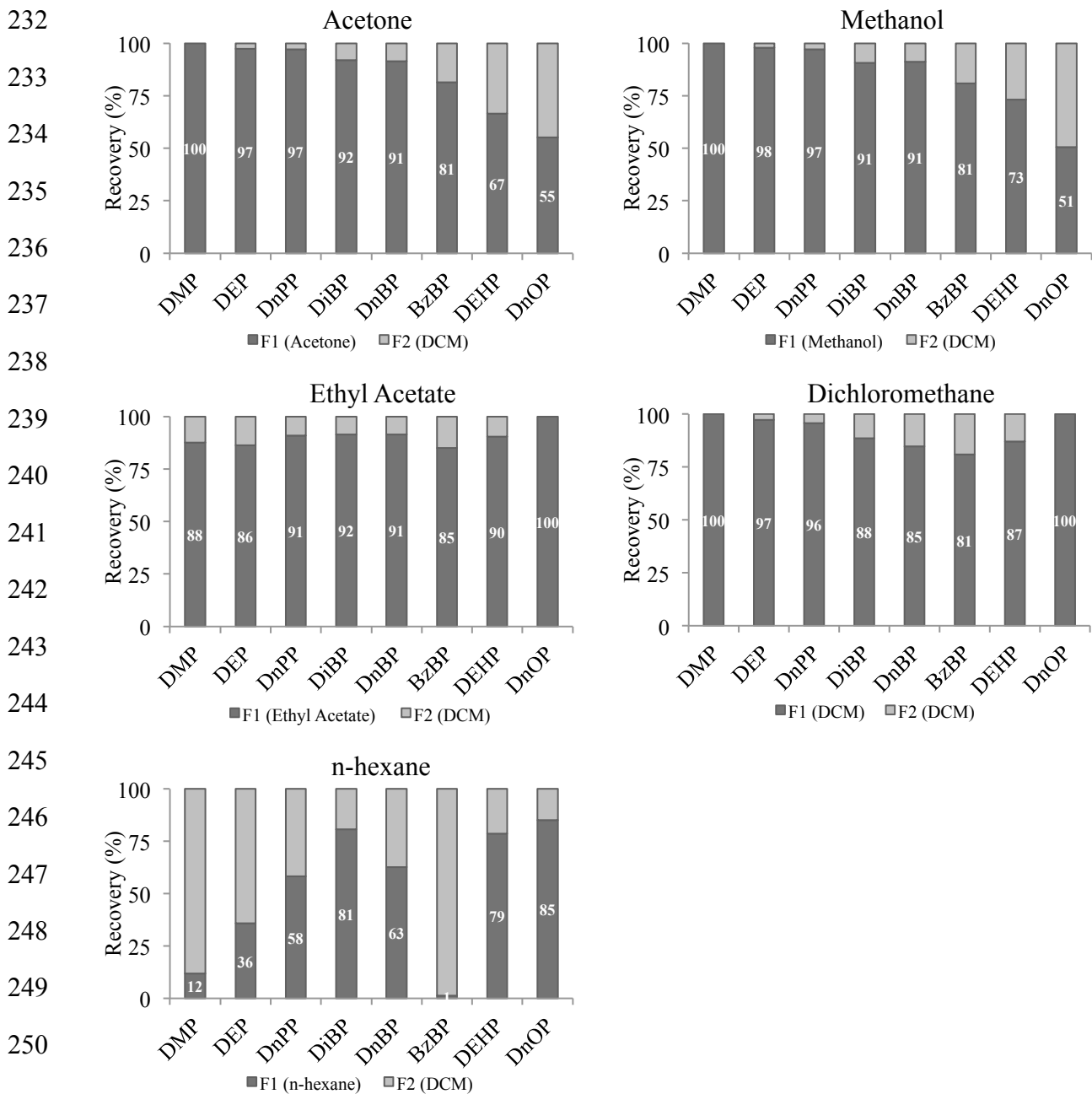


Figure 2. Recovery of PAEs obtained in two elution fractions carried out with five different organic solvents (F1) and dichloromethane (F2).

a) Elution solvent effect

First, 10 μ L of 20 mg/L PAE solution was passed through the cartridges, and two fractions of elution were collected. The first fraction was eluted with 5 mL of either acetone, dichloromethane, ethyl acetate, methanol or n-hexane, and the second fraction was systematically eluted with 5 mL of dichloromethane in order to elute the remaining PAEs off the cartridge. Internal standards were spiked directly in the eluted fraction. The repartition of PAEs between the two fractions is given in Fig. 2.

261 Acetone, dichloromethane, ethyl acetate and methanol yielded acceptable recoveries ranging from 55
262 to 100 % while n-hexane was satisfactory only for DEHP and DnOP (79-85 %), with 12 % for DMP,
263 36 % for DEP and 1 % for BzBP. n-Hexane is a better solvent for aliphatic compounds, unlike DMP,
264 DEP (short carbon chain) and BzBP (higher aromaticity).

265 Acetone and methanol showed a similar elution strength with recoveries of 80-100 % for
266 DMP, DEP, DPP, DiBP, DnBP and BzBP in the first fraction and 70 % and 50 % for DEHP and
267 DnOP, respectively. This result is consistent with the studies of Liou et al. (2014) and Yang et al.
268 (2014), which performed the elution with 6 mL of methanol. Dichloromethane and ethyl acetate both
269 indicated high recoveries for all PAEs in the first fraction and were the most appropriate elution
270 solvents. Dichloromethane was the most appropriate elution solvent for DMP (100 %), DEP (98 %)
271 and DPP (97 %), whereas ethyl acetate was the most appropriate for the elution of DiBP (91 %),
272 DnBP (92 %), BzBP (85 %) and DEHP (90 %). DnOP was efficiently eluted (100 %) with both
273 solvents. Previously, published protocols used 10 mL of dichloromethane/n-hexane (He et al., 2013) or
274 10 mL of acetone/dichloromethane (Sanchez-Avila et al., 2011; 2012) as the elution solvent. When
275 comparing their recoveries and those from our developed protocol, the combination of
276 dichloromethane with acetone seems to be more effective for PAE elution than its combination with n-
277 hexane. In these protocols, the ability to elute PAEs from the cartridges was improved by increasing
278 the elution solvent volume to 10 and 20 mL, respectively. According to our results, ethyl acetate is the
279 best solvent for PAE elution.

280 *b) Seawater volume and sorbent mass effects*

281 As high SPE enrichment factors are required to compensate for the likely low PAE levels in
282 seawater, it is important to ensure that the analytes do not breakthrough the sorbent at high percolation
283 volumes. Increasing volumes (100 mL, 200 mL, 500 mL and 1000 mL) of seawater collected at
284 Sunmex station at a 1.5 m depth were spiked with 10 µL of 20 mg/L PAEs and percolated through
285 either 200 mg (Liou et al., 2014) or 400 mg of sorbent. For each experimental condition, two
286 additional samples were kept unspiked and were processed in the same conditions (blank samples).
287 The recoveries were calculated as the difference between the concentration in the spiked and unspiked
288 samples. In this experiment, the deuterated internal standards DEP-d4, DnBP-d4 and DEHP-d4 were

289 spiked after the elution of the solvent. The recoveries of the extractions obtained with 4 different
 290 volumes of spiked seawater for 200 and 400 mg of HLB sorbent are given in Table 2.

291

292 Table 2. Recoveries of PAE extractions obtained with 4 different volumes of spiked seawater and 200
 293 and 400 mg of HLB sorbent.

294

Compounds	Recoveries (%)							
	200 mg				400 mg			
	100 mL	200 mL	500 mL	1000 mL	100 mL	200 mL	500 mL	1000 mL
DMP	99	108	100	105	114	107	109	110
DEP	61	93	88	89	95	93	95	100
DPP	105	117	113	120	122	121	119	114
DiBP	96	122	111	123	121	100	107	115
DnBP	79	75	63	66	136	77	67	82
BzBP	102	125	94	106	126	104	87	97
DEHP	14	13	8	10	11	7	21	6
DnOP	8	10	15	10	10	6	24	6

295

296

297 The volume of the sample does not significantly affect the recovery of most of the PAEs,
 298 indicating the absence of breakthrough. Only DEP and DnBP were marginally affected by increasing
 299 sample volumes. The recovery of DEP increased from 60 % with 100 mL to 93 % with all other
 300 volumes on 200 mg of sorbent. However, the recovery was stable between 93 and 100 % on 400 mg.
 301 DnBP showed the opposite trend, where the recovery decreased from 75-79 % with 100-200 mL to 63-
 302 66 % with 500-1000 mL on both 200 mg and 400 mg of sorbent. Acceptable recoveries (93-120 %)
 303 were determined for DMP, DPP, DiBP and BzBP under all experimental conditions. The two most
 304 hydrophobic PAEs DEHP and DnOP exhibited lower recoveries regardless of the experimental
 305 conditions, attributed to their potential for adsorption onto glassware. Such losses were corrected for
 306 by the use of deuterated DEHP as an internal standard (Table 3).

307 Above all, the volume of the sample did not significantly influence the performance of the
 308 HLB sorbent toward PAEs. Similar results were observed for the sorbent mass. The DnOP
 309 concentration in seawater is expected to be low, indicating the need to increase the enrichment factor

310 by extracting 1 L of sample. BzBP is also characterized by low concentrations in seawater and by
311 relatively high instrumental detection limits, confirming the need for a large volume of sample. As the
312 differences between 200 and 400 mg of sorbent did not justify the use of double the amount of sorbent,
313 200 mg of sorbent was selected to reduce the price and time for a single extraction. Previously,
314 published protocols reported the use of 1 L of matrix, but different amounts of solid phase, from 200
315 mg (Liou et al., 2014) to 500 mg (Yang et al., 2014). According to the results, our optimized
316 conditions were 200 mg of HLB phase prepared in a combusted glass tube, 1 L of sample volume, and
317 6 mL of ethyl acetate as the elution solvent, split in two steps.

318

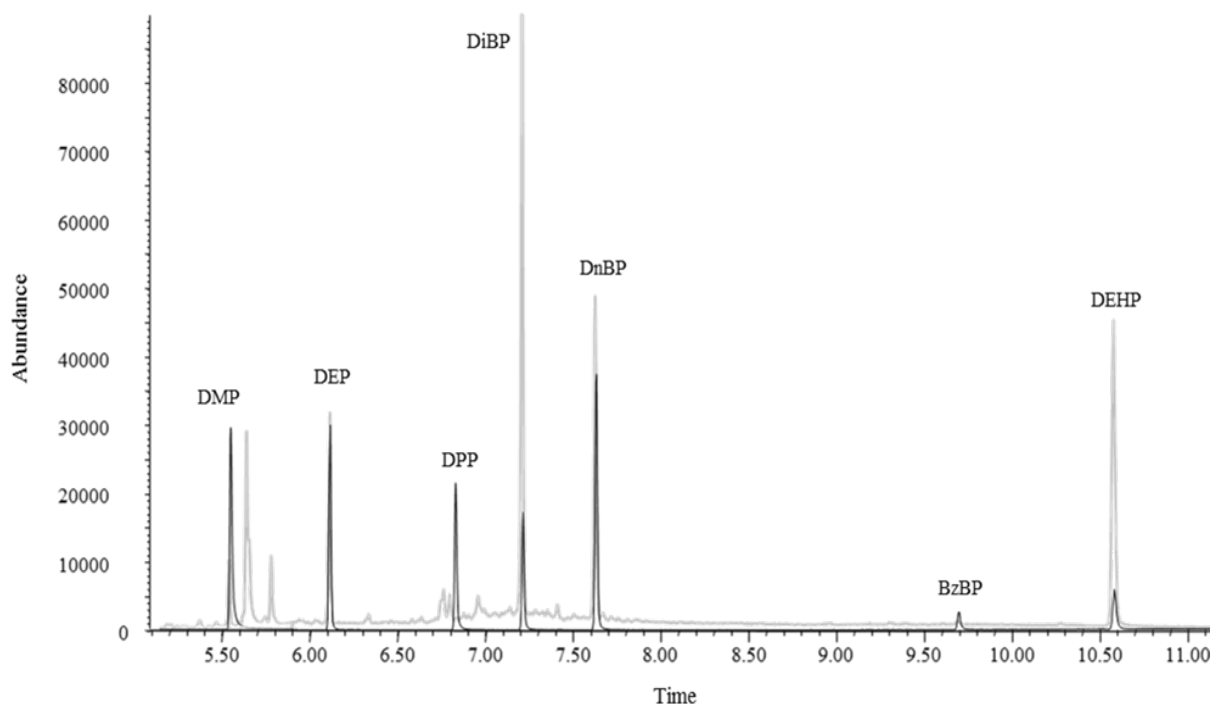
319 **3.2 Method validation**

320 *Blank, response linearity and extraction validation*

321 Procedural blank samples were examined by passing dry air and ethyl acetate directly through
322 the precleaned cartridge and measuring the PAEs directly in the elution solution. Linearity was
323 investigated over an eight-point calibration ranging from 50 to 6000 pg injected. Instrumental
324 repeatability was measured through the relative standard deviation of 10 injections of the same
325 standard solution, corresponding to 100 pg injected, whereas instrumental detection limits (IDLs) were
326 extrapolated for a signal-to-noise ratio of 3. The noise region was equivalent to the width of the peak
327 before the peak, and the noise value was taken as the average height (“RMS” mode). The recoveries
328 were calculated from the spiked seawater samples (10 μ L of 20 mg/L of PAEs in 1 L) after subtraction
329 of the concentrations measured for the unspiked seawater samples (n=2). Such experiments were
330 repeated with half the spiked concentration (5 μ L of 20 mg/L of PAEs) in seawater samples (n=2) to
331 study the influence of the concentration level.

332 The method was applied to 1 L seawater samples to minimize contamination and to allow the
333 detection of low concentrations of BzBP and DnOP in seawater. The samples were passed through 200
334 mg of HLB sorbent, and the cartridges were eluted twice with 3 mL of ethyl acetate. A typical gas
335 chromatogram of the PAEs isolated from the seawater samples collected in the Marseilles Bay is given
336 in Figure 3. Identification of the PAEs was confirmed by GC/MS. Despite the precautions taken to
337 prevent contamination, DEP, DiBP and occasionally DnBP were detected in the blanks at levels that

338 remained below 0.4-2 %, 2-3 % and 0-4 % of the masses measured in different seawater samples,
339 respectively. The average recoveries of PAEs spiked in the seawater samples (Table 3) showed
340 acceptable recoveries for all the phthalates, ranging from 97 % of DEP to 110 % of DiBP. The high
341 recoveries obtained at low spiking levels also validated the appropriate performance of the protocol.
342 To assess the influence of the concentration level on the recoveries, 1 L aliquots of seawater collected
343 in the Bay of Marseilles were spiked at increasing concentration levels (standard addition technique).
344 Different ranges of spiked concentrations were used depending on the initial concentration of the
345 compound in seawater (from 5 to 40 ng/L for DMP, DEP, DPP, BzBP and DnOP and from 40 to 800
346 ng/L for DiBP, DiBP and DEHP). Excellent correlation coefficients ($R^2 > 0.96$) and recoveries
347 (between 95 and 116 %) indicate that the method is linear and accurate for all compounds, including
348 DEHP ($R^2=0.987$; $n=7$) and DnOP ($R^2=0.962$; $n=5$), in a range of environmentally realistic
349 concentrations.



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351 Figure 3. Typical capillary gas chromatogram of PAEs (DMP, DEP, DiBP, DnBP, BzBP and DEHP)
352 isolated from the seawater sample (grey line) collected from the Marseilles Bay (northwestern
353 Mediterranean Sea) in October 2014 and typical PAEs standard solution chromatogram (black line, 0.1
354 mg/L). The identification of PAEs was confirmed by GC/MS. The mass spectra of the PAEs (not
355 presented here) were in accordance with those published in the literature. DPP and DnOP were not
356 detected in this sample.

357 Table 3. Statistical data of the linear regression between the spiked concentration of PAEs and the
 358 measured peak areas. PAE recovery was reported.

Compound	spiked concentration (ng L ⁻¹)	n	R ²	slope (μVs/ng L ⁻¹)	y-intercept (μVs)	Recovery (%)	MDL (ng/L)
DMP	0, 5, 10, 20, 40	5	0.98	11022	20671	101.2	0.11
DEP	0, 5, 10, 20, 40, 100	6	0.99	13978	40049	98.1	0.21
DPP	0, 5, 10, 20, 40	5	0.98	12072	2893	115.5	0.31
DiBP	0, 40, 100, 200,400,600,800	7	0.99	11006	5477511	110.4	0.23
DnBP	0, 40, 100, 200,400,600	6	0.99	19424	1965660	99.7	0.28
BzBP	0, 5, 10, 20, 40	5	0.99	2718	1845	98.7	1.67
DEHP	0, 40, 100, 200,400,600,800	7	0.99	1638	23076	103.8	0.93
DnOP	0, 5, 10, 20, 40	5	0.96	268	323	95.0	2.73

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362 3.3 Concentration of PAEs in the northwestern Mediterranean Sea and Rhone River

363 All PAEs, excepted DPP and DnOP, were detected in the Mediterranean seawater and Rhone
 364 River samples (Table 4). We found that offshore (Antares site), the concentration of total PAEs ranged
 365 from 75.3 ng/L at the surface to 310.2 ng/L in the deepest water, from 244.5 ng/L to 1207.1 ng/L a few
 366 meters above the bottom in the Marseilles Bay and from 182.2 to 212.6 ng/L in the Rhone River
 367 plume and close to the Marseilles Cortiou water treatment plant outlet. Although particulate PAEs
 368 were not measured in this study, previous study indicated that DnBP, BzBP and DEHP particulate
 369 concentration may represent the 2, 29 and 43 %, respectively, of the dissolved concentration in coastal
 370 seawater (North Sea, Xie et al., 2005). Very limited data are available for the occurrence and fate of
 371 dissolved PAEs in marine water, especially in the Mediterranean Sea. DEHP concentrations detected
 372 in the seawater samples in our study were in the range of 50.7-296.5 ng/L. These concentrations were
 373 in the same range reported by Sanchez-Avila et al. (2012) for Catalan coastal water (Spain, NW
 374 Mediterranean Sea) and by Turner and Rowling (2000) for the south coast of the UK, respectively,
 375 with range of 31-617 ng/L and 99-2200 ng/L. However, our results were 10 times lower than those
 376 reported by Brossa et al. (2005) for the Spanish coast. Lower concentrations have been only reported
 377 for North Sea with concentration ranges of 0.5-5.3 ng/L for DEHP and 0.02-4.0 ng/L for DMP and
 378 DEP (Xie et al., 2005).

379 Table 4. PAEs concentrations detected in different Mediterranean Sea samples: Surface Rhone River
 380 and associated Plume (March, 2014), Bay of Marseilles (October, 2014) and surface 'Cortiou' station
 381 (November, 2014).
 382

Compounds	Rhone	Rhone River Plume			Bay of Marseilles				Cortiou
	River								
	S=0	S=10	S=18	S=28	1.5 m	5 m	15 m	30 m	
DMP	5.7	5.7	4.8	2.7	1.8	1.7	1.4	6.4	7.3
DEP	30.5	29.0	30.2	16.4	12.7	6.9	7.1	50.0	33.5
DPP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiBP	125.9	48.6	67.0	136.4	215.7	56.5	77.0	383.4	81.7
DnBP	40.5	21.8	21.7	22.8	87.0	63.4	138.9	466.0	28.8
BzBP	5.4	2.7	2.6	2.5	3.5	3.2	4.0	4.8	5.1
DEHP	406.8	101.2	55.9	39.2	103.5	112.8	102.9	296.5	56.2
DnOP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	615.1	209.0	182.2	220.0	424.2	244.5	331.3	1207.1	212.6

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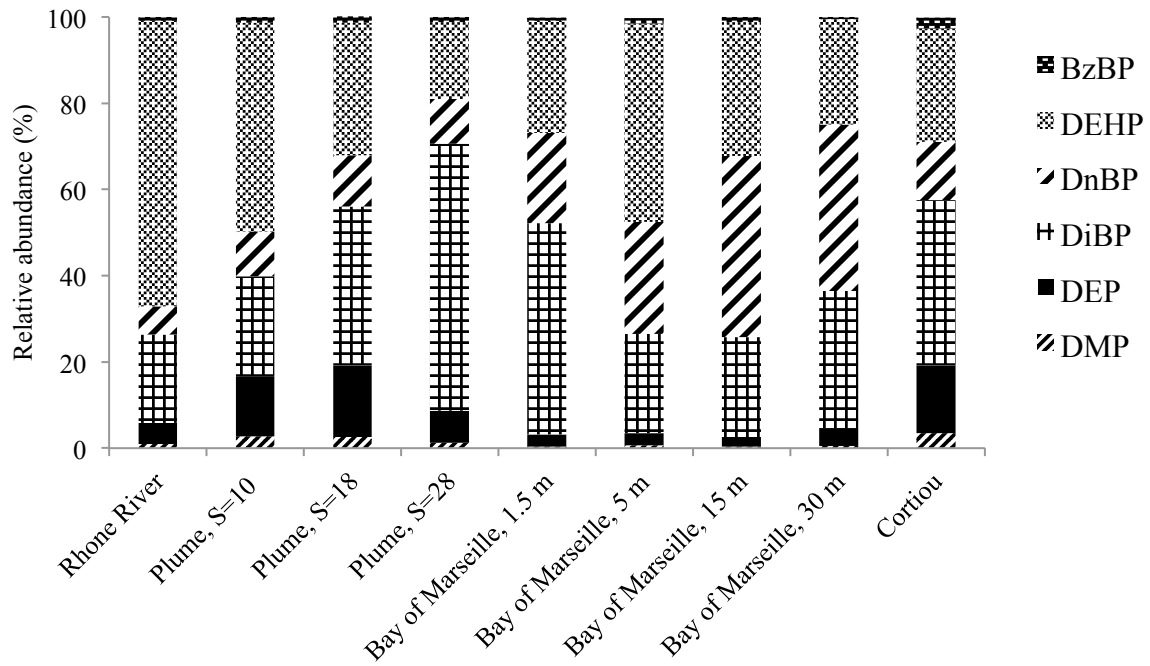
In Marseilles Bay, the samples were characterized by high concentrations of PAEs in the surface waters, a slight decrease with the depth, probably because of microbial degradation, and then an increase close to the bottom (Table 4). DiBP was the most abundant at the surface (47.3 % of total PAEs) followed by DEHP (22.7 %) and DnBP (19.1 %), whereas DnBP was predominant (38.6 %) followed by DiBP (31.8 %) and DEHP (24.6 %) a few meters above the bottom. DMP, DEP and BzBP were detected as 0.5, 2.5 and 0.8 % of total PAEs, respectively (Table 5; Figure 4). The high concentration at the bottom suggests the resuspension of PAE-rich sediments. As marine litter has already been reported to be abundant near the bottom (M I O unpublished observations) of the Marseilles Bay, it is likely that PAEs in the surficial sediment were released during plastic debris aging through physico-chemical and biotic processes (Bakir et al., 2014). This seems to be more evident for DnBP, whose relative fraction showed a stronger variation. Different PAEs are usually used by industry during plastic polymer production in order to increase flexibility, transparency, durability and longevity of the different materials produced (Net et al., 2015b, and reference therein). DEHP and DnBP are used as plasticizer for soft PVC, PVA and rubber as well as fixative in paint and especially DnBP is essentially used for its viscosity reducing properties and compatibility with non-PVC mixture (ECHA, 2013). It is likely that the PAEs detected above the bottom depend on the type

401 of material that accumulates in the sediment after sinking. In addition, microplastics could come from
 402 larger plastic debris that degrades into smaller pieces and microbeads, a type of microplastic that are
 403 added as exfoliants to health and beauty products and easily pass through water filtration systems and
 404 end up in the sea, could transfer attached pollutants and additive chemicals (Browne et al., 2007) in the
 405 water column. At Cortiou station, near the Marseilles wastewater treatment plant, the surface water
 406 PAE concentration averaged at 212.6 ng/L (sd \pm 3.4) (Table 4), detected 5-6 m from the Cortiou water
 407 treatment plant outlet, which was quite low during the studied period. DiBP was the most abundant
 408 PAE (38.4 %), followed by DEHP (26.4 %), DEP (15.7 %) and DnBP (13.5 %), with DMP and BzBP
 409 representing minor fractions (3.5 and 2.5 %, respectively) (Table 5; Figure 4). The relative abundance
 410 was not very different from what we detected in Marseilles Bay, with the only exception of DEP.

411
 412 Table 5. PAE relative abundances (%) detected in different Mediterranean Sea samples: Surface
 413 Rhone River and associated Plume (March, 2014), Bay of Marseilles (October, 2014) and surface
 414 'Cortiou' station (November, 2014).

Compound	Rhone River	Rhone River Plume			Bay of Marseilles				Cortiou
	S=0	S=10	S=18	S=28	1.5 m	5 m	15 m	30 m	
DMP	0.9	2.7	2.6	1.2	0.4	0.7	0.4	0.5	3.4
DEP	5.0	13.9	16.6	7.5	2.8	2.8	2.1	4.1	15.7
DPP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DiBP	20.5	23.3	36.8	62.0	47.3	23.1	23.3	31.8	38.4
DnBP	6.6	10.4	11.9	10.4	19.1	25.9	42.0	38.6	13.5
BzBP	0.9	1.3	1.4	1.1	0.8	1.3	1.2	0.4	2.4
DEHP	66.1	48.4	30.7	17.8	22.7	46.0	31.1	24.6	26.4
DnOP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

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 419 Figure 4. PAE relative abundances (%) detected in different Mediterranean Sea samples: Surface
 420 Rhone River and associated Plume (March, 2014), Bay of Marseilles (October, 2014) and surface
 421 ‘Cortiou’ station (November, 2014).
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423 Five PAEs were detected offshore in the ‘Antares station’ water column (Figure 5-6; Table 6).
 424 DnBP was characterized by a large range of concentrations (8.2-225.5 ng/L), which reached a
 425 maximum in deep water (Figure 6). With the exception of DnBP, the vertical profiles seem to have
 426 similar trends. The surface samples were characterized by high concentrations that slightly decrease
 427 down to 200-500 m. Then, the concentration started to increase down to 1500 m, where the
 428 concentration was similar to that detected in the surface samples. The DnBP vertical profile was
 429 characterized by the same trend in the first meter of the water column, decreasing from 13.4 to 8.2
 430 ng/L, but it was followed by a strongly increasing concentration in the deepest samples (87.3-225.5
 431 ng/L). DEHP was the dominant species in the first 50 m (66.9-76.7 % of total PAEs) followed by
 432 DnBP (10.9-15.2 %). Between 100 and 250 m, the relative abundance of DEHP was slightly lower
 433 (56.9-65.4 %), whereas that of DnBP was higher (25.8-28.5 %) up to the maximum salinity and
 434 minimum temperature. Under 500 m, DnBP was the most abundant PAE (57.0-72.6 %) followed by
 435 DEHP (20.1-33.1 %). DiBP, DMP and DEP were detected as minor fractions of the total PAEs
 436 detected in offshore waters, representing 7.9, 5.7 and 4.4 % of total PAEs at the surface and 3.5, 2.3
 437 and 1.5 % in deepest waters, respectively (Fig. 5; Tab. 6). Note that this offshore station area is

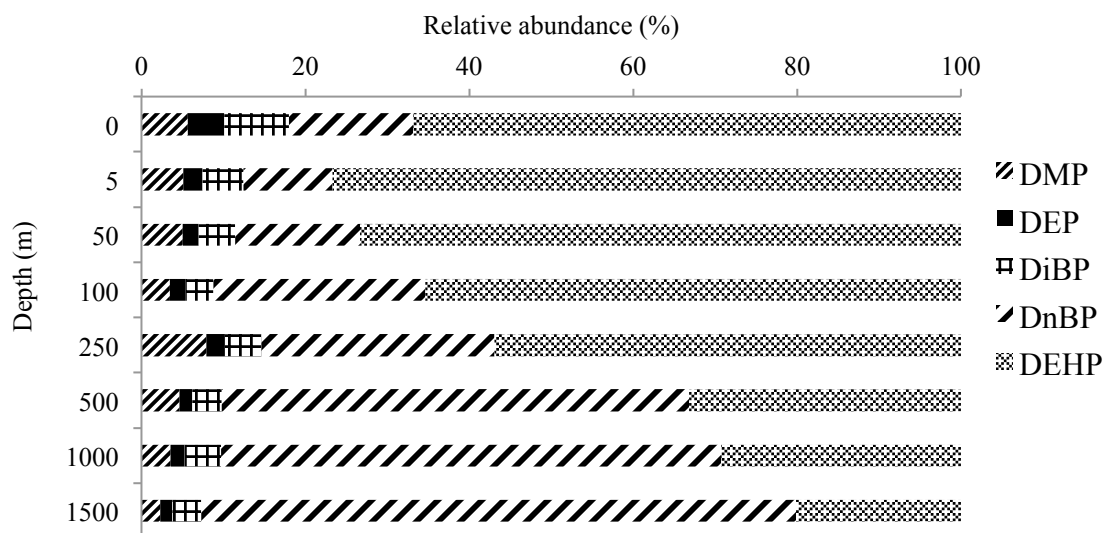
438 influenced by the shallow depth Northern Current (NC) with high seasonal variability (Milot, 1991),
 439 which may penetrate into the shelf of the Gulf of Lions (Barrier et al., 2016 and references therein).

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441 Table 6. PAE relative abundances (%) detected in offshore Antares station-samples collected at the
 442 surface and at depths of 5, 50, 100, 250, 500,1000 and 1500 m in March 2015.

Compounds	Depth (m)							
	0	5	50	100	250	500	1000	1500
DMP	5.7	5.1	5.0	3.5	7.9	4.6	3.5	2.3
DEP	4.4	2.3	2.0	1.9	2.3	1.6	1.7	1.5
DiBP	7.9	5.1	4.5	3.5	4.4	3.6	4.5	3.5
DnBP	15.2	10.9	15.2	25.8	28.5	57.0	61.0	72.6
DEHP	66.9	76.7	73.3	65.4	56.9	33.1	29.2	20.1

443



444 Figure 5. PAE relative abundances (%) detected in offshore Antares station-samples collected at the
 445 surface and at depths of 5, 50, 100, 250, 500,1000 and 1500 m in March 2015.

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449 This water originating eastward from the Italian coasts may carry plastic debris (Schmidt et
 450 al., submitted) and dissolved PAEs. The decrease in concentration in the first meter of the water
 451 column could be explained by biodegradation processes. The increase in concentration at 200-500 m
 452 suggests the production of PAEs from the aging of plastic debris, as was observed in Marseilles Bay.
 453 Indeed, the behavior of DnBP in the deepest water seems to reflect the same production observed close

454 to the bottom of the Marseilles area. These data were the first attempt to investigate the vertical
 455 distribution of PAEs in the water column.

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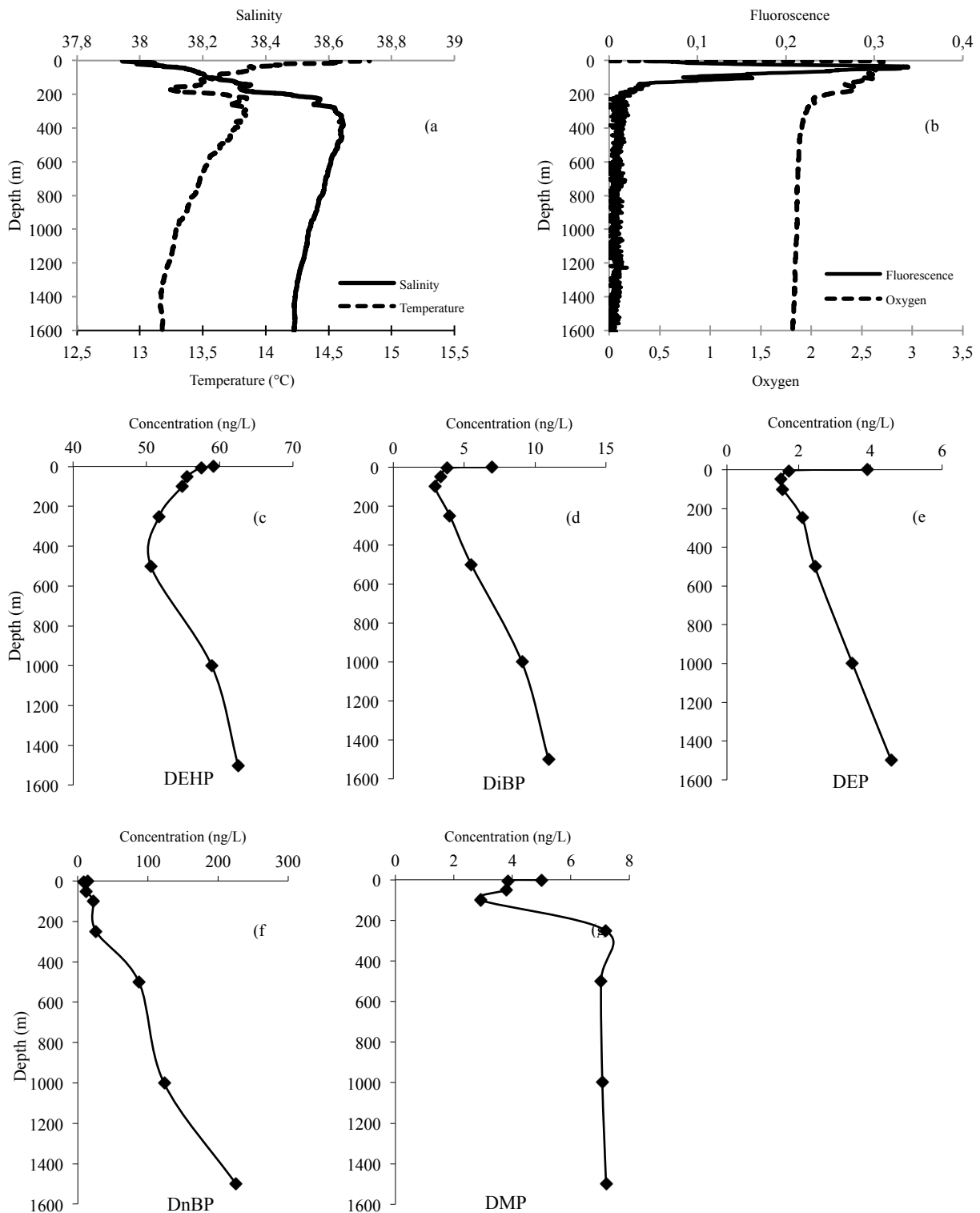


Figure 6. Vertical profile (0-1500 m) of 5 PAEs, along with salinity, temperature (a), fluorescence and oxygen (b) detected at offshore Antares station in March 2015. The compounds detected were: (c) diethylhexyl phthalate (DEHP), (d) di-iso-butyl phthalate (DnBP), (e) diethyl phthalate (DEP), (f) di-n-butyl phthalate (DnBP) and (g) dimethyl phthalate (DMP).

494 *Rhone River and river mouth*

495 The total PAE concentrations ranged from 615.1 ng/L to 182.2-220.0 ng/L in the Rhone River plume
496 (Table 4) suggesting that the Rhone is clearly a source of PAEs in the coastal area. Previous studies
497 dealing with individual PAEs reported DMP concentrations in the range of 2.5 – 5.5 ng/L (Sanchez-
498 Avila et al., 2012) in six rivers in Spain (Muga, Fluvià, Ter, Besòs, Llobregat and Ebro River). DEP
499 was detected in different studies in the Seine River (France) in a range slightly higher than our data,
500 from 46-208 ng/L (Teil et al., 2007) to 71-181 ng/L (Dargnat et al., 2009), as well as 52-284 ng/L in
501 Spain (Sanchez-Avila et al., 2012). BzBP was also reported at low concentration in the range of 5-23
502 ng/L (Dargnat et al., 2009; Sanchez-Avila et al., 2012). Dargnat et al. (2009) and Teil et al. (2007)
503 found similar concentrations of DEHP, 161-314 ng/L and 323-779 ng/L, respectively, in the Seine
504 River. We found that DEHP was the most abundant PAE (66.1 %) in the Rhone River followed by
505 DiBP (20.5 %) and DnBP (6.6 %), whereas DMP, DEP and BzBP represented the smallest fractions
506 (Table 5; Figure 4). The relative abundance of DEHP decreased from 66.1 % of the total PAEs in the
507 river (S=0) to 48.4, 30.7 and 17.8 % in the plume stations (S=10-28). The opposite trend was observed
508 for all other PAEs, especially for DnBP and DiBP, with DnBP values increasing from 6.6 to 11.9 %
509 and from 20.5 to 62.0 % for DiBP. A similar trend was also observed for DMP and DEP. Changes in
510 the relative abundance from the river to the plume could be due to different selective bacterial
511 degradation and/or photodegradation processes occurring in the salty water. Only DiBP showed, after
512 the concentration first decreased probably due to dilution effect, an increase in concentration and in
513 relative abundance. This could be caused by some atmospheric input of this compound as it was
514 already supposed in the Bay of Marseilles.

515

516 **4. Conclusion**

517 A reliable analytical technique for measuring PAEs in natural seawater samples at trace levels
518 was slightly improved and allowed determination of PAEs in seawater. This technique consists of the
519 adsorption on PAEs from acidified seawater samples onto 200 mg Oasis HLB glass cartridges

520 followed by elution with ethyl acetate and determination by GC/MS. The recoveries of the PAEs from
521 spiked seawater samples were found to be between 95-115 %, and the contamination was strongly
522 reduced by using a number of precautions. Our results showed that the Rhone River was a source of
523 PAE in estuary and in coastal area. Although DEHP was predominant in the river, its relative
524 abundance decreased with increasing salinities, DiBP being predominant in the plume for a salinity of
525 28. We reported, for the first time, the PAE vertical profile (0-1500 m) from the offshore water, which
526 had lower concentrations compared to the Bay of Marseilles. We observed that DEHP was also
527 predominant in surface marine waters including offshore (0-250 m) and below the surface in Marseille
528 Bay (5m). The opposite trend was found for DnBP for which concentrations significantly increased in
529 mesopelagic waters. High concentrations of DnBP, DEHP and DiBP were detected close to the bottom
530 (30 m) of the Bay of Marseilles. Such feature might be related to diffusion of PAEs released from the
531 sediment surface very likely induced from plastic debris degradation and previously accumulated in
532 the bottom. Precise origin of such material still needs to be determined. Other processes such as
533 photochemical oxidation reactions in surface waters, bacterial degradation, are certainly important
534 processes that regulate as well the distribution of PAEs in the water column. High PAE concentrations
535 found in coastal area near Marseilles city strongly suggest that these organic compounds are very
536 likely abundant in the whole Mediterranean that is surrounded by urbanized areas and that is submitted
537 to relatively high inputs of rivers and atmospheric deposition compared to the global Ocean.

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