1 **This is the accepted manuscript version of the article:**

2 A holistic assessment of the sources, prevalence, and distribution of bisphenol A and

3 analogues in water, sediments, biota and plastic litter of the Ebro Delta (Spain) by Gil-Solsona,

4 R.; Castaño-Ortiz, J.M.; Muñoz-Mas, R.; Insa, S.; Farré, M.; Ospina-Alvarez, N.; Santos,

5 L.H.M.L.M.; García-Pimentel, M.; Barceló, D. and Rodríguez-Mozaz, S. *Environmental Pollution*

6 (2022) 314, 120310

7 **available online at https://doi.org/10.1016/j.envpol.2022.120310**

8 **A holistic assessment of the sources, prevalence, and distribution of bisphenol A and**

9 **analogues in water, sediments, biota and plastic litter of the Ebro Delta (Spain)**

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Abstract

Bisphenol A (BPA) is one of the main ubiquitous compounds released from plastics in the environment. This compound, considered an endocrine disruptor, poses a risk to aquatic wildlife and human population, and is thus included in multiple environmental monitoring programmes. Following the regulations restricting BPA use in the last years, BPA-like chemicals have been produced and used as BPA substitutes. However, they are not commonly included in monitoring programs yet and their presence is thus misrepresented, despite showing similar endocrine disrupting potential. In this work, an analytical method for analysing bisphenol A and five of its analogues (Bisphenol S, B, F, AF and Tetrabromobisphenol A) is described, validated for water (riverine, sea and wastewater), sediment, and biota (fish and biofilm) and applied to monitor their presence in the Ebro River Delta (NE Spain). In addition, plastic litter was also collected to evaluate their role as potential source of bisphenols. All compounds except BPF were detected in the analysed samples. Wastewater treatment plants (WWTPs) were discarded as sources of BPs into the natural aquatic environment, as no BPs were detected in treated effluents. Indeed, the high levels of BPs seemed to be related with direct discharge of raw wastewater from small rural population nucleus. The analysis of riverine plastic leachates yielded 4 out of the 6 BPs analysed, strengthening the hypothesis that plastic debris are also a source of BPs in the natural environment. Whereas Bisphenol S and BPA were detected in water and, to a limited extent, in biota, less polar analogues (mainly BPAF and TBBPA) were not found in any of the water samples. Instead, these hydrophobic BPs were found in fish tissues and biofilm, pointing out plastics and microplastics as their possible vectors. Finally, biofilm demonstrated its potential as sentinel of chemical contamination in freshwater environment.

Keywords: Bisphenols, HPLC-MS/MS, emerging contaminants, biofilm, bioaccumulation, plastic litter

46

47 **Highlights**:

48 - Bisphenol A and 5 analogues analysed in different environmental compartments

49 - Polar bisphenols detected in water, sediment and biota

50 - Less polar bisphenol analogues were found only in fish tissues and fluvial biofilm

51 - Fluvial biofilm has shown its potential as a sentinel for bisphenols monitoring

52

53 **1. Introduction**

54 Plastics are inherent right to modern daily life and have been incorporated to almost all
55 industrial processes. Due to their properties, they are widely used in fields ranging from food
56 packaging, manufacturing, construction, and clothing (among multiple applications). Plastic
57 materials end up very often in the environment, and the degradation of this through different
58 pathways is the principal source of microplastic particles (secondary microplastics).
59 Microplastics can also enter the environment through their direct use, for instance as
60 components of personal care products (primary microplastics). Such extended widespread
61 usage makes our life easier but comes with a huge environmental impact. Plastic debris reaching
62 the aquatic medium can act as a source and vector of pollutants, eventually releasing plasticizers
63 and other adsorbed pollutants into organisms following ingestion (Basak et al., 2020). Both
64 (micro)plastics and the chemical compounds leaching from them have been demonstrated as a
65 potential contamination source to the environment, negatively impacting environmental and
66 human health (Barboza et al., 2020). From this wide variety of chemicals inherently present
67 some in plastic materials, one of the most ubiquitous compounds are bisphenols.

68 Bisphenols (BPs), and more specifically Bisphenol A (BPA), have been intensively used in
69 the manufacturing of polycarbonate plastics and epoxy resins (Mustieles et al., 2020). BPA has
70 been considered an endocrine-disrupting compound (Basak et al., 2020), previously reported in

71 food and water (Naveira et al., 2021) and, as a result, in human tissues and biofluids (Gao et al.,
72 2021). During the last decade, due to the new legislations, the manufacture and use of new
73 bisphenol analogues as substitutes of BPA have been boosted (e.g. Bisphenol B (BPB), Bisphenol
74 F (BPF), Bisphenol S (BPS), Bisphenol AF (BPAF)). In addition other BPA analogues such as
75 Tetrabromobisphenol A (TBBPA), are widely used as flame retardant in epoxy resins and
76 polycarbonates (Alexander et al., 2011).

77 These chemicals, far from being safer compared to BPA, have shown similar or more
78 hazardous effects(Bahelka et al., 2021; Sendra et al., 2021). BPS, for example, has been used as
79 an alternative to BPA, supported by its higher stability and theoretically lower toxicity. However,
80 some studies have reported that BPS also affects the central nervous system, disrupts estrogenic
81 production and alters thyroid hormone signalling (Naderi and Kwong, 2020). BPF and BPB are
82 also likely to alter hormone production (Ijaz et al., 2020), whereas BPAF yielded alterations in
83 mammary gland development in mice (Tucker et al., 2018). TBBPA has also demonstrated
84 neurodevelopmental toxicity and thyroid hormones alterations in zebrafish (*Danio rerio*) larvae
85 (Zhu et al., 2018). These findings question the suitability of these compounds as safe BPA
86 substitutes. Actually, some of them have demonstrated higher potential to bioaccumulate under
87 laboratory conditions (Wang et al., 2020) or even to be biomagnified across trophic chains (Li et
88 al., 2021). Considering the potential harmful effects of BPs and their ability to accumulate in
89 biological tissues, their monitoring in different environmental compartments is very relevant to
90 better understand their impact.

91 Bisphenol A and some of their analogues have been extensively monitored in surface
92 water in several countries. In China, considered one of the main plastic producers around the
93 world, concentrations in river water have reached ranges of 40-180 $ng \cdot L^{-1}$ as well as in river and
94 lake sediments, with comparable concentrations ranging from 4 to 270 $ng \cdot g^{-1}$ (Jin and Zhu, 2016;
95 Liu et al., 2017; Zhang et al., 2019; Zhao et al., 2020, 2019). On the other hand, though BPA has

96 been monitored in fish samples in some studies (Di Marco Pisciottano et al., 2020; Lv et al., 2019;
97 Yang et al., 2020), their analogues and substitutes are generally omitted. Only a few authors, as
98 Barboza et al. (Barboza et al., 2020), performed a comprehensive screening of BPA and its
99 analogues in fish tissues. The authors concluded that the muscle of fish can act as a vector of
100 BPA to humans through ingestion. However, an integrative monitoring of these compounds
101 including water, sediment and different types of biota samples have not been performed to
102 date.

103 With the main objective of investigating the sources and fate of BPs in the aquatic
104 environment the following goals were set up in the present work: a) to establish a reliable
105 analytical methodology to determine the concentrations of BPA and five relevant analogues
106 (BPS, BPB, BPF, BPAF, TBBPA) in river/sea water, associated sediments, fish and biofilms from
107 the Ebro River delta (NE Spain) as a case study; b) to investigate possible sources of BPs into
108 watercourses, including WWTPs and leachates from plastic litter; c) to explore the partition of
109 BPs into different fish tissues (plasma, muscle and liver); and d) to consider the potential of river
110 biofilm as a possible alternative to fish in biomonitoring studies. To the best of our knowledge,
111 this is the first integrative study tackling the occurrence of six BPs in so many different
112 environmental compartments.

113 **2. Materials and methods**

114 *2.1. Standards and chemicals*

115 All analytical standards were acquired from Merck (Darmstadt, Germany) with a purity grade
116 higher than 98%, including BPA, BPB, BPS, BPF, BPAF, TBBPA. BPA–d_4, BPB–d_8 and BPS-d_8 were
117 also purchased and used as internal labelled standards (ILS). Individual stock solutions of the
118 standards and internal standards were prepared in methanol (LC/MS-grade from Merck,
119 Darmstadt, Germany) at concentrations of 1000 $mg \cdot L^{-1}$. Two different mixtures at 1 $mg \cdot L^{-1}$ were
120 prepared with labelled and non-labelled standards. Acetonitrile (LC/MS grade), Water (LC/MS

121 grade), EDTA 0.1 M aqueous solution and filters were also acquired from Merck (Darmstadt,
122 Germany). OASIS HLB® SPE cartridges used for solid-phase extraction were purchased from
123 Waters Corporation (Milford, MA, U.S.A.).

124 Sepra ZT (30 µm, 85 ˚A) powder, Sepra ZTL-WCX (100 µm, 300 ˚A) powder and Sepra ZTL-WAX
125 (115 µm, 330 ˚A) powder were purchased from Phenomenex. Isolute ENV+ powder was
126 purchased from Biotage.

127 *2.2. Sample collection*

128 A sampling campaign was performed in the lower stretch of the Ebro River (NE Spain), as well as
129 in the two shallow bays in the Ebro Delta in the Mediterranean Sea (**Figure 1** shows sampling
130 locations, sample types and WWTPs present in the area). Samples of river and sea water,
131 sediment, fluvial biofilm, fish tissues, plastic debris, and water from wastewater treatment
132 plants (WWTPs) in the area, influent (WWI) and effluent (WWE) wastewater samples, were
133 collected in two consecutive weeks during March 2019.

134 Surface grab water samples were taken by directly immersing a 2.5 L glass bottle
135 (previously cleaned with HPLC-grade water, ethanol, and hexane) in the sampling points (river
136 or sea). Surface sediment was sampled with a dredge and stored in aluminium trays (also
137 cleaned with the same solvents). Fluvial biofilm was obtained from different rocks near each
138 selected sampling point, scratching them with a wire brush in an aluminium tray and then
139 pouring it into a clean 1 L glass bottle. WWTP samples (WWI and WWE) were taken by operators
140 (1-day composite sample) and picked up from their facilities. Fish individuals were captured by
141 electrofishing and transported to the edge of the river for processing. Blood samples were taken
142 from caudal vessels with a heparinized glass syringe, centrifuged in Eppendorf tubes for 5
143 minutes (at 2000 g) and the plasma was immediately transferred to glass vials. Sampling
144 procedure was also performed with HPLC-grade water (1 blank for each 10 individuals) to obtain
145 field blank samples and account for any bisphenol migration from field material to samples. All

146 plasma samples were then stored in dry ice. Once blood was taken, animals were euthanised
147 and dissected to take liver and muscle samples, which were directly stored in dry ice, covered
148 with aluminium foil. All these samples were stored in a portable freezer with dry ice during field
149 work and kept frozen until reaching lab facilities. Plastic debris were collected, stored in
150 aluminium foil, and transported to the lab.

151 All procedures were approved by the "Fisheries Department of Catalonia Government"
152 and "University of Girona" ethics and Animal Welfare Committee according to national (Royal
153 Decree RD53/2013) and EU legislation (2010/63/EU) on the handling of animals for experiments.

154 *2.3. Sample treatment*

155 *2.3.1. Water*

156 Water samples were thawed overnight at 4°C and filtered through a 1 µm glass fibre filter
157 followed by a 0.45 µm PVDF membrane filter (Whatman, U.K.). Multi-layer cartridges, described
158 elsewhere (Gago-Ferrero et al., 2020) were previously conditioned (for all the cartridges used in
159 this work, conditioning process consisted on passing 6 mL MeOH and 6 mL HPLC-grade water)
160 and employed for water matrices. Different volumes were loaded depending on the type of
161 sample: 500 mL in the case of sea water, 100 mL for river water, 50 mL for WWE and 25 mL of
162 WWI. Procedural blanks were performed with HPLC-grade water, passing 500 mL through the
163 cartridge for all matrices. SPE cartridges were eluted with 6 mL MeOH, led to dryness with a
164 gentle stream of N_2, reconstituted with 1 mL MeOH:H_2O (20:80) and stored at -20°C until
165 analysis. ILS was added prior to injection, adding 50 µL from a mix of 1 $mg \cdot L^{-1}$ for a final
166 concentration of 50 $\mu g \cdot L^{-1}$ in the extract. For recovery tests, different volumes of 1 $\mu g \cdot mL^{-1}$ non-
167 labelled standard mixtures were added to water samples and then vigorously shaken before
168 passing through the cartridge.

169 *2.3.2. Sediments*

170 Sediment samples were freeze-dried (during 7 days) and sieved at 0.25 mm. Samples (1 g) were
171 weighed and transferred to a falcon tube and 10 mL of ACN:Citrate buffer (pH:4) (1:1, v/v) were
172 added, vortexed and sonicated using a Branson digital sonifier (30 seconds, 20% amplitude).
173 Extract was centrifuged (10,000 g, 5 min) and supernatant was collected in a glass tube. Process
174 was repeated three times to ensure a complete extraction. Full sample extract was dried to half
175 of the initial volume with a TurboVap (Biotage®), evaporating almost the totality of the ACN, and
176 then mixed with 85 mL HPLC-grade water. SPE extraction was adapted from a previously
177 published work of our group (Gros et al., 2012). Extracts were passed through an Oasis HLB
178 cartridges (200 mg, 6 mL) previously conditioned. Procedural blanks were performed following
179 the whole sample extraction process without the addition of 1 g of sediment. SPE cartridges
180 were eluted with 6 mL MeOH, led to dryness with a gentle stream of N_2, reconstituted with 1
181 mL MeOH:H_2O (20:80) and stored at -20°C until analysis. ILS was added prior to injection, adding
182 50 µL from a mix of 1 $mg \cdot L^{-1}$ for a final concentration of 50 $\mu g \cdot L^{-1}$ in the extract. For recovery
183 tests, different volumes of 1 $mg \cdot L^{-1}$ non-labelled standard mixtures were added to sediment
184 samples, let 30 minutes for solvent evaporation and then extracted with the same procedure
185 followed for the remining samples.

186 *2.3.3. Biofilm*

187 Biofilm samples were thawed overnight at 4°C, transferred to falcon tubes and centrifuged (5000
188 g, 10 min). Pellets were transferred to aluminium trays (previously cleaned with HPLC-grade
189 water, ethanol, and hexane) and freeze-dried for 24 hours. Freeze-dried samples were extracted
190 following Santos et al. procedure (Santos et al., 2019). Briefly, 100 mg of freeze-dried biofilm
191 were extracted in a MP Biomedicals FastPrep tissuelyzer with 1 g of glass beads (previously acid
192 washed) and 1 mL of ACN:Citrate buffer (pH:4) (1:1, v/v), centrifuged (10000g, 10 min) and
193 transferred to a glass tube. The process was repeated by triplicate to ensure a thorough
194 extraction. Final volume (approx. 3 mL) was reduced to approx. 1.5 mL under a gentle stream of

195 N_2 and mixed with 100 mL HPLC-water. Sample was passed through an Oasis HLB cartridges
196 previously conditioned. Procedural blanks were performed following the whole sample
197 extraction process without the addition of 100 mg of biofilm. SPE cartridges were eluted with 6
198 mL MeOH, led to dryness with a gentle stream of N_2, reconstituted with 1 mL MeOH:H_2O (20:80)
199 and stored at -20°C until analysis. ILS was added prior to injection, adding 50 µL from a mix of 1
200 mg·L^{-1} for a final concentration of 50 µg·L^{-1} in the extract. For recovery tests, different volumes
201 of 1 mg·L^{-1} non-labelled standard mixtures were added to biofilm samples, let 30 minutes for
202 solvent evaporation and then extracted with the same procedure as the rest of samples.

203 *2.3.4. Fish*

204 *2.3.4.1. Plasma*

205 Plasma sample treatment was adapted from a previous work by Gil-Solsona et. al. (Gil-Solsona
206 et al., 2017). Briefly, plasma was thawed at room temperature, 0.25 mL were transferred to an
207 Eppendorf vial and 0.75 mL ACN were added. Sample was vortexed, centrifuged (12,500 g, 10
208 min) and supernatant was transferred to a glass vial. Field blanks were treated with the same
209 procedure as samples, using them also as procedural blanks. Samples were then stored at -80°C
210 until analysis. For recovery tests, different volumes of 1 mg·L^{-1} non-labelled standard mixtures
211 were added to plasma samples, vigorously shaken and then ACN was added, and same
212 procedure was followed as for the rest of plasma samples. ILS was added prior to injection,
213 adding 50 µL from a mix of 1 mg·L^{-1} for a final concentration of 50 µg·L^{-1} in the extract

214 *2.3.4.2. Liver and muscle*

215 Liver and muscle samples were freeze-dried (for 7 days) and then powdered and homogenised
216 with a ceramic mortar (previously cleaned with HPLC-grade water, ethanol, and hexane). Then,
217 the extraction protocol described elsewhere was applied (Gil-Solsona et al., 2021). Briefly, 150
218 mg of liver or muscle sample were weighed and extracted with 1 g of zirconium beads and 1 mL

219 of ACN:Citrate buffer (pH:4) (1:1, v/v) in a MP Biomedicals FastPrep tissuelyzer, centrifuged
220 (10,000 g, 10 min) and transferred to a glass tube. The process was repeated by triplicate to
221 ensure a thorough extraction. Volume was reduced to approx. 1.5 mL under a gentle steam of
222 N_2 and mixed with 100 mL HPLC-water. Sample was passed through an Oasis HLB cartridges
223 previously conditioned. Procedural blanks were performed following the whole sample
224 extraction process without the addition of 150 mg of matrix. SPE cartridges were eluted with 6
225 mL MeOH, led to dryness with a gentle stream of N_2, reconstituted with 1 mL MeOH:H_2O (20:80)
226 and stored at -20°C until analysis. ILS was added prior to injection, adding 50 µL from a mix of 1
227 mg·L^{-1} for a final concentration of 50 µg·L^{-1} in the extract. For recovery tests, different volumes
228 of 1 mg·L^{-1} non-labelled standard mixtures were added to fish tissue samples, led 30 minutes for
229 solvent evaporation and then extracted with the same procedure as the rest of the samples.

230 *2.3.5. Plastics leachates sample preparation*

231 Plastic leachates from litter samples were generated following a published method method by
232 León et al. (León et al., 2018). Briefly, ultrasonic extractions with MeOH were performed in a
233 glass tube with MeOH, using a volume enough to cover previously weighed plastic (mass ranged
234 from 0.02-0.45 g, **Table S1**) completely (approx. 10 mL), and performed by triplicate to ensure a
235 thorough extraction. Total volume of solvent was transferred to a glass tube and evaporated
236 until 5 mL using a rotary evaporator. Finally, 2 mL aliquot was concentrated until near dryness
237 and reconstituted with 200 µL of MeOH:H_2O (50:50). Extracts were then stored at -20°C until
238 analysis.

239 *2.4. Instrumental analysis*

240 The acquisition was performed using a PAL autosampler (CTC Analysis) and a UHPLC pump
241 (Accela 1250) coupled to a TSQ Vantage triple quadrupole mass spectrometer (Thermo Fisher
242 Scientific) with an electrospray turbo spray ionization (ESI) interface working in negative
243 ionization mode. Chromatographic separation was performed with a Phenomenex Luna Omega

244 C18 column (100 x 2.1 mm, 1.6 µm) from Phenomenex. Pure water (B) and methanol (A) were
245 used as mobile phases. Chromatographic gradient was as follows (%A): Initial 20% during 1 min
246 to 50% at 2.75 min, 100% at 6.5 min, maintained at 100% until 8 min and reduced to 20% at 9.5
247 min with a total run time of 10.5 min. Injection volume was 20 µL. Chromatographic column was
248 always kept at 40 °C in a column oven. Two selected reaction monitoring (SRM) transitions
249 (previously optimized with analytical standard mixture) were recorded for each compound
250 (**Table S2**), one of them for quantitation and the other for identity confirmation using Q/q ratio.

251 *2.5. Method performance evaluation*

252 To evaluate method performance, compounds were spiked at two concentration levels before
253 sample treatment for each matrix, as described previously. They were used to evaluate recovery
254 and repeatability, (calculated as relative standard deviation (RSD (%) of the three replicates at
255 each spiked concentration) in each matrix.

256 Calibration curves were prepared from 0.001 to 500 $\mu g \cdot L^{-1}$ in final extract solvent, to
257 evaluate linearity, detection, and quantitation limits (estimated from s/n ratio greater than 3
258 and 10 respectively). Matrix matched calibrations curves were prepared for each type of sample
259 to reduce matrix effects in quantitation. Results for all these parameters have been summarized
260 in **Table S2** and **Table S3**.

261

262 **3. Results and discussion**

263 3.1. Method performance

264 Linear response for all target compounds (with R^2 values always higher than 0.99) ranged from
265 0.05 to 500 $ng \cdot L^{-1}$ in water samples, except for BPB which values ranged from 0.1 to 500 $ng \cdot L^{-1}$
266 (see **Table S3**). For biological tissues and sediment, calibration curves ranged from 0.1 (for BPS
267 and BPAF) or 0.5 (BPA, BPB, BPF and TBBPA) to 100 $ng \cdot g^{-1}$ (dry weight) and, in case of plasma, it

268 ranged from 0.01 to 100 ng·mL^{-1} except for BPF and TBBPA, where it ranged from 0.1 to 100
269 ng·mL^{-1}.

270 Detection limits (LOD) showed, as expected, a strong dependence on the selected matrix
271 (**Table S3).** They ranged from 0.01 to 0.07 ng·L^{-1} in river and sea water samples, 3 to 151 ng·mL^{-}
272 ^{1} in plasma, 0.02 to 1.09 ng·g^{-1} for sediment, from 0.06 to 9.2 ng·g^{-1} in muscle, from 0.31 to 28.6
273 ng·g^{-1} in fish liver, which had the higher LOD for most compounds and from 0.22 to 10.0 ng·g^{-1}
274 in biofilm. BPS and BPAF were the compounds exhibiting the highest detection sensitivity, while
275 BPF showed 20-100 times lower sensitivity.

276 Recoveries were calculated at two different spiking concentrations for each matrix.
277 However, for compounds with high LOD in specific matrices (e.g., biofilm, fish plasma, liver, and
278 muscle), only recovery values for the highest spiking concentration could be calculated (biofilm
279 and plasma, liver, and fish muscle). Recovery values were between 60-130% for almost every
280 compound in the different matrices. For river and sea water, acceptable recoveries were
281 obtained (i.e. between 62-128% and 71-130% respectively). For WWE, recoveries ranged from
282 61-120% and for WWI from 19 – 121%. In the case of both WW matrices, TBBPA recovery at 100
283 ng·L^{-1} was quite low (only 16% in WWE and 27% in WWI), because the concentration was
284 probably too close to the LOD, making us decide that LOD for this compound, in both WW
285 matrices, should be set at a higher level, 1000 ng·L^{-1}. In the case of WWI, BPB also exhibited poor
286 recovery (25% and 43%), as well as for BPAF (25% and 19%), showing the matrix effects, which
287 are not corrected even with the addition of labelled internal standards, so the use of matrix-
288 matched calibrations seemed necessary for this matrix. However, in the analysed samples none
289 of these compounds were present. For sediment samples, acceptable recoveries were obtained
290 (47-108%), although they were occasionally below the 60%. Biofilm also showed acceptable
291 recoveries (41-144%), as well as fish plasma (65-185%) and muscle (63-122%), although for some
292 compounds recovery at the lowest concentration was not available. In the case of fish liver, only

293 BPS (111%) and BPAF (80%) were correctly recovered. BPA and TBBPA showed low recovery
294 values (15% and 3% respectively), probably due to sample characteristics, whereas BPB and BPF
295 could not be recovered with the proposed analytical methodology. Additional clean-up step was
296 applied in order to obtain better recoveries, consisting on the extraction of fat content with
297 Chloroform:Methanol:Water (1:2:0.8), as proposed by other authors for tissue clean-up (Wu et
298 al., 2008). However, the most lipophilic compounds (BPB, TBBPA and BPAF) were not recovered
299 either in the polar fraction (data not shown), so we decided to maintain the whole fraction. Due
300 to the low recovery value and low TBBPA concentration, the reported concentrations in the
301 collected samples can only be considered informative.

302

303 *3.2. Occurrence in environmental matrices*

304 Among the six bisphenols analysed, BPS was ubiquitous in all the environmental compartments,
305 while BPF was never detected in any of them. The other BPs were found at variable
306 concentrations depending on the considered matrix. Only two compounds, BPA and BPS were
307 detected at a detection frequency (DF) of 100% of water samples, both in surface and sea water
308 (see **Table 1** and **Table S4** for individual concentrations). Concentrations ranged from 10.8 to
309 45.3 $ng \cdot L^{-1}$ for BPA and from 7.0 to 20.4 $ng \cdot L^{-1}$ for BPS, except for one sample in Ebro River that
310 contained >500 $ng \cdot L^{-1}$. Values found in Ebro River were approximately one order of magnitude
311 lower than those found in different studies in China: up to 173 $ng \cdot L^{-1}$ for BPA, 160 $ng \cdot L^{-1}$ for BPS,
312 46 $ng \cdot L^{-1}$ for BPB or 110 $ng \cdot L^{-1}$ for BPAF. High concentrations have also been reported in other
313 Spanish rivers in 2015, up to 649 $ng \cdot L^{-1}$ in water from the high polluted Llobregat River (NE
314 Spain), and at similar concentrations in Ebro River, up to 229 $ng \cdot L^{-1}$ and up to 84 $ng \cdot L^{-1}$ in Júcar
315 River (E Spain) (Gorga et al., 2015). A similar profile was found in sea water, with BPA and BPS
316 detected in all samples at concentrations ranging from 3.3-49.5 $ng \cdot L^{-1}$ and 0.8-25.3 $ng \cdot L^{-1}$,
317 respectively. Additionally, BPB exclusively occurred in sea water (0.8-25.3 $ng \cdot L^{-1}$). Higher

318 concentrations have been observed for this compound in sand beaches in Spain (up to 1200 ng·L^{-1}), although these were obtained near touristic areas like Barcelona, Fuengirola or Cádiz (Kwon et al., 2020).

321 BPA was found in all sediment samples at concentrations ranging from 8.0 to 9.1 ng·g^{-1} in those collected in Ebro River and from 6.1 to 30.4 ng·g^{-1} in marine sediments. BPS was only found in 25% of marine sediments at very low values; up to 1.1 ng·g^{-1} and was not detected in any of the river sediment samples. BPAF was found in river sediments in 100% samples at 0.4 ng·g^{-1} and in 75% of marine sediments ranging from 0.3 to 0.6 ng·g^{-1}. Finally, TBBPA was found in 100% of river sediment samples at values ranging from 47.1 to 67.5 ng·g^{-1} but in 87.5% of marine sediments at values up to 84.7 ng·g^{-1}. These chemicals have been found in China, at concentrations of 270 ng·g^{-1} for BPA, 47 ng·g^{-1} for BPS, 4 ng·g^{-1} for BPB, 27.5 ng·g^{-1} for BPF, 57.1 ng·g^{-1} for BPAF, 2.5 ng·g^{-1} for BPZ or 0.40 ng·g^{-1} for BPAP (Jin and Zhu, 2016; Liu et al., 2017; Zhao et al., 2020) in sediments. High values have also been reported in the United States or Japan (106 and 23 ng·g^{-1} for BPA, 4.65 and 4.46 ng·g^{-1} for BPS, 27.5 and 9.11 ng·g^{-1} for BPF or 0.36 ng·g^{-1} for BPAF respectively (Liao et al., 2012)) as well as in sediments collected in sand beaches around the world (up to 670 ng·g^{-1} for BPA) (Kwon et al., 2020). Lower concentrations were detected in Ebro Delta sediments compared with the literature, which is in accordance with our work.

336 In biofilm samples up to 59.3, 1.17, 1.4 and 47.6 ng·g^{-1} were detected for BPA, BPS, BPAF and TBBPA, respectively, with a detection frequency (DF) of 50% for BPA, BPAF and TBBPA and 75% for BPS (**Table 1** and **Table S4**). Biofilm is a rarely studied matrix, but it has recently been included in singular studies of river contamination of pharmaceuticals and endocrine disrupting compounds (Huerta et al., 2016a; Ruhí et al., 2016; Zind et al., 2021). Furthermore, BPA and BPS have been found in biofilm of rivers in London at 8.27 ng·g^{-1} for BPA and 0.56 ng·g^{-1} for BPS (Wilkinson et al., 2018), both in the range of those presented in our study.

343 Concentrations detected in fish plasma and liver and muscle tissues varied (**Table 2**) and
344 presented certain variability by species (**Table S4**). Prevalence of BPS was generally higher but
345 maximum concentration varied depending on the analysed fish sample. Therefore, BPA was
346 found at high concentrations in fish plasma (up to 216.2 ng·mL^{-1}, DF=70%), and muscle (up to
347 1.5 ng·g^{-1}, DF=4%), but not in fish liver. The latter may be masked by the higher detection limits
348 for this compound in liver (i.e. 19 ng·g^{-1}). BPS was also found in all fish matrices, at
349 concentrations up to 30.1 ng·mL^{-1} in plasma (DF=90%), from 0.21 to 1.6 ng·g^{-1} in liver (DF: 100%
350 of samples) and up to 4.2 ng·g^{-1} in muscle (DF=80%). BPAF was found in fish plasma (up to 6.7
351 ng·mL^{-1}, DF=25%), fish muscle (up to 11.6 ng·g^{-1}, DF= 10%) and liver (up to 0.17 ng·g^{-1}, DF=21%).
352 BPA have been reported in fish from contaminated sites in China at variable concentrations
353 ranging from 2 to 40,000 ng·g^{-1} in liver, up to 141 ng·g^{-1} in plasma and up to 65.5 ng·g^{-1} in muscle
354 (Lv et al., 2019). In fish captured in Persian gulf, BPA was found at lower concentrations (ranging
355 from 1.15 to 21.45 ng·g^{-1} in muscle) (Akhbarizadeh et al., 2020), who also reported BPF (up to
356 6.7 ng·g^{-1}) and BPAF (up to 11.92 ng·g^{-1}). In Tagus estuary (Central Portuguese coasts), BPA was
357 also found in golden grey mullet (*Chelon auratus*) at similar concentrations (ranging from 5.6 to
358 9.1 ng·g^{-1}) (Álvarez-Muñoz et al., 2015). In line with results found in water and sediment samples
359 of Ebro Delta, BPs concentrations in fish samples were lower than those reported in literature,
360 except by TBBPA, not reported before in the literature and detected in this study in all fish
361 samples. Specifically, TBBPA occurred in plasma (up to 44.8 ng·mL^{-1}, DF=35%), muscle (up to 5.9
362 ng·g^{-1}, DF=30%) and liver (up to 57.9 ng·g^{-1}, DF=26%). It was a hundredfold more concentrated
363 in fish liver than the rest of analogues and, tenfold more concentrated than BPS or BPA in fish
364 muscle. This compound, TBBPA, should be considered in future studies due to its apparent
365 bioaccumulation potential. The fact that it was not detected in grab water samples, but
366 prevailed in other environmental matrices, highlights the importance of biomonitoring.

367 For assessing possible BPs sources, plastic debris collected in most of the river sites and
368 wastewater (both WWI and WWE) from the 7 WWTPs were also analysed. BPS was the only

369 compound detected in WWI from the selected WWTPs, up to 360 ng·L^{-1} in WWTP1 (up to 30
370 times more concentrated than it was in river water), whereas none of the analysed BPs were
371 detected in WWE (**Table S4**). In contrast, BPA, BPS, BPAF and TBBPA were measured in plastic
372 leachates at concentrations up to 46.8 ng·g^{-1} for BPA, 1.39 ng·g^{-1} for BPS, 2.36 ng·g^{-1} for BPAF
373 and 1.50 ng·g^{-1} for TBBPA (**Table S1)**.

374 *3.3. Sources of contamination and spatial distribution*

375 Urban wastewater (both, raw and treated) as well as plastic litter were hypothesized as main
376 sources of BPs into the aquatic environment in the Ebro Delta area. Treated urban wastewater
377 have demonstrated to be the main entrance for some chemicals (e.g., pharmaceuticals) to water
378 bodies (Gros et al., 2012), and similar results were expected in the case of BPs, which are
379 contaminants related to anthropogenic activities. However, among the 6 BPs analysed, only BPS
380 was detected in the influent (WWI) of the two WWTPs under study (WWTP 2 and WWTP 3)
381 (**Table S4**). By contrast, they were completely removed in the WWTP as they were not detected
382 in the effluents (WWE), which is in line with the efficiency reported in previous studies where
383 nearly 100% BPS, BPF and BPAF removal was achieved (Česen et al., 2018). Conversely, the
384 highest concentration for BPA and BPS in river water was found in DE 3. River section DE 1-3
385 corresponds to a rural area where people live in small villages, (Miravet: 706 inhab., Benifallet:
386 723 inhab. Xerta: 1154 inhab. (source: https://www.idescat.cat/, last accessed December 2021),
387 some of which do not have operational WWTPs (e.g. Miravet). Consequently, the discharge of
388 raw urban wastewater from Miravet (placed upstream DE 3) into the river might be pointed out
389 as an important contribution to the increase of the concentration of BPA (from 11.8 to 28.6 and
390 40.7 ng·L^{-1}) and BPS, from 8.3 to 16.1 and 20.4 ng·L^{-1}) observed from DE 1 to DE 3 (see **Table S4**).

391 Moreover, attenuation of the concentrations of BPA and BPS was observed downstream
392 DE 3, along the river, from 40.7 and 20.4 to ng·L^{-1} in DE 4 to 11.3 ng·L^{-1} and 7.0 ng·L^{-1} in DE 7
393 (lower stretch of Ebro River) for BPA and BPS respectively despite the discharge of the effluents

394 of 3 larger WWTP effluents (WWTP1_1, WWTP1_2 and WWTP1_3, see **Figure 1**). Fish were
395 sampled in Ebro River, in the section between DE 4_1 and DE 4_2, where two different WWTPs
396 are placed (WWTP 1_2 and 1_3, see **Figure 1**) to study differences in BPs concentrations
397 between both points. However, no remarkable differences were observed between fish sampled
398 in DE 4_1 and 4_2 (see **Table S5**). As no impact of treated wastewater discharges in the water
399 bodies can be depicted, WWTPs were thus discarded as entrance route of these compounds,
400 but raw urban wastewater.

401 Nevertheless, plastic litter could also be considered as a potential source of BPs, as these
402 chemicals are used in plastic production as plastic additives, which can be further be released
403 into the environment after contact with water and UV radiation as it has been demonstrated
404 e.g. additives from polyvinyl chloride, polyethylene (Suhrhoff and Scholz-Böttcher, 2016) or
405 polycarbonate (Staniszewska et al., 2016). Plastic debris collected in Ebro River were extracted
406 with MeOH to favour the leaching of plastic additives (León et al., 2019) and further analysed to
407 provide a BPs profile. BPA, BPS, BPF, BPAF and TBBPA were detected in these extracts (**Table**
408 **S1**), confirming that the presence of BPs in the Ebro Delta ecosystem can be directly originated
409 from plastic litter itself. Actually, as indicated before, the most polar compounds, BPA and BPS,
410 were detected in the natural water bodies (**Figure 3**). Interestingly, BPAF and TBBPA, the less
411 polar ones, were not found in water, but in biofilm as well as in fish tissues, as can be seen in
412 **Table 1** and **Table 2**. Plastic litter, but also microplastics, can not only be the source of BPs in
413 water phase, but also can act as vectors of these BPs into aquatic biota. In fact, microplastics
414 found in these sites showed presence of bisphenol (Bisphenol AP) (Llorca et al., 2021), with
415 similar polarity of studied BPAF (LogP: 4.4).

416 Surprisingly, high concentrations of BPS were found in DE 8 (>500 $ng \cdot L^{-1}$), as well as an
417 increase on BPA concentrations (from 11.3 to 45.3 $ng \cdot L^{-1}$) compared to the rest of the sampling
418 sites (**Table S4**). Such high concentration, considering previous concentrations observed in the

419 river, may be linked with the presence of different plastic sources near this point (e.g., eel-fishing
420 plastic lines). Anthropogenic activities near DE 8 may be contributing locally to high BPS
421 contamination in water. Although grab water samples represent only snapshots of river
422 pollution, these findings point out human activities intimately related with plastic usage (and
423 not only through plastic litter) as sources of BPs. This is also the case of BPB, which was only
424 found once in sea water (together with BPA and BPS). Common activities in Fangar Bay (DE 10-
425 13) and Alfacs Bay (DE 14-17), such as mussel farming (placed near DE 15 and DE 10) or tourism,
426 may also contribute to BPs pollution in the area. This finding is again in concordance with the
427 hypothesis that the most important anthropogenic sources of BP contamination in the area are
428 not WWTPs, but other related human activities. Furthermore, similar concentrations were
429 detected within each bay, which can be attributed to the effect of currents and topography that
430 isolates the bay from open sea, which causes high residence times in the water Bay (e.g. in Alfacs
431 bay) (Cerralbo et al., 2018), implying high residence time of pollutants. It makes these zones
432 more vulnerable to contamination than the open sea. Despite our results suggest that
433 anthropogenic activities may be intimately linked with BPs presence in the Ebro Delta rather
434 than the WWTP discharges, more studies should be performed to finally confirm this hypothesis.

435 *3.4. Environmental distribution*

436 As it can be seen in **Table 1** and **Table 2**, BPA and BPS were found in almost all the matrices at
437 high occurrence (**Figure 3A**). BPS (logKow: 1.65) was detected in river water at concentrations
438 ranging from 7.0 to 20.4 $ng{\cdot}L^{-1}$, in sea water at concentrations from 0.8 to 25.3 $ng{\cdot}L^{-1}$ and in fish
439 plasma at concentrations up to 30.1 $ng{\cdot}mL^{-1}$ (illustrated in **Figure 3B**). However, BPS showed low
440 levels in fish tissues, such as muscle, at concentrations up to 4.2 $ng{\cdot}g^{-1}$, in liver from 0.21 to 1.60
441 $ng{\cdot}g^{-1}$ or in biofilm at concentrations up to 1.17 $ng{\cdot}g^{-1}$ but it was not detected in river sediments
442 (with LOD: 0.04 $ng{\cdot}g^{-1}$).

443 Considering bioconcentration factor (BCF) as: BCF: C_B/C_{WD} (Arnot and Gobas, 2006);
444 where C_B is the concentration of the analyte in biota (at $g \cdot Kg^{-1}$, dry weight) and C_{WD} is the
445 dissolved concentration of the analyte in water (at $g \cdot L^{-1}$), in our case, logBCF for BPS in common
446 carp (*Cyprinus carpio*) was around 0.9 in liver and muscle. These values are in the same order as
447 reported in fish muscle and liver (0.1-1.3) from a laboratory-scale study performed at similar
448 water concentrations (ca. 15 $ng \cdot L^{-1}$) (Wang et al., 2020). These authors used concentration in
449 biological matrices in wet weight, which implies lower BCF, as the total content of water in
450 *Cyprinus carpio* body have been reported from 70 to 80% (Martemyanov, 2013), which makes
451 our result more similar to the one reported in the literature. BCFs for BPS have also been
452 estimated for other fish species, as zebrafish larvae (Moreman et al., 2017), at values around
453 0.067, confirming that BPS had low bioconcentration factor compared with other bisphenol
454 analogues, such as BPA, BPF or BPAF.

455 BPA (logKow: 3.32) was detected at similar concentration levels than BPS in river water,
456 ranging from 10.8 to 45.3 $ng \cdot L^{-1}$ as well as in sea water, ranging from 3.3 to 49.5 $ng \cdot L^{-1}$. However,
457 it was found at high concentrations in fish plasma with concentrations up to 216 $ng \cdot mL^{-1}$. Such
458 pattern may indicate that BPA is more easily bioaccumulated compared to BPS, supported by
459 the higher logBCF (1.08) assigned in the literature to BPA (Wang et al., 2020). Unfortunately, we
460 were unable to detect BPA in fish liver, and it was only found in one muscle sample at a low
461 concentration (0.08 $ng \cdot g^{-1}$) so we did not calculate BCF for BPA. In contrast with that was
462 observed for BPS, BPA was also found in sediment samples (at concentrations around 9 $ng \cdot g^{-1}$),
463 which is probably due to its higher hydrophobicity relative to BPS.

464 BPAF (logKow: 4.47) was not found in water (**Figure 3**). Conversely it was found in
465 sediments at concentrations of 0.4 $ng \cdot g^{-1}$. In addition, BPAF was quantified in fish plasma, at
466 concentrations up to 6.7 $ng \cdot L^{-1}$, as well as in fish liver and muscle and in biofilms at
467 concentrations up to 0.17, 11.6 and 1.4 $ng \cdot g^{-1}$. Literature indicates that BPAF has a BCF higher

than 500 for liver, around 1000 for plasma and around 50 in muscle (Wang et al., 2020). Considering these BCF, concentrations in water samples might have been below LOD (0.01 ng·L^{-1}), which may be the main reason why BPAF was found in animal tissues but not in water. Notwithstanding, BPAF was found in sediments and plastic leachates (**Table S1** and **Figure 3**) reinforcing the fact that BPAF enters the ecosystem via plastic debris and microplastics, and accumulates in sediments and biota.

A similar environmental distribution was observed for TBPPA (logKow: 7.29), with higher concentrations in biofilm (levels around 47.6 ng·g^{-1}) and fish plasma (up to 44.8 ng·L^{-1}), muscle (<LOD – 5.9 ng·g^{-1}) and liver levels around 57.9 ng·g^{-1}, despite the low recovery obtained for liver). Similar concentrations were found in river sediments (ranging from 54.0 to 67.5 ng·g^{-1}) and its occurrence was also confirmed in plastic leachates. In this case, a higher hydrophobicity of TBBPA could led to greater accumulation compared with BPAF in both sediments and aquatic

Considering results for BPAF and TBBPA in fish individuals (see **Table S5**), those species with feeding habits closely related with sediment (benthic feeders) (e.g. mullets *Liza sp.*, flathead grey mullet *Mugil cephalus* and common carp *Cyprinus carpio*) yielded detectable concentrations of both compounds in plasma compared with other carnivorous species such as wels catfish (*Silurus glanis*) where TBBPA was only detected in tissues. In addition, BPS and BPA, coming directly from water, are also present in plasma. Plasma, being a biofluid with a high turnover rate, can reflect recent contamination (Briels et al., 2019).The fact that benthic feeders are in close contact with sediment, due to their feeding habits, joined with the high concentration of these compounds found in sediments (and not in water) makes sediment and also microplastics a possible entrance of less polar BPs (BPAF and TBBPA) in aquatic organisms. As a result, the replacement of BPA by more lipophilic analogues, such as BPAF, may constitute an environmental risk due to its potential to accumulate in river sediments and biofilm, readily being incorporated in aquatic trophic webs.

493 We encourage to include TBBPA and other lipophilic BPA analogues in lists of concerning
494 substances, as it has shown its potential to bioaccumulate in biological tissues, and shows
495 endocrine disrupting effects comparable to the ones of BPA and BPS (Bahelka et al., 2021;
496 Sendra et al., 2021).

497

498 *3.5. Biofilm role as sentinel matrix in rivers*

499 Biofilm is a biological matrix easy-to-collect from river systems, avoiding challenging sampling
500 campaigns of fish and their tissues, and minimizing the unnecessary sacrifice of individuals.
501 Biofilm, has a key ecological role in primary production, nutrient cycling, and detoxification,
502 being an important part of aquatic ecosystems but, in unison, it also accumulates contaminants
503 which can end up incorporated to higher trophic levels (Sabater et al., 2007).

504 Biofilm concentrations of BPS, BPAF and TBBPA corresponded well with findings in fish
505 tissues (**Table S4** and **S5**): Similar concentrations of BPS (up to 1.31 $ng{\cdot}g^{-1}$ in fish liver and 1.17
506 $ng{\cdot}g^{-1}$ in biofilm), BPAF (up to 0.2 $ng{\cdot}g^{-1}$ in fish liver and 1.4 $ng{\cdot}g^{-1}$ in biofilm) and TBBPA (up to 58
507 $ng{\cdot}g^{-1}$ in fish liver and 47.6 $ng{\cdot}g^{-1}$ in biofilm) were observed in both biotic matrices. In addition,
508 BPA was also detected in biofilm (up to 59.3 $ng{\cdot}g^{-1}$) at higher concentrations than those found
509 in fish muscle (1.5 $ng{\cdot}g^{-1}$). Moreover, biofilm was the only biological matrix where these four
510 compounds (BPA, BPS, BPAF and TBBPA) were detected in contrast with water, where only BPA
511 and BPS were observed or with sediment, where BPA, BPAF and TBBPA were detected. Previous
512 studies have also been published demonstrating that biofilm can be used as a local exposure
513 sentinel for emerging contaminants in rivers and reinforce biofilm as a suitable candidate for the
514 screening of aquatic pollution (Huerta et al., 2016b; Mastrángelo et al., n.d.; Sabater et al., 2007;
515 Valdés et al., 2021). In accordance, it can be concluded that biofilms are of great potential as
516 sentinels for BPs and analogues pollution monitoring.

517

518 **4. Conclusions**

519 The occurrence, distribution, and possible sources of BPA and five of its analogues (BPS, BPB,
520 BPF, BPAF, TBBPA) has been assessed, taking samples in the in the Ebro River and Delta. BPA
521 and BPS were observed in almost every selected compartment, highlighting their widespread
522 presence in freshwater and marine coastal environments, while BPAF and TBBPA were only
523 found in biota at higher concentrations than the other analogues, presumably due to their
524 higher lipophilicity. Although BPS was detected in WWI (up to 360 $ng·L^{-1}$), it was not present in
525 WWE because of their efficient removal in WWTPs. Therefore, its presence in natural waters
526 cannot be attributed to WWTP discharges, but to diffuse and uncontrolled sources such as
527 untreated wastewater discharges, the leaching from plastic litter present in the environment
528 and other anthropogenic sources. Considering our findings and their potential impact in aquatic
529 life, the monitoring of less polar BPA analogues (such as BPAF or TBBPA) in watercourses must
530 be considered for future surveillance programmes. River biofilm has demonstrated its role as
531 sentinel for river contamination being a more integrative compartment than water, sediments,
532 or fish for the evaluation of the contamination by BPA and its analogues.

533

534 **Acknowledgements**

535 Authors acknowledge Spanish Ministry of Economy and Competitiveness (project PLAS-MED;
536 CTM2017-89701-C3-2-R) for its financial support. Authors acknowledge Maria Guzman for her
537 support in the sample analysis and method development. J.M. Castaño-Ortiz acknowledges the
538 predoctoral grant from the Agency for Management of University and Research Grants (AGAUR)
539 (2019 FI_B 00881). R. Muñoz-Mas benefitted from a postdoctoral Juan de la Cierva fellowship
540 from the Spanish Ministry of Science (FJCI-2016-30829).

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542 **Bibliography**

543 Akhbarizadeh, R., Moore, F., Monteiro, C., Fernandes, J.O., Cunha, S.C., 2020. Occurrence,
544 trophic transfer, and health risk assessment of bisphenol analogues in seafood from the
545 Persian Gulf. Mar. Pollut. Bull. 154, 111036.
546 https://doi.org/10.1016/j.marpolbul.2020.111036

547 Alexander, J., Benford, D., Boobis, A., Ceccatelli, S., Cottrill, B., Cravedi, J.-P., Di Domenico, A.,
548 Doerge, D., Dogliotti, E., Edler, L., Farmer, P., Filipič, M., Fink-Gremmels, J., Fürst, P.,
549 Guerin, T., Katrine Knutsen, H., Machala, M., Mutti, A., Rose, M., Schlatter, J., van
550 Leeuwen, R., Bergman, Å., Johansson, N., Knutsen, H., Merletti, F., Päpke, O., Schrenk, D.,
551 Van Leeuwen, R., Van Leeuwen, S., Zeilmaker, M., Carletti, A., Cioacata, G., Ramos
552 Bordajandi, L., Scaravelli, E., 2011. Scientific Opinion on Tetrabromobisphenol A (TBBPA)
553 and its derivatives in food. EFSA J. 9, 2477. https://doi.org/10.2903/J.EFSA.2011.2477

554 Álvarez-Muñoz, D., Rodríguez-Mozaz, S., Maulvault, A.L., Tediosi, A., Fernández-Tejedor, M.,
555 Van den Heuvel, F., Kotterman, M., Marques, A., Barceló, D., 2015. Occurrence of
556 pharmaceuticals and endocrine disrupting compounds in macroalgaes, bivalves, and fish
557 from coastal areas in Europe. Environ. Res. 143, 56–64.
558 https://doi.org/10.1016/J.ENVRES.2015.09.018

559 Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and
560 bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms.
561 Environ. Rev. https://doi.org/10.1139/A06-005

562 Bahelka, I., Stupka, R., Čítek, J., Šprysl, M., 2021. The impact of bisphenols on reproductive
563 system and on offspring in pigs – A review 2011–2020. Chemosphere.
564 https://doi.org/10.1016/j.chemosphere.2020.128203

565 Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O., Guilhermino, L., 2020. Bisphenol A

566 and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation
567 to microplastic contamination. Exposure and risk to human consumers. J. Hazard. Mater.
568 393, 122419. https://doi.org/10.1016/j.jhazmat.2020.122419

569 Basak, S., Das, M.K., Duttaroy, A.K., 2020. Plastics derived endocrine-disrupting compounds
570 and their effects on early development. Birth Defects Res. 112, 1308–1325.
571 https://doi.org/10.1002/bdr2.1741

572 Briels, N., Torgersen, L.N., Castaño-Ortiz, J.M., Løseth, M.E., Herzke, D., Nygård, T., Bustnes,
573 J.O., Ciesielski, T.M., Poma, G., Malarvannan, G., Covaci, A., Jaspers, V.L.B., 2019.
574 Integrated exposure assessment of northern goshawk (Accipiter gentilis) nestlings to
575 legacy and emerging organic pollutants using non-destructive samples. Environ. Res. 178,
576 108678. https://doi.org/10.1016/j.envres.2019.108678

577 Cerralbo, P., Espino, M., Grifoll, M., Valle-Levinson, A., 2018. Subtidal circulation in a microtidal
578 mediterranean bay. Sci. Mar. 82, 231–243. https://doi.org/10.3989/scimar.04801.16A

579 Česen, M., Lenarčič, K., Mislej, V., Levstek, M., Kovačič, A., Cimrmančič, B., Uranjek, N., Kosjek,
580 T., Heath, D., Dolenc, M.S., Heath, E., 2018. The occurrence and source identification of
581 bisphenol compounds in wastewaters. Sci. Total Environ. 616–617, 744–752.
582 https://doi.org/10.1016/j.scitotenv.2017.10.252

583 Di Marco Pisciottano, I., Mita, G.D., Gallo, P., 2020. Bisphenol A, octylphenols and
584 nonylphenols in fish muscle determined by LC/ESI-MS/MS after affinity chromatography
585 clean up. Food Addit. Contam. Part B 13, 139–147.
586 https://doi.org/10.1080/19393210.2020.1740335

587 Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H.P.,
588 Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of >2000 emerging
589 contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation

590 of its performance through the validation of 195 selected representative analytes. J.
591 Hazard. Mater. 387, 121712. https://doi.org/10.1016/j.jhazmat.2019.121712

592 Gao, Q., Niu, Y., Wang, B., Liu, J., Zhao, Y., Zhang, J., Wang, Y., Shao, B., 2021. Estimation of
593 lactating mothers' daily intakes of bisphenol A using breast milk. Environ. Pollut. 286,
594 117545. https://doi.org/10.1016/J.ENVPOL.2021.117545

595 Gil-Solsona, R., Nácher-Mestre, J., Lacalle-Bergeron, L., Sancho, J.V., Calduch-Giner, J.A.,
596 Hernández, F., Pérez-Sánchez, J., 2017. Untargeted metabolomics approach for
597 unraveling robust biomarkers of nutritional status in fasted gilthead sea bream (Sparus
598 aurata). PeerJ 2017. https://doi.org/10.7717/peerj.2920

599 Gil-Solsona, R., Rodriguez-Mozaz, S., Diaz-Cruz, M.S., Sunyer-Caldú, A., Luarte, T., Höfer, J.,
600 Galbán-Malagón, C., Gago-Ferrero, P., 2021. A protocol for wide-scope non-target
601 analysis of contaminants in small amounts of biota using bead beating tissuelyser
602 extraction and LC-HRMS. MethodsX 8, 101193.
603 https://doi.org/10.1016/j.mex.2020.101193

604 Gorga, M., Insa, S., Petrovic, M., Barceló, D., 2015. Occurrence and spatial distribution of EDCs
605 and related compounds in waters and sediments of Iberian rivers. Sci. Total Environ. 503–
606 504, 69–86. https://doi.org/10.1016/J.SCITOTENV.2014.06.037

607 Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue
608 analysis of a broad range of human and veterinary pharmaceuticals and some of their
609 metabolites in surface and treated waters by ultra-high-performance liquid
610 chromatography coupled to quadrupole-linear ion trap tandem. J. Chromatogr. A 1248,
611 104–121. https://doi.org/10.1016/j.chroma.2012.05.084

612 Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., Barcelo,
613 D., 2016a. Determination of a broad spectrum of pharmaceuticals and endocrine

614 disruptors in biofilm from a waste water treatment plant-impacted river. Sci. Total
615 Environ. 540, 241–249. https://doi.org/10.1016/j.scitotenv.2015.05.049

616 Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., Barcelo,
617 D., 2016b. Determination of a broad spectrum of pharmaceuticals and endocrine
618 disruptors in biofilm from a waste water treatment plant-impacted river. Sci. Total
619 Environ. 540, 241–249. https://doi.org/10.1016/J.SCITOTENV.2015.05.049

620 Ijaz, S., Ullah, A., Shaheen, G., Jahan, S., 2020. Exposure of BPA and its alternatives like BPB,
621 BPF, and BPS impair subsequent reproductive potentials in adult female Sprague Dawley
622 rats. Toxicol. Mech. Methods 30, 60–72.
623 https://doi.org/10.1080/15376516.2019.1652873

624 Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and
625 sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351.
626 https://doi.org/10.1016/j.watres.2016.07.059

627 Kwon, B.G., Chung, S.Y., Saido, K., 2020. Sandy beaches as hotspots of bisphenol A. Environ.
628 Res. 191, 110175. https://doi.org/10.1016/j.envres.2020.110175

629 León, V.M., García-Agüera, I., Moltó, V., Fernández-González, V., Llorca-Pérez, L., Andrade,
630 J.M., Muniategui-Lorenzo, S., Campillo, J.A., 2019. PAHs, pesticides, personal care
631 products and plastic additives in plastic debris from Spanish Mediterranean beaches. Sci.
632 Total Environ. 670, 672–684. https://doi.org/10.1016/j.scitotenv.2019.03.216

633 León, V.M., García, I., González, E., Samper, R., Fernández-González, V., Muniategui-Lorenzo,
634 S., 2018. Potential transfer of organic pollutants from littoral plastics debris to the marine
635 environment. Environ. Pollut. 236, 442–453.
636 https://doi.org/10.1016/j.envpol.2018.01.114

637 Li, H., Zhang, Z., Sun, Y., Wang, W., Xie, J., Xie, C., Hu, Y., Gao, Y., Xu, X., Luo, X., Mai, B., 2021.

638 Tetrabromobisphenol A and hexabromocyclododecanes in sediments and biota from two
639 typical mangrove wetlands of South China: Distribution, bioaccumulation and
640 biomagnification. Sci. Total Environ. 750, 141695.
641 https://doi.org/10.1016/j.scitotenv.2020.141695

642 Liao, C., Liu, F., Moon, H.B., Yamashita, N., Yun, S., Kannan, K., 2012. Bisphenol analogues in
643 sediments from industrialized areas in the United States, Japan, and Korea: Spatial and
644 temporal distributions. Environ. Sci. Technol. 46, 11558–11565.
645 https://doi.org/10.1021/es303191g

646 Liu, Y., Zhang, S., Song, N., Guo, R., Chen, M., Mai, D., Yan, Z., Han, Z., Chen, J., 2017.
647 Occurrence, distribution and sources of bisphenol analogues in a shallow Chinese
648 freshwater lake (Taihu Lake): Implications for ecological and human health risk. Sci. Total
649 Environ. 599–600, 1090–1098. https://doi.org/10.1016/j.scitotenv.2017.05.069

650 Llorca, M., Vega-Herrera, A., Schirinzi, G., Savva, K., Abad, E., Farré, M., 2021. Screening of
651 suspected micro(nano)plastics in the Ebro Delta (Mediterranean Sea). J. Hazard. Mater.
652 404, 124022. https://doi.org/10.1016/J.JHAZMAT.2020.124022

653 Lv, Y.Z., Yao, L., Wang, L., Liu, W.R., Zhao, J.L., He, L.Y., Ying, G.G., 2019. Bioaccumulation,
654 metabolism, and risk assessment of phenolic endocrine disrupting chemicals in specific
655 tissues of wild fish. Chemosphere 226, 607–615.
656 https://doi.org/10.1016/j.chemosphere.2019.03.187

657 Martemyanov, V.I., 2013. Assessment of the physiological state of the common carp Cyprinus
658 carpio L. by the water content in the organism. Inl. Water Biol. 6, 80–84.
659 https://doi.org/10.1134/S1995082912030091

660 Mastrángelo, M.M., M. E. Valdés, B.E., Ossana, N.A., Barceló, D., Sabater, S., Rodríguez-Mozaz,
661 S., Giorgi., A.D.N., n.d. Occurrence and accumulation of pharmaceutical products in water

662 and biota of urban lowland rivers. Sci. Total Environ.

663 Moreman, J., Lee, O., Trznadel, M., David, A., Kudoh, T., Tyler, C.R., 2017. Acute Toxicity,
664 Teratogenic, and Estrogenic Effects of Bisphenol A and Its Alternative Replacements
665 Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish Embryo-Larvae.
666 https://doi.org/10.1021/acs.est.7b03283

667 Mustieles, V., D'Cruz, S.C., Couderq, S., Rodríguez-Carrillo, A., Fini, J.B., Hofer, T., Steffensen,
668 I.L., Dirven, H., Barouki, R., Olea, N., Fernández, M.F., David, A., 2020. Bisphenol A and its
669 analogues: A comprehensive review to identify and prioritize effect biomarkers for
670 human biomonitoring. Environ. Int. https://doi.org/10.1016/j.envint.2020.105811

671 Naderi, M., Kwong, R.W.M., 2020. A comprehensive review of the neurobehavioral effects of
672 bisphenol S and the mechanisms of action: New insights from in vitro and in vivo models.
673 Environ. Int. https://doi.org/10.1016/j.envint.2020.106078

674 Naveira, C., Rodrigues, N., Santos, F.S., Santos, L.N., Neves, R.A.F., 2021. Acute toxicity of
675 Bisphenol A (BPA) to tropical marine and estuarine species from different trophic groups.
676 Environ. Pollut. 268, 115911. https://doi.org/10.1016/J.ENVPOL.2020.115911

677 Ruhí, A., Acuña, V., Barceló, D., Huerta, B., Mor, J.R., Rodríguez-Mozaz, S., Sabater, S., 2016.
678 Bioaccumulation and trophic magnification of pharmaceuticals and endocrine disruptors
679 in a Mediterranean river food web. Sci. Total Environ. 540, 250–259.
680 https://doi.org/10.1016/j.scitotenv.2015.06.009

681 Sabater, S., Guasch, H., Ricart, M., Romaní, A., Vidal, G., Klünder, C., Schmitt-Jansen, M., 2007.
682 Monitoring the effect of chemicals on biological communities. The biofilm as an interface.
683 Anal. Bioanal. Chem. 387, 1425–34. https://doi.org/10.1007/s00216-006-1051-8

684 Santos, L.H.M.L.M., Freixa, A., Insa, S., Acuña, V., Sanchís, J., Farré, M., Sabater, S., Barceló, D.,
685 Rodríguez-Mozaz, S., 2019. Impact of fullerenes in the bioaccumulation and

686 biotransformation of venlafaxine, diuron and triclosan in river biofilms. Environ. Res. 169,
687 377–386. https://doi.org/10.1016/j.envres.2018.11.036

688 Sendra, M., Pereiro, P., Figueras, A., Novoa, B., 2021. An integrative toxicogenomic analysis of
689 plastic additives. J. Hazard. Mater. 409, 124975.
690 https://doi.org/10.1016/j.jhazmat.2020.124975

691 Staniszewska, M., Graca, B., Nehring, I., 2016. The fate of bisphenol A, 4-tert-octylphenol and
692 4-nonylphenol leached from plastic debris into marine water - experimental studies on
693 biodegradation and sorption on suspended particulate matter and nano-TiO2.
694 Chemosphere 145, 535–542. https://doi.org/10.1016/j.chemosphere.2015.11.081

695 Suhrhoff, T.J., Scholz-Böttcher, B.M., 2016. Qualitative impact of salinity, UV radiation and
696 turbulence on leaching of organic plastic additives from four common plastics - A lab
697 experiment. Mar. Pollut. Bull. 102, 84–94.
698 https://doi.org/10.1016/j.marpolbul.2015.11.054

699 Tucker, D.K., Hayes Bouknight, S., Brar, S.S., Kissling, G.E., Fenton, S.E., 2018. Evaluation of
700 Prenatal Exposure to Bisphenol Analogues on Development and Long-Term Health of the
701 Mammary Gland in Female Mice. Environ. Health Perspect. 126, 087003.
702 https://doi.org/10.1289/EHP3189

703 Valdés, M.E., Santos, L.H.M.L.M., Rodríguez Castro, M.C., Giorgi, A., Barceló, D., Rodríguez-
704 Mozaz, S., Amé, M.V., 2021. Distribution of antibiotics in water, sediments and biofilm in
705 an urban river (Córdoba, Argentina, LA). Environ. Pollut. 269, 116133.
706 https://doi.org/10.1016/J.ENVPOL.2020.116133

707 Wang, Q., Chen, M., Qiang, L., Wu, W., Yang, J., Zhu, L., 2020. Toxicokinetics and
708 bioaccumulation characteristics of bisphenol analogues in common carp (Cyprinus
709 carpio). Ecotoxicol. Environ. Saf. 191, 110183.

710 https://doi.org/10.1016/j.ecoenv.2020.110183

711 Wilkinson, J.L., Hooda, P.S., Swinden, J., Barker, J., Barton, S., 2018. Spatial (bio)accumulation
712 of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites
713 in river sediment, aquatic plants and benthic organisms. Environ. Pollut. 234, 864–875.
714 https://doi.org/10.1016/j.envpol.2017.11.090

715 Wu, H., Southam, A.D., Hines, A., Viant, M.R., 2008. High-throughput tissue extraction protocol
716 for NMR- and MS-based metabolomics. Anal. Biochem. 372, 204–212.
717 https://doi.org/10.1016/j.ab.2007.10.002

718 Yang, J., Chan, K.M., Gong, J., 2020. Seasonal variation and the distribution of endocrine-
719 disrupting chemicals in various matrices affected by algae in the eutrophic water
720 environment of the pearl river delta, China. Environ. Pollut. 263, 114462.
721 https://doi.org/10.1016/j.envpol.2020.114462

722 Zhang, H., Zhang, Y., Li, J., Yang, M., 2019. Occurrence and exposure assessment of bisphenol
723 analogues in source water and drinking water in China. Sci. Total Environ. 655, 607–613.
724 https://doi.org/10.1016/j.scitotenv.2018.11.053

725 Zhao, X., Qiu, W., Zheng, Y., Xiong, J., Gao, C., Hu, S., 2019. Occurrence, distribution,
726 bioaccumulation, and ecological risk of bisphenol analogues, parabens and their
727 metabolites in the Pearl River Estuary, South China. Ecotoxicol. Environ. Saf. 180, 43–52.
728 https://doi.org/10.1016/j.ecoenv.2019.04.083

729 Zhao, X., Zhang, H., Chen, Z. lin, Wang, X. chun, Shen, J. min, 2020. Spatial and temporal
730 distributions of bisphenol analogues in water and sediment from the Lanzhou section of
731 the Yellow River, China. Arab. J. Geosci. 13, 1–8. https://doi.org/10.1007/s12517-020-
732 06122-y

733 Zhu, B., Zhao, G., Yang, L., Zhou, B., 2018. Tetrabromobisphenol A caused neurodevelopmental

734 toxicity via disrupting thyroid hormones in zebrafish larvae. Chemosphere 197, 353–361.
735 https://doi.org/10.1016/J.CHEMOSPHERE.2018.01.080

736 Zind, H., MONDAMERT, L., REMAURY, Q.B., CLEON, A., LEITNER, N.K.V., LABANOWSKI, J., 2021.
737 Occurrence of carbamazepine, diclofenac, and their related metabolites and
738 transformation products in a French aquatic environment and preliminary risk
739 assessment. Water Res. 196. https://doi.org/10.1016/j.watres.2021.117052

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Table 1: Median and range of concentrations, and total occurrence of BPA and analogues in river water, sea water, river sediment, sea sediment, biofilm (dry weight) and plastic litter.

	RIVER WATER ($ng \cdot L^{-1}$)			SEA WATER ($ng \cdot L^{-1}$)			RIVER SEDIMENT ($ng \cdot g^{-1}$)			SEA SEDIMENT ($ng \cdot g^{-1}$)			BIOFILM ($ng \cdot g^{-1}$)			PLASTIC LITTER ($ng \cdot g^{-1}$)		
	Median	Range	Detection frequancy	Median	Range	Detection frequancy	Median	Range	Detection frequancy	Median	Range	Detection frequancy	Median	Range	Detection frequancy	Median	Range	Detection frequancy
BPS	**16.1**	**7 - >500**	**100%**	**5.1**	**0.8 - 25.3**	**100%**	<LOD	<LOD	0%	**0.14**	**<LOD – 1.1**	**25%**	**0.25**	**<LOD - 1.17**	**75%**	**0.27**	**<LOD - 1.39**	**94%**
BPF	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	**<LOD**	0%
BPA	**21.0**	**10.8 - 45.3**	**100%**	**13.7**	**3.3 - 49.5**	**100%**	**8.2**	**7.9 – 9.1**	**100%**	**7.6**	**6.1 – 30.4**	**100%**	**14.7**	**<LOD - 59.3**	**50%**	**4.7**	**<LOD – 46.8**	**50%**
BPB	<LOD	<LOD	0%	**4**	**2.1 - 8.7**	**100%**	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	<LOD	0%	<LOD	**<LOD**	0%
BPAF	<LOD	<LOD	0%	<LOD	<LOD	0%	**0.4**	**0.4**	**100%**	**0.3**	**<LOD – 0.6**	**75%**	**0.9**	**<LOD - 1.4**	**50%**	**0.42**	**<LOD – 2.36**	**69%**
TBBPA	<LOD	<LOD	0%	<LOD	<LOD	0%	**56.9**	**47.1 – 67.5**	**100%**	**45.4**	**<LOD – 84.7**	**87.5%**	**23.8**	**<LOD - 47.6**	**50%**	**0.36**	**<LOD - 1.50**	**25%**

Table 2: Median and range of concentrations, and total occurrence of BPA and analogues in fish plasma, liver, and muscle (dry weight).

	FISH PLASMA ($ng \cdot mL^{-1}$)			FISH LIVER ($ng \cdot g^{-1}$)			FISH MUSCLE ($ng \cdot g^{-1}$)		
	Median	Range	Occurrence	Median	Range	Occurrence	Median	Range	Occurrence
BPS	**9.8**	**<LOD - 30.1**	**90%**	**0.55**	**0.21 – 1.6**	**100%**	**0.03**	**<LOD - 4.2**	**80%**
BPF	<LOD	<LOD	0%	-	-	-	<LOD	<LOD	0%
BPA	**65.0**	**<LOD - 216.2**	**70%**	<LOD	<LOD	0%	-	**<LOD - 1.5**	**4%**
BPB	<LOD	<LOD	0%	-	-	-	<LOD	<LOD	0%
BPAF	**5.2**	**<LOD - 6.7**	**25%**	**0.2**	**<LOD – 0.17**	**21%**	**0.62**	**<LOD - 11.6**	**10%**
TBBPA	**28.3**	**<LOD - 44.8**	**35%**	**44.3**	**<LOD – 57.9**	**26%**	**0.10**	**<LOD - 5.9**	**30%**

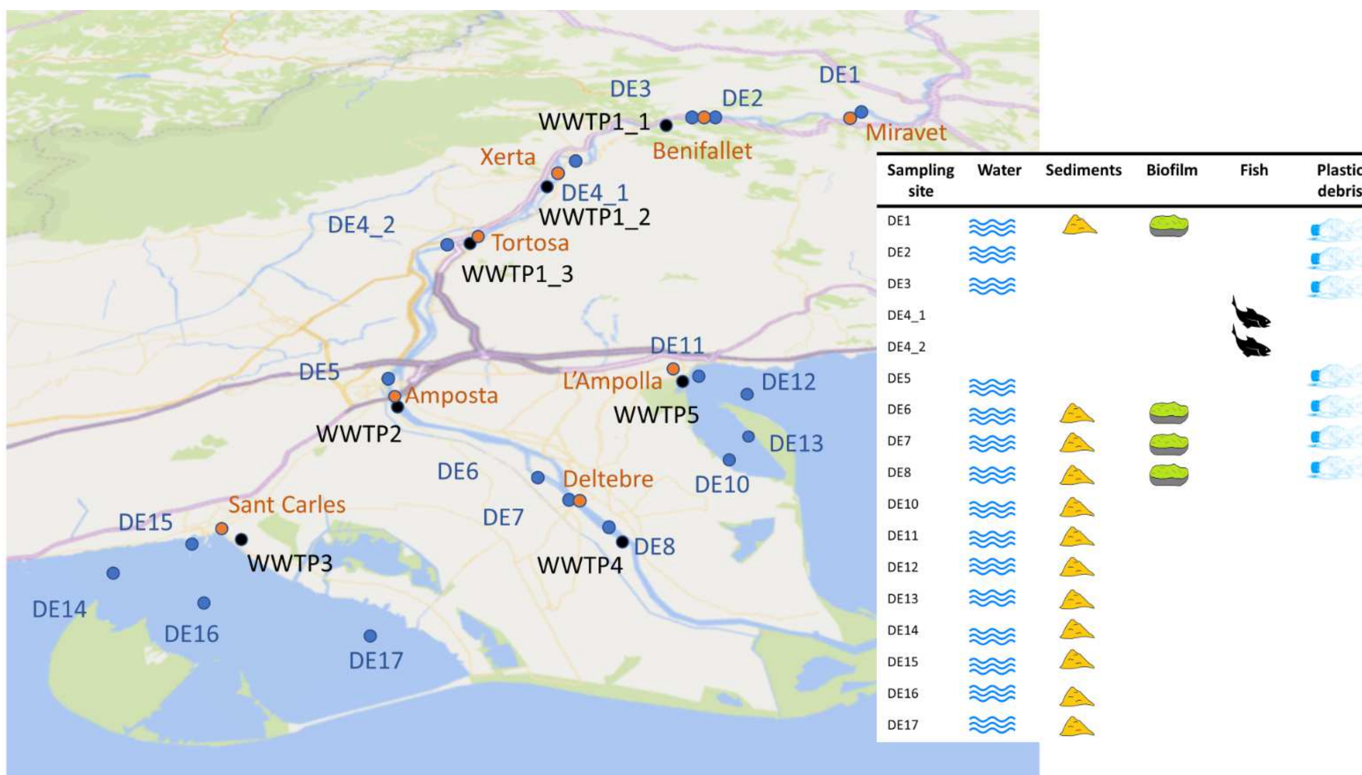

Figure 1: Ebro sampling points (in blue), towns (in orange) and wastewater treatment plants (WWTP, in black). Type of sample taken from each sampling site are indicated in the annexed table. DE 2 and DE 3 samples were taken upstream and downstream *Benifallet*. Fish were captured upstream *Xerta* (DE 4_1) and downstream Tortosa WWTP1 discharge point (DE 4_2). DE 5 samples were taken upstream WWTP2 and *Amposta*. DE 6 samples were taken upstream *Deltebre*, DE 7 at the town centre and DE 8 downstream. DE 10-13 samples were taken in Fangar bay, while DE 14-17 were sampled in Alfacs Bay.

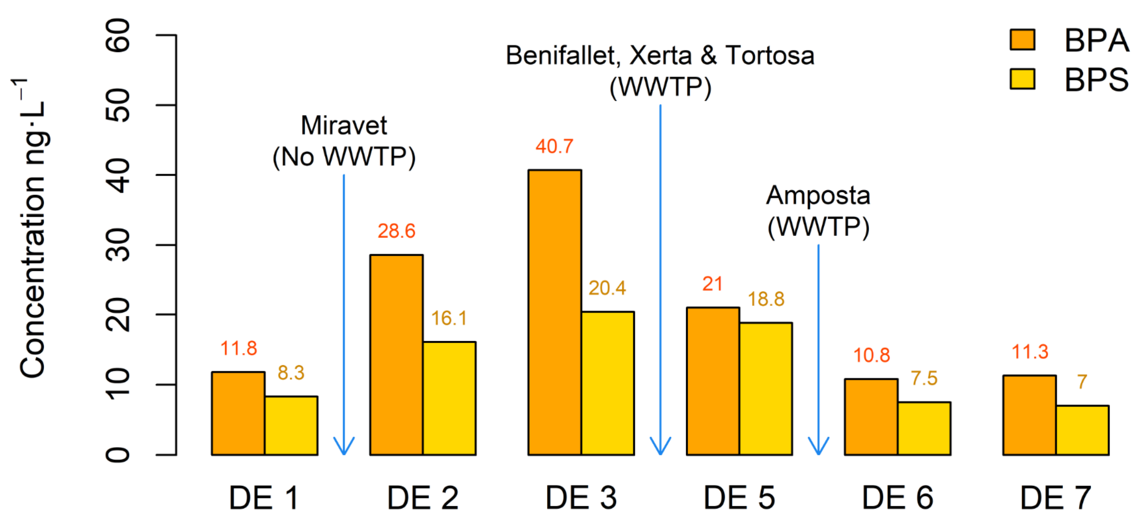

Figure 2: Spatial distribution of BPA (left) and BPS (right) in river water throughout the studied area of the Ebro River (DE 1 to DE 7).

A)

DETECTION FREQUENCY	BPS	BPA	BPB	BPAF	TBBPA
Log Kow	1.65	3.32	4.13	4.47	7.29
Infl Water					
Efl Water					
River Water					
Sea Water					
Fish Plasma					
Fish Muscle					
Fish Liver					
River Biofilm					
River Sediment					
Sea Sediment					
River Plastic litter					

B)

MEDIAN	BPS	BPA	BPB	BPAF	TBBPA
Log Kow	1.65	3.32	4.13	4.47	7.29
Infl Water					
Efl Water					
River Water					
Sea Water					
Fish Plasma					
Fish Muscle					
Fish Liver					
River Biofilm					
River Sediment					
Sea Sediment					
Rivrer Plastic litter					

Figure 3: Heatmap showing the occurrence levels using the corresponding frequency of detection (A) and median values (B) of the bisphenols found in the different environmental compartments (table 1 and 2). Colour intensity depends on the median value found in each kind of sample.