**MEDIUM TO HIGHLY POLAR PESTICIDES IN SEAWATER: ANALYSIS
AND FATE IN CATALONIA COASTAL AREAS (NE-SPAIN)**

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25 **Abstract**

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27 Pollution has been less investigated in marine and coastal environments than in inland waters. The
28 low levels at which pollutants are expected to be present in seawater calls for the use of reliable and
29 sensitive analytical methodologies. In this context, this work presents the optimization and
30 validation of an analytical method to determine 26 medium to highly polar pesticides in seawater
31 based on solid phase extraction and liquid chromatography-tandem mass spectrometry detection.
32 The developed methodology was linear, accurate (relative recoveries within 80-120% for most
33 analytes), repeatable (relative standard deviations <18% for most analytes), and sensitive (limits of
34 determination <1 ng/L for 89% of the compounds). The use of isotopically labeled compounds as
35 surrogate standards compensated for low analyte recoveries and matrix effects. The method was
36 applied to the analysis of seawater samples collected along the coastline of Catalonia (NE Spain).
37 Overall, total pesticide loads were higher inside the marinas than outside, being the booster biocides
38 diuron and irgarol used in antifouling paintings and different triazine pesticides the most abundant
39 compounds. Irgarol was present above the maximum allowable concentration set in European
40 regulations in 70% of the samples collected inside the marinas. A different pesticide pollution
41 pattern, with MCPA and bentazone presenting the highest concentrations, was observed at the Ebro
42 Delta area due to the impact of the agricultural activities carried out there. To the authors'
43 knowledge, 4 out of the 26 target pesticides, namely, chlorfenvinphos, fenthion oxon, fenthion
44 sulfone, and fenthion sulfoxide, have not been previously investigated in seawater.

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48 **Keywords:** herbicides, biocides, marine pollution, isotope dilution, LC-MS/MS, irgarol

49 **1. Introduction**

50 The fact that pesticide residues remain in the environment after their application is not
51 unknown. The potential risk that their occurrence may have on ecosystems and human health has
52 motivated their study in the different environmental compartments worldwide. After risk
53 assessment, the production and use of selected pesticides have been banned or regulated in different
54 countries. For instance, maximum allowable concentrations (MAC) have been set for 21 pesticides
55 in surface water (Directive 2013/39/EU) (Pinto et al., 2016), and for individual and total pesticides
56 and transformation products in groundwater (Directive 2006/118/EC) in Europe.

57 While a large number of studies have focused on the fate and pollution effects of pesticides
58 in continental environments, such knowledge in marine and coastal aquatic ecosystems is still
59 relatively scarce (Brumovský et al., 2017). The behavior of these compounds in marine
60 environments may differ indeed from that observed inland. There is evidence in the literature that
61 salinity could reduce water solubility of organic compounds and enhance their partitioning potential
62 (Mézin and Hale, 2004; Saranjampour et al., 2017). This may affect the sorption, bioavailability,
63 and toxicity of organic pollutants present in seawater and consequently deserves further
64 investigation.

65 To date, persistent organochlorine pesticides, and booster biocides, such as diuron and
66 irgarol, are among the pesticides most investigated in aquatic marine environments (Konstantinou
67 and Albanis, 2004). Overall, pesticide concentrations offshore are usually lower than in coastal
68 areas (Brumovský et al., 2017; Carafa et al., 2007; Orlikowska et al., 2015) due to seaward dilution,
69 degradation and/or sorption on the particulate matter of riverine inputs, urban and industrial
70 activities at the coastline, and agricultural runoffs. Pesticide pollution in coastal areas is related to
71 coastal landforms. Semi-enclosed bays, coastal lagoons, natural and artificial harbors present low
72 flushing rates of pollutant inputs, whereas estuaries and deltas may present several pollution
73 scenarios because they are directly impacted by riverine discharges. Additionally, deltas are usually

74 areas of intensive agricultural farming. Inland seas, such as the Baltic Sea, the Black Sea, the
75 Mediterranean Sea, and the Seto Inland Sea of Japan, are considered highly vulnerable areas to
76 anthropogenic pollution due to the high urban, agricultural and industrial pressures at their basin
77 coastlines and the limited water circulation and reduced dilution within their basin (Orlikowska et
78 al., 2015).

79 Pesticide concentrations in aquatic marine environments are usually in the low ng/L level.
80 Therefore, their detection requires the use of very reliable, selective and sensitive analytical
81 methodologies that include a pre-concentration step of the target analytes from the seawater sample
82 so that such low levels can be reached with current analytical techniques. For this, different
83 approaches have been followed: conventional solid phase extraction (SPE) (Ali et al., 2014; Catalá-
84 Icardo et al., 2014; Cavas et al., 2016; Di Landa et al., 2009; Gatidou et al., 2005; Loos et al., 2013;
85 Orlikowska et al., 2015; Sánchez-Rodríguez et al., 2011) and liquid-liquid extraction (LLE) (Said et
86 al., 2015) of large volumes of samples (500 mL to 1000 mL), on-line SPE (Gimeno et al., 2001;
87 Rodríguez-González et al., 2016); on-site solid phase microextraction (Huang et al., 2015), liquid-
88 liquid microextraction (Habedank et al., 2017; Rodríguez-González et al., 2015), stir-bar sorptive
89 extraction (Pérez-Carrera et al., 2007; Sánchez-Avila et al., 2010), microfunnel liquid-phase
90 microextraction (Saleh et al., 2014), and modified nanoparticles-based SPE (Mehdinia et al., 2016a;
91 Mehdinia et al., 2016b). The extract analysis is usually performed by gas chromatography or liquid
92 chromatography coupled to mass spectrometry. The analysis of specific pesticides in marine
93 environments has also been carried out using sensor and bioassay-based methodologies, e.g., an
94 enzyme-based biosensor for the detection of carbofuran (Gao et al., 2017), an electrochemical
95 sensor based on reduced graphene oxide-gold nanoparticles for the selective detection of diuron
96 (Shams et al., 2016), and a green microalgae-based optical bioassay (Moro et al., 2018).

97 In this context, this work aimed at developing and validating a selective and sensitive multi-
98 residue method based on SPE followed by liquid chromatography-electrospray-tandem mass

99 spectrometry (LC-ESI-MS/MS) for the determination of 26 medium to highly polar (Log K_{ow} from
100 -0.83 to 3.95) pesticides in seawater. Another objective was to apply this method to assess the
101 contamination levels of selected pesticides in coastal and offshore seawater of Catalonia (NE of
102 Spain, west of the Mediterranean Sea) so that their presence could be related to the geographical
103 and functional characteristics of the different sampled areas.

104 Target pesticides were selected according to the extent of their use in the investigated area,
105 information obtained from water authorities (priority substances and candidates to be included in
106 future regulations), and their amenability to LC-MS analysis. The list consists of seven triazines
107 (atrazine (ATR), simazine (SIMA), cyanazine (CYA), desethylatrazine (DEA), terbutylazine
108 (TER), and the booster biocides irgarol (IRG) and terbutryn (TBT)), four phenylureas (diuron
109 (DIU), isoproturon (ISO), linuron (LINU), and chlortoluron (CHLO)), nine organophosphates
110 (azinphos ethyl (AZET), chlorfenvinphos (CFP), diazinon (DIAZ), dimethoate (DIME),
111 fenitrothion (FEN), and the transformation products fenthion oxon (FENOX), fenthion sulfone
112 (FENSN), and fenthion sulfoxide (FENSX)), one anilide (propanil (PROP)), two chloroacetanilides
113 (alachlor (ALA) and metolachlor (METO)), and four acid herbicides (mecoprop (MECO), 2,4 D,
114 bentazone (BEN), and MCPA). The use of some of the target pesticides is banned in the EU
115 territory (the s-triazines atrazine, simazine, cyanazine and terbutryn, the organophosphates
116 chlorfenvinphos, diazinon and fenitrothion, the anilide propanil, and the chloroacetanilides alachlor
117 and metolachlor). Moreover, some of these substances (atrazine, simazine, alachlor,
118 chlorfenvinphos, and terbutryn) together with diuron, isoproturon and irgarol are considered priority
119 substances in surface waters (Directive 2013/39/EU). To the authors' knowledge, 4 out of the 26
120 target pesticides, namely, chlorfenvinphos, fenthion oxon, fenthion sulfone, and fenthion sulfoxide
121 have not been previously investigated in marine environments.

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123 2. Materials and methods

124 **2.1. Standards and solvents**

125 High purity (96-99.9 %) standards of the selected 26 pesticides and 24 isotopically labeled
126 analogs used as surrogate standards (SS) were supplied by Fluka (Sigma-Aldrich, Steinheim,
127 Germany) or Dr. Ehrenstorfer (LGC Standards, Teddington, UK). Target compounds, and relevant
128 physical-chemical properties (average molecular mass, solubility, and octanol-water partition
129 coefficient (Log Kow), among others) are shown in Table 1. Stock solutions of the individual
130 analytes were prepared in methanol (MeOH) and were further diluted with this solvent to prepare
131 working solutions of the individual analytes and analyte mixtures at various concentrations. A
132 methanolic solution containing only the SS (500 ng/mL) to be used in sample analyses was also
133 prepared. All standard solutions were stored at -20 °C in the dark. Pesticide-grade MeOH,
134 acetonitrile (ACN) and dichloromethane (DCM) and LC-grade water were purchased from Merck
135 (Darmstadt, Germany).

137 **2.2. Sample pre-treatment and extraction**

138 Analyte pre-concentration and extraction was carried out by means of SPE using a 12-port
139 vacuum manifold system (J.T. Baker®, Thermo Fisher Scientific). Before extraction, the SS
140 mixture was added to the sample at a concentration of 50 ng/L. Then the unfiltered seawater sample
141 (1000 mL) was loaded at a flow-rate of approximately 5 mL/min into a previously conditioned (5
142 mL of MeOH:DCM, 1:1, v/v) and equilibrated (5 mL of LC-grade water) Oasis HLB cartridge (500
143 mg, 6 mL) (Waters, Milford, MA, USA). After sample loading, the salt was washed out from the
144 cartridge with 7.5 mL of LC-grade water, and the washed cartridge was vacuum-dried. Analytes
145 were eluted from the cartridge with 4 mL of MeOH:DCM (1:1, v/v). The eluate was blown down to
146 dryness using a gentle stream of nitrogen and finally reconstituted in 0.5 mL of MeOH (resulting in
147 a 2000x volumetric concentration factor) for subsequent LC-ESI-MS/MS analysis.

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Table 1. Target pesticides and relevant physical-chemical properties.

Pesticide / Surrogate	Abbrev	Average molecular mass ^c (Da)	Type	Substance group	Solubility ^c (mg/L)	Octanol-water partition coefficient ^c (Log Kow)
2,4-Dichlorophenoxyacetic acid	2,4-D	221.0	herbicide	acid	24300	-0.82
<i>2,4-Dichlorophenoxyacetic acid d₃</i>	<i>2,4-D-d₃</i>	224.1				
Alachlor	ALA	269.8	herbicide	chloroacetanilide	240	3.09
<i>Alachlor d₁₃</i>	<i>ALA-d₁₃</i>	282.9				
Atrazine	ATR	215.7	herbicide	triazine	35	2.7
<i>Atrazine d₅</i>	<i>ATR-d₅</i>	220.7				
Azinphos Ethyl	AZET	345.4	insecticide/acaricide	organophosphate	4.5	3.18
<i>Azinphos Ethyl d₁₀</i>	<i>AZET-d₁₀</i>	355.4				
Bentazone	BEN	240.3	herbicide	benzothiazinone	7112	-0.46
<i>Bentazone d₆</i>	<i>BEN-d₆</i>	246.3				
Chlorfenvinphos	CFP	359.6	insecticide/acaricide	organophosphate	145	3.80
<i>Chlorfenvinphos d₁₀</i>	<i>CFP-d₁₀</i>	369.6				
Chlortoluron	CHLO	212.7	herbicide	phenylurea	74	2.50
<i>Chlortoluron d₆</i>	<i>CHLO-d₆</i>	218.7				
Cyanazine	CYA	240.7	herbicide	acid	171	2.10
<i>Cyanazine d₅</i>	<i>CYA-d₅</i>	245.7				
Desethylatrazine	DEA	187.6	metabolite	triazine	2700	1.51
<i>Desethylatrazine d₆</i>	<i>DEA-d₆</i>	193.6				
Diazinon	DIAZ	304.4	insecticide	organophosphate	40	3.81
<i>Diazinon d₁₀</i>	<i>DIAZ-d₁₀</i>	314.4				
Dimethoate	DIME	229.3	insecticide	organophosphate	25900	0.75
<i>Dimethoate d₆</i>	<i>DIME-d₆</i>	235.3				
Diuron	DIU	233.1	herbicide	phenylurea	35.6	2.87
<i>Diuron d₆</i>	<i>DIU-d₆</i>	239.1				
Fenitrothion	FEN	277.2	insecticide	organophosphate	19	3.32
<i>Fenitrothion d₆</i>	<i>FEN-d₆</i>	283.3				
Fenthion oxon ^a	FENOX	262.3	metabolite	organophosphate	214	2.31

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151 **Table 1 (continued).** Target pesticides and relevant physical-chemical properties.

Pesticide / Surrogate	Abbrev	Average molecular mass ^c (Da)	Type	Substance group	Solubility ^c (mg/L)	Octanol-water partition coefficient ^c (Log P)
Fenthion sulfone	FENSN	310.3	metabolite	organophosphate	190.4	2.25
<i>Fenthion sulfone d₆</i>	<i>FENSN-d₆</i>	316.4				
Fenthion sulfoxide	FENSX	294.3	metabolite	organophosphate	3.72	1.92
<i>Fenthion sulfoxide d₆</i>	<i>FENSX-d₆</i>	300.4				
Irgarol	IRG	253.4	algicide/herbicide	triazine	7.0	3.95
<i>Irgarol d₉</i>	<i>IRG-d₉</i>	262.4				
Isoproturon	ISO	206.3	herbicide	phenylurea	70.2	2.5
<i>Isoproturon d₆</i>	<i>ISO-d₆</i>	212.3				
Linuron	LINU	249.1	herbicide	phenylurea	63.8	3.00
<i>Linuron d₆</i>	<i>LINU-d₆</i>	255.1				
MCPA	MCPA	200.6	herbicide	acid	29390	-0.81
<i>MCPA d₃</i>	<i>MCPA-d₃</i>	203.6				
Mecoprop	MECO	214.7	herbicide	acid	250000	-0.19
<i>Mecoprop d₃</i>	<i>MECO-d₃</i>	217.7				
Metolachlor	METO	283.8	herbicide	chloroacetanilide	530	3.4
<i>Metolachlor d₆</i>	<i>METO-d₆</i>	289.8				
Propanil ^b	PROP	218.1	herbicide	anilide	95	2.29
Simazine	SIMA	201.7	herbicide	triazine	5.0	2.30
<i>Simazine d₁₀</i>	<i>SIMA-d₁₀</i>	211.7				
Terbuthylazine	TER	229.7	herbicide	triazine	6.6	3.40
<i>Terbuthylazine d₅</i>	<i>TER-d₅</i>	234.7				
Terbutryn	TBT	241.4	herbicide	triazine	25	3.66
<i>Terbutryn d₅</i>	<i>TBT-d₅</i>	246.4				

^a *FENSX-d₆* used as surrogate standard

^b *FEN-d₆* used as surrogate standard

^c Data extracted from Agriculture & Environment Research Unit (AERU). The PPDB, Pesticide Properties Database. University of Hertfordshire, UK (Online available at <http://sitem.herts.ac.uk/aeru/footprint/index2.htm>>)

157 2.3. LC-ESI-MS/MS analysis

158 Seawater extracts were analyzed by LC-ESI-MS/MS with an Acquity UPLC® system
159 connected in series with a TQD triple-quadrupole mass spectrometer equipped with a ZsprayTM
160 electrospray source (Waters). The analytical method applied here is a variation of a previous one
161 developed for the analysis of pesticides in sediments (Köck-Schulmeyer et al., 2013), modified to
162 include six new target compounds, i.e., azinphos ethyl, fenthion oxon, fenthion sulfone, fenthion
163 sulfoxide, irgarol and terbutryn, and their corresponding isotopically labelled analogues (except for
164 fenthion oxon) for isotope dilution quantification.

165 Chromatographic separation was performed using a Purospher STAR RP-18e column (125 x
166 2 mm, 5 µm) preceded by a guard column (4 x 4 mm, 5µm) of the same packing material (Merck,
167 Darmstadt, Germany) and an organic linear gradient of a mobile phase consisting of ACN and
168 water. During the chromatographic run (40 min), the ACN content of the mobile phase gradient
169 increased from 10% to 50% in 5 min, then to 80% in the next 20 min and to 100% in one more
170 minute at a constant flow rate of 0.2 mL/min. This composition was maintained for 5 min to clean
171 the column before returning to initial conditions (10% of ACN). The injection volume was set to 5
172 µL.

173 MS/MS detection was carried out in the selected reaction monitoring (SRM) mode recording
174 two SRM transitions per compound and one SRM transition per SS. All target analytes were
175 measured in a single run alternating positive and negative electrospray ionization. Source and
176 desolvation temperatures were 150°C and 450°C, respectively. The capillary voltage was set to 3.5
177 kV and the extractor voltage to 3 V. Nitrogen was used as both nebulizing and desolvation gas.
178 Instrument control, data acquisition, and evaluation were performed using Masslynx 4.1 software
179 (Micromass, Manchester, UK).

180 Table 2 shows the LC retention time and the optimum (most abundant and characteristic)
181 SRM transitions and ionization conditions selected for MS analysis of each analyte.

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Table 2. Relevant parameters for LC-ESI-MS/MS analysis of the target pesticides.

Pesticide / Surrogate	Abbrev	Rt (min)	ESI mode	SRM1 / SRM2 (m/z)	Cone (V) SRM1/ SRM2	CE (eV) SRM1/ SRM2	SRM1/ SRM2 ratio
Bentazone	BEN	4.97	-	239>132/239>197	35/35	25/20	1.5
<i>Bentazone d₆</i>	<i>BEN-d₆</i>		-	245>132	35	25	
Desethylatrazine	DEA	5.50	+	188>146/188>79	30/30	15/25	3.5
<i>Desethylatrazine d₆</i>	<i>DEA-d₆</i>		+	194>147	25	20	
MCPA	MCPA	5.68	-	199>141/199>155	20/15	15/15	32.2
<i>MCPA-d₃</i>	<i>MCPA-d₃</i>		-	204>146	25	20	
Dimethoate	DIME	5.72	+	230>125/230>199	25/25	15/15	0.8
<i>Dimethoate d₆</i>	<i>DIME-d₆</i>		+	236>131	30	15	
2,4-D	2,4-D	5.85	-	219>125/219>161	15/15	30/10.	15.6
<i>2,4-D d₃</i>	<i>2,4-D d₃</i>		-	224>127	20	25	
Mecoprop	MECO	6.54	-	213>141/213>71	15/15	15/15	17.4
<i>Mecoprop d₃</i>	<i>MECO-d₃</i>		-	218>146	25	15	
Simazine	SIMA	7.09	+	202>124/202>71	30/30	20/25	1.0
<i>Simazine d₁₀</i>	<i>SIMA-d₁₀</i>		+	212>137	35	15	
Cyanazine	CYA	7.12	+	241>214/241>174	30/30	15/20	10.1
<i>Cyanazine d₅</i>	<i>CYA-d₅</i>		+	246>219	30	20	
Fenthion sulfoxide	FENSX	7.40	+	295>109/295>125	40/40	30/35	3.9
<i>Fenthion sulfoxide d₆</i>	<i>FENSX-d₆</i>		+	301>108	35	30	
Chlortoluron	CHLO	7.98	+	213>72/213>140	25/25	15/30	33.2
<i>Chlortoluron d₆</i>	<i>CHLO-d₆</i>		+	219>78	35	15	
Isoproturon	ISO	8.33	+	207>72/207>165	35/35	20/15	6.6
<i>Isoproturon d₆</i>	<i>ISO-d₆</i>		+	213>171	30	20	
Atrazine	ATR	8.46	+	216>174/216>132	35/35	15/20	6.7
<i>Atrazine d₅</i>	<i>ATR-d₅</i>		+	221>179	35	15	
Diuron	DIU	8.66	+	233>72/235>72	25/25	15/15	1.8
<i>Diuron d₆</i>	<i>DIU-d₆</i>		+	239>78	25	25	
Fenthion oxon ^a	FENOX	8.88	+	263>231/263>216	35/35	20/25	1.8
Fenthion sulfone	FENSN	9.51	+	311>125/311>109	35/35	30/40	1.8
<i>Fenthion sulfone d₆</i>	<i>FENSN-d₆</i>		+	317>115	45	30	
Propanil ^b	PROP	10.30	-	216>160/218>162	30/30	15/15	1.6
Terbutylazine	TER	10.86	+	230>174/230>96	25/25	15/25	5.2
Terbutylazine d ₅	TER-d ₅		+	235>179	30	20	
Linuron	LINU	11.27	+	249>160/249>192	25/25	15/15	1.3
<i>Linuron d₆</i>	<i>LINU-d₆</i>		+	255>184	30	30	
Irgarol	IRG	12.81	+	254>108/254>125	35/30	30/25	2.8
<i>Irgarol d₉</i>	<i>IRG-d₉</i>		+	263>199	40	20	
Terbutryn	TBT	12.93	+	242>71/242>91	30/30	30/30	1.1
<i>Terbutryn d₅</i>	<i>TBT-d₅</i>		+	247>191	35	20	
Metolachlor	METO	13.55	+	284>176/284 73	25/25	25/25	8.4
<i>Metolachlor d₆</i>	<i>METO-d₆</i>		+	290>258	25	15	
Alachlor	ALA	13.72	+	270>238/270>162	30/30	15/15	2.3
<i>Alachlor d₁₃</i>	<i>ALA-d₁₃</i>		+	283>251	15	10	
Fenitrothion	FEN	13.98	-	262>152/262>122	25/25	30/20	19.9
<i>Fenitrothion d₆</i>	<i>FEN-d₆</i>		-	265>152	30	15	
Azinphos Ethyl	AZET	14.34	+	346>132/346>104	15/15	20/35	4.2
<i>Azinphos Ethyl d₁₀</i>	<i>AZET-d₁₀</i>		+	356>132	15	20	
Chlorfenvinphos	CFP	14.58	+	359>155/359>170	25/25	15/40	1.3
<i>Chlorfenvinphos d₁₀</i>	<i>CFP-d₁₀</i>		+	369>101	25	30	
Diazinon	DIAZ	17.24	+	305>153/305>97	35/35	20/30	1.7
<i>Diazinon d₁₀</i>	<i>DIAZ-d₁₀</i>		+	315 >170	35	20	

^a FENSX-d₆ used as surrogate standard

^b FEN-d₆ used as surrogate standard

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188 **2.4. Method performance**

189 The performance of the optimized analytical method in seawater was validated in-house in
190 terms of linearity, accuracy (analyte recovery), precision (repeatability), matrix effects, and
191 sensitivity, using one of the seawater samples collected in the monitoring study (see section 2.5).

192 Twelve methanolic calibration solutions within the range 0.005 - 800 ng/mL (equivalent to
193 0.0025 - 400 ng/L in sea water) for all analytes except azinphos ethyl (0.003-480 ng/mL) and
194 fenthion oxon (0.004-640 ng/mL) were used to evaluate method linearity and to construct
195 calibration curves. Linear calibration curves were generated using least square linear regression
196 analysis following the classical isotope dilution approach, i.e., considering the ratio of the analyte
197 signal to that of the corresponding SS (see Tables 1 and 2) at the different calibration points.

198 Method recovery and intra-day reproducibility for the different target analytes were
199 evaluated at three different levels (5, 50 and 100 ng/L) in seawater. These fortification levels are
200 near or below the environmental quality standards (EQS) set by the EU for the priority substances
201 included in the methodology (2.5-1000 ng/L) (Directive 2013/39/EU). Absolute recoveries (AR) of
202 the target analytes and SS were calculated by comparing the LC-MS/MS peak areas obtained in
203 fortified seawater samples vs. methanolic standard solutions at equivalent concentrations. Relative
204 recoveries (RR) were determined by comparing the AR of each target analyte (AR_{analyte}) with that of
205 its corresponding SS (AR_{ss}). Background pesticide levels in the seawater sample used in the
206 recovery study and in procedural blanks (a methanolic SS solution) were considered, if existing, in
207 the calculation of method recoveries.

208 Intra-day reproducibility of method performance was evaluated by performing five different
209 replicates at each investigated level.

210 Evaluation of matrix effects was carried out by comparing the peak areas of the target
211 analytes and SS in seawater extracts reconstituted with the standard mixture vs. methanolic standard
212 solutions at equivalent concentrations (100 ng/mL).

213 Method sensitivity in terms of limit of detection (LOD) and quantification (LOQ) was
214 experimentally estimated from the analysis of seawater samples fortified at the lowest level (5 ng/L)
215 in the recovery study based on the concentrations of the analyte giving a signal-to-noise (S/N) ratio
216 of 3 and 10, respectively. Sensitivity was also evaluated with the limit of determination (LDet) that
217 is the minimum concentration that could be quantified (LOQ of the SRM1) and confirmed (LOD of
218 the SRM2).

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220 ***2.5. Sample collection***

221 Seawater samples were collected from 20 different sampling locations distributed along the
222 coastline of Catalonia in February and March 2015: 4 in the province of Girona, 9 in the province of
223 Barcelona, and 7 in the province of Tarragona. It is important to highlight that sampling location 19
224 was at the Ebro River delta where water is rather more similar to fresh water than seawater. The
225 samples collected in this location presented conductivity values below 1.2 mS/cm, whereas
226 conductivity values of the remaining water samples were in the range 32-61 mS/cm (see Table 3).

227 One-liter grab samples were collected in amber polyethylene terephthalate (PET) bottles
228 inside (IN) a marina and in a nearby beach/accessible location (OUT) at each sampling location.
229 Thus, a total of forty samples of surface marine water (50-cm depth) were available for analysis. An
230 extra volume of water was collected from sampling location 3 (OUT) for method development and
231 validation. Upon collection, samples were transported to the laboratory under cool conditions and
232 stored at 4°C in the dark until extraction, which was performed within less than 24 hours from
233 collection.

234

235 **Table 3.** Conductivity and pH value of the seawater samples analyzed.
236

Sample code	pH value	Conductivity ($\mu\text{S}/\text{cm}$)
1-in	8.02	51,351
1-out	8.00	48,835
2-in	7.90	50,606
2-out	7.80	60,692
3-in	n/a	n/a
3-out	n/a	n/a
4-in	7.94	49,471
4-out	7.69	50,961
5-in	7.95	47,360
5-out	8.11	50,423
6-in	8.05	48,823
6-out	8.07	50,491
7-in	8.05	50,271
7-out	8.00	50,817
8-in	7.97	49,772
8-out	7.97	50,545
9-in	8.13	50,537
9-out	8.20	50,623
10-in	8.17	50,676
10-out	8.14	50,300
11-in	n/a	n/a
11-out	n/a	n/a
12-in	8.09	49,823
12-out	7.93	50,428
13-in	7.96	49,867
13-out	8.04	50,575
14-in	7.73	33,660
14-out	8.10	50,044
15-in	7.97	49,223
15-out	8.01	49,821
16-in	8.09	49,909
16-out	8.10	50,507
17-in	8.00	49,386
17-out	8.06	50,006
18-in	7.91	34,932
18-out	8.22	32,141
19-in	7.71	1,198
19-out	7.64	563
20-in	7.92	38,571
20-out	7.91	45,271

n/a: data not available.

237
238

239

240 3. Results and discussion

241 3.1. Optimization of the analytical procedure

242 The compounds selected for analysis present a wide variety of physical-chemical properties
243 (see Table 1) and therefore the use of a single methodology for their simultaneous analysis is a
244 challenge. As discussed in the introduction, extraction of pesticides from aqueous marine samples
245 using SPE is an extended practice. In this approach, a generic purpose sorbent (Oasis HLB) was
246 used based on the results obtained in a previous study (Köck-Schulmeyer et al., 2013), in which
247 various SPE sorbents were tested for the extraction of 77% of the target pesticides (Oasis HLB,
248 Evolute ABN, LiChrolut EN, and Carbograph). The elution step of the SPE process was also
249 optimized in that work (six different solvents tested: MeOH, MeOH:DCM 9:1, MeOH:DCM 1:1,
250 MeOH:DCM 1:9, acetone, and acetone:hexane 1:1), being MeOH:DCM 1:1 the best option (Köck-
251 Schulmeyer et al., 2013).

252 Since pesticide concentrations in seawater are expected to be very low, two aspects affecting
253 method sensitivity, matrix effects, and analyte breakthrough were optimized in the present study:
254 the volume of sample extracted (500, 1000, 2000, and 3000 mL) and the volume of LC-grade water
255 used to wash the SPE cartridge (7.5 mL and 15 mL). Signal losses due to analyte breakthrough or
256 matrix effects were evaluated by comparing the pesticide AR obtained after testing in triplicate each
257 sample volume and wash solvent volume at three different concentration levels (10, 50 and 100
258 ng/L). Seawater blanks were always processed in triplicate so that background concentrations of the
259 target pesticides could be considered in each case.

260 The effect of the sample volume on the average analyte recovery (n=3) observed at the
261 different fortification levels evaluated is summarized in Figure 1. In the light of the results, the
262 extraction of one liter of seawater provided overall the highest levels of recovery and the least
263 dispersion of the data. On the contrary, the extraction of lower and higher sample volumes provided
264 very variable recoveries, showing either extremely poor recoveries (desethylatrazine and simazine)

265 or high recoveries for some of the target pesticides (fenitrothion, dimethoate, propanil, and
266 alachlor).

267 As for the cartridge wash volume, the average relative response of the analyte recovery
268 observed after extraction in triplicate of 1000 mL of fortified seawater at different levels (10, 50 and
269 100 ng/L) using different volumes (7.5 mL and 15 mL) for the cartridge wash step is shown in
270 Figure 2a. As it can be observed, better recoveries were obtained with 7.5 mL than with 15 mL for
271 highly polar pesticides, i.e., 2,4-D, MCPA and mecoprop (solubility >20000 mg/L and Log Kow<0
272 (at pH 7), see Table 1), as they may partially be washed out from the cartridge at high wash
273 volumes. On the contrary, high wash volumes enhanced the recovery of the least polar target
274 pesticides, i.e., chlorfenvinphos, diazinon, and fenitrothion (solubility <145 mg/L and Log
275 Kow>3.3, see Table 1). This could be explained by an increased removal from the cartridge of
276 unwanted matrix components when using high wash volumes, as was confirmed upon evaluation of
277 the extent of matrix effects for each analyte (shown in Figure 2b). This figure indicates that the
278 response was relatively higher in reconstituted extracts of unfortified seawater that were processed
279 using a wash volume of 15 mL as compared to 7.5 mL for most analytes. The smaller variation
280 observed in matrix effect removal (less than 10%) as compared with that seen in analyte
281 breakthrough (more than 10%) justified the selection of 7.5 mL as wash volume.

282

283

Figure 1. Effect of the extracted sample volume on analyte breakthrough (each fortification level, i.e., R1=10 ng/L, R2=50 ng/L, and R3=100 ng/L, was evaluated in triplicate).

Figure 2. Absolute recoveries normalized to 1 to evaluate the effect of wash volumes on pesticide recovery a) after extraction in triplicate of seawater fortified at different concentrations (10, 50 and 100 ng/L) and b) in seawater extracts obtained after extraction in triplicate of unfortified sea water and reconstituted with the standard solution mixture at two different concentration levels (10 and 50 ng/mL) (sample volume = 1000 mL).

The LC-MS/MS method employed is a variation of a previously published methodology for the analysis of 26 pesticides in sediment samples (Köck-Schulmeyer et al., 2013) that shared 20 target analytes in common with the present method. The expansion of target analytes with six

new pesticides and five isotopically labeled compounds required optimization of their two major SRM transitions and associated cone and collision energy voltages. Moreover, it was necessary to adjust the acquisition windows (and acquisition times) so that chromatographic peak signals are maximized without affecting reproducibility of analyte peak areas. Figure 3 shows the signal obtained for the quantification SRM transition after LC-ESI-MS/MS analysis of a seawater sample fortified at a concentration of 50 ng/L.

3.2. Method performance

The optimized analytical methodology was validated as described in section 2.4. Table 4 shows the results obtained in seawater fortified with the target pesticides at a concentration of 50 ng/L. The performance of the method at 5 ng/L and 100 ng/L is provided in Table 5.

The linearity range of the proposed methodology for the different analytes expanded between their specific LOQ and the upper limit of the linearity range. Considering that the linearity is based on the response of calibration curve solutions prepared in MeOH, where target analytes are not subject to extraction losses or matrix effects, and method sensitivity (LOD and LDet) is based on the response of seawater samples spiked at different levels, the linearity range of the method expands below the lowest analyte concentrations that can be quantified in seawater (see Table 4). The shortest linearity ranges (about two orders of magnitude) were observed for azinphos ethyl, fenitrothion, and fenthion sulfone. A minimum of 6 calibration points were used to construct the linearity models based on least-square linear regression. Coefficients of determination (r^2) above 0.99 were obtained in all cases.

Figure 3. Extracted SRM chromatograms for the target analytes obtained after analysis of a seawater sample fortified at a concentration of 50 ng/L.

As explained in section 2.4, the absolute and relative recoveries in seawater were calculated at three levels (5, 50 and 100 ng/L). Method ARs were comparable at all concentrations tested, except in the case of alachlor, bentazone, fenitrothion, fenthion sulfone, and metolachlor, for which

relative variations over the three concentration levels were between 21 and 43 %. However, these variations were well compensated by the use of isotopically labeled compounds as SS, as the RRs of these pesticides varied less than 9% over the evaluated concentrations, with the exception of fenitrothion, for which the recovery variation decreased from 28% to 13% when corrected by its corresponding SS..

Table 4. Method performance.

Pesticide	Linearity		Recovery (n=5, 50 ng L ⁻¹)			LOD (ng L ⁻¹)	LDet (ng L ⁻¹)
	r ²	Range (ng/L)	Absolute (%)	Relative (%)	RSD (%)		
2,4-D	0.9994	0.25-400	61	81	21	0.05	0.70
ALA	0.9938	0.5-400	61	121	14	0.35	1.20
ATR	0.9966	0.025-400	69	103	2	0.02	0.10
AZET	0.9985	1.5-240	82	101	6	0.32	2.29
BEN	0.9978	0.05-400	58	99	15	0.04	0.13
CFP	0.9975	0.25-400	63	105	4	0.11	0.36
CHLO	0.9979	0.05-400	62	108	12	0.03	0.27
CYA	0.9989	0.025-400	44	97	5	0.03	0.28
DEA	0.9996	0.25-400	30	123	11	0.16	0.55
DIAZ	0.9997	0.025-400	63	103	3	0.03	0.06
DIME	0.9951	0.5-400	57	104	10	0.23	0.77
DIU	0.9972	0.025-400	68	95	18	0.02	0.08
FEN	0.9977	2.5-400	120	108	11	1.76	27.0
FENOX ^a	0.9946	0.2-320	66	99	21	0.10	0.32
FENSN	0.9996	5-400	77	121	21	3.71	14.5
FENSX	0.9996	0.25-400	82	122	18	0.10	0.38
IRG	0.9999	0.05-400	87	102	2	0.05	0.17
ISO	0.9967	0.5-400	69	101	4	0.01	0.51
LINU	0.9950	0.5-400	86	108	9	0.26	0.97
MCPA	0.9994	0.25-400	68	111	15	0.07	7.69
MECO	0.9993	0.25-400	78	99	11	0.03	0.51
METO	0.9987	0.25-400	59	100	2	0.03	0.31
PROP ^a	0.9998	0.05-400	102	91	13	0.10	0.32
SIMA	0.9982	0.025-400	51	109	7	0.05	0.15
TER	0.9999	0.005-400	86	101	5	0.01	0.05
TBT	0.9998	0.05-400	88	103	1	0.03	0.09

^a compound quantified using a structurally related or closely eluting compound as surrogate standard.

Table 5. Recovery and method repeatability at the lowest and the highest levels tested (5 ng/L and 100 ng/L), and instrumental sensitivity for the target analytes.

Pesticide	Recovery (n=5, 5 ng/L) ^a			Recovery (n=5, 100 ng/L) ^a			Instrumental sensitivity (ng/L) ^b	
	AR (%)	RR (%)	RSD (%)	AR (%)	RR (%)	RSD (%)	LOD	LDet
2,4-D	74	104	20	55	107	29	0.04	0.61
ALA	115	112	12	78	102	5	0.17	0.57
ATR	81	110	3	64	104	5	0.01	0.07
AZET	58	64	22	80	117	7	0.27	0.93
BEN	83	109	22	60	117	18	0.03	0.09
CFP	79	84	12	68	98	4	0.11	0.37
CHLO	67	92	10	68	103	10	0.03	0.17
CYA	44	99	10	42	102	2	0.02	0.14
DEA	26	105	12	21	99	2	0.08	0.26
DIAZ	59	99	3	66	103	3	0.01	0.04
DIME	53	90	21	58	93	9	0.30	1.01
DIU	77	129	28	67	91	10	0.02	0.07
FEN	< LDet	< LDet	< LDet	81	130	13	1.15	13.2
FENOX ^c	58	66	30	67	83	19	0.03	0.10
FENSN	< LDet	< LDet	< LDet	49	128	17	1.16	3.85
FENSX	81	93	28	82	103	26	0.05	0.26
IRG	118	103	3	86	103	2	0.04	0.29
ISO	89	103	12	81	104	18	0.01	0.49
LINU	63	89	10	74	101	9	0.48	1.60
MCPA	< LDet	< LDet	< LDet	56	96	9	0.05	3.59
MECO	106	105	12	77	99	6	0.03	0.54
METO	117	101	5	81	102	3	0.03	0.50
PROP ^c	86	160	23	72	116	11	0.05	0.15
SIMA	56	117	21	44	108	1	0.02	0.08
TER	69	101	4	62	105	11	0.01	0.04
TBT	113	98	2	86	104	1	0.02	0.06

^a AR: absolute recovery, RR: relative recovery, RSD: relative standard deviation of n=5 replicate analysis

^b Lowest concentration of the analyte that can be detected (LOD) and quantified (SRM1>LOQ and SRM2>LOD), LDet) by LC-ES-MS/MS analysis.

^c analyte quantified using a structurally related or closely eluting deuterated compound.

The lowest AR (average of three levels) was observed for desethylatrazine (26%) which is a very polar pesticide (Log Kow = 1.5) and present high solubility (2,700 mg/L) and may be partially washed out from the cartridge when extracting sample volumes above 500 mL (see Figure 1). On the contrary, 52% of the target pesticides presented average AR above 70%. Low average ARs were also well corrected by the use of isotopically labeled analogs as SS. In this regard, as shown in

33 Table 4, RRs were satisfactory (between 80 and 120%) for almost all compounds. Slight deviations
34 from the acceptable RR range were observed at 5 ng/L for diuron, at 50 ng/L for alachlor,
35 desethylatrazine, fenthion sulfone, and fenthion sulfoxide, and at 100 ng/L for fenitrothion and
36 fenthion sulfone. The strongest deviations were registered at the lowest concentration tested (5
37 ng/L) for azinphos ethyl (64%), fenthion oxon (66%), and propanil (160%), (see Tables 4 and 5).
38 Note that isotopically labeled analogues were available for 92% of the target compounds and, in the
39 absence of them, structurally related and/or closely eluting deuterated compounds with similar ARs
40 were used as SS for fenthion oxon and propanil. The use of isotopically labeled analogues as SS in
41 similar SPE and LC-MS-based methodologies described in the peer-reviewed literature for the
42 analysis of polar pesticides in seawater covered only 20% (Brumovský et al., 2017) to 30% (Loos et
43 al., 2013) of the target compounds.

44 Method repeatability, expressed as the relative standard deviations (RSD) of the peak areas,
45 obtained after replicate analysis of n=5 fortified seawater samples at each of the evaluated
46 concentrations, was below 18% at high levels (50 ng/L and 100 ng/L) for all analytes, with the
47 exception of fenthion sulfone, fenthion oxon, fenthion sulfoxide, and 2,4-D that presented RSD
48 values up to 29%. These pesticides also showed high variability in the analysis of low
49 concentrations (5 ng/L), together with azinphos ethyl, bentazone, dimethoate, diuron, propanil, and
50 simazine (RSD values between 20 and 30%). These figures comply with the minimum performance
51 criteria (maximum uncertainty of 50% at the level of EQS) required for analytical methods intended
52 for monitoring water status (Directive 2009/90/EC).

53 As for sensitivity, the methodology allowed detection and quantification of the target
54 pesticides in seawater at the pg or low ng/L level (81% of the target pesticides presented average
55 LDet below 1 ng/L). The poorest sensitivity was observed for fenitrothion, fenthion sulfone, and
56 MCPA, with average LDet of 27 ng/L, 15 ng/L, and 8 ng/L, respectively. The sensitivity achieved
57 with this methodology is similar to that reported by other SPE and MS-based methods in the

58 literature, and slightly better than that obtained with different detection techniques (GC-ECD or
59 HPLC-UV). The LDet obtained for the priority substances included in the methodology are 1 to 4
60 orders of magnitude lower than the EQS set for these compounds in other (than inland) surface
61 waters (transitional, coastal and territorial waters) (Directive 2013/3/EU) (e.g., LDet of simazine is
62 0.15 ng/L and its EQS is 1000 ng/L).

63 As for selectivity, the LC-MS/MS approach presented provides the minimum number of
64 identification points (IP) required in the legislation for the analysis of residues of banned substances
65 in animal products (4 IPs), i.e., 1 point per precursor ion and 1.5 points for each product ion
66 recorded (Commission Decision 2002/657/EC), which is very often applied to the environmental
67 analysis field.

68 The extent of the matrix effects observed in the analysis of seawater samples has been
69 summarized in Figure 4. As can be observed, all compounds were affected by ion suppression
70 effects. However, for the compounds that signal suppression was below 20%, i.e., diazinon, diuron,
71 fenitrothion, fenthion sulfone, irgarol, propanil, and terbutryn, the effect of the matrix components
72 in the MS analysis could be considered negligible. The pesticides that were affected the most by
73 matrix components (with signal suppression between 45 and 77%) were cyanazine,
74 desethylatrazine, dimethoate, MCPA, and simazine. This may explain the low AR obtained for
75 these compounds. In fact, taking into consideration these matrix effects, analyte extraction
76 recoveries would be in the range 94 - 121%. Based on the method performance figures
77 obtained and the performance criteria set in the legislation (Directive 2009/90/EC) for analytical
78 methods intended to measure (priority) pollutants in the environment (water, sediments and biota),
79 the methodology presented is suitable for the determination of all target pesticides in coastal waters.

80

Figure 4. Extent of matrix effects observed for each target analyte in the analysis of seawater.

3.3. Application to real samples

After validation, the analytical method developed was used to monitor the presence of the target pesticides in different seawater samples collected along the Catalan coast. Concentrations of pesticides positively identified in the samples have been summarized in Table 6. As detection frequencies were in general very low (below 50% for all analytes except atrazine (65%) and irgarol (70%)), non-detects were not considered for the calculation of mean and median concentrations, and in the case of concentrations below the LDet, half of the corresponding LDet was used.

Dimethoate and linuron were the only currently allowed pesticides that were not detected in the investigated aquatic marine environments. Most of the target banned pesticides were also not detected, which suggests a positive impact of policy measurements on water quality. The most ubiquitous banned pesticides in the area were terbutryn and atrazine that were present in 70 and

65% of the samples, respectively, and the currently allowed pesticides irgarol (70%) and diuron (50%). As for the levels of concentrations measured, currently allowed pesticides presented median and average concentrations in the area above those registered for banned substances. The most abundant banned pesticides were simazine and metolachlor with maximum concentrations of 24 ng/L and 5.3 ng/L, respectively. MCPA, bentazone, and irgarol presented the highest maximum (672 ng/L, 514 ng/L, and 85 ng/L, respectively) and median concentrations (94 ng/L, 19 ng/L, and 20 ng/L, respectively) of all investigated pesticides.

Table 6. Pesticides concentration in aquatic marine environments investigated along the Catalan coast.

Pesticide	Frequency of detection (%) (n=40)	Median conc (ng/L) ^c	Mean conc (standard deviation) (ng/L) ^c	Max conc (ng/L)
<i>EC legal status: banned</i>				
Atrazine (ATR)	65	0.98	1.06 (0.57)	3.75
Azinphos Ethyl (AZET)	3	<LDet ^b	<LDet ^b	<LDet ^b
Diazinon (DIAZ)	43	0.19	0.22 (0.17)	0.71
Fenthion sulfoxide (FENSX) ^a	8	0.69	1.01 (0.56)	1.67
Metolachlor (METO)	20	0.86	1.72 (1.80)	5.28
Simazine (SIMA)	43	1.26	2.85 (5.64)	24.2
Terbutryn (TBT)	70	0.22	0.29 (0.22)	0.91
<i>EC legal status: allowed</i>				
2,4-D	8	11.0	15.3 (7.95)	24.5
Bentazone (BEN)	23	19.2	134 (211)	514
Chlortoluron (CHLO)	3	6.54	6.54 (-)	6.54
Diuron (DIU)	50	2.19	4.25 (5.39)	20.1
Irgarol (IRG)	70	20.2	25.0 (25.1)	85.4
Isoproturon (ISO)	15	9.49	24.2 (24.3)	56.2
MCPA	13	93.9	199 (267)	672
Mecoprop (MECO)	3	1.12	1.12 (-)	1.12
Terbuthylazine (TER)	40	0.78	5.34 (14.6)	59.4

^a Transformation product from EC banned or priority pesticide

^b LDet: 2.29 ng/L

^c Median and mean concentrations were calculated considering only positive samples.

Banned pesticides that were not detected in any sample: alachlor, chlorfenvinphos, cyanazine, desethylatrazine, fenitrothion, and its transformation products (fenthion oxon and fenthion sulfone), and propanil

Allowed pesticides that were not detected in any sample: dimethoate and linuron

113 Attending to their distribution in the investigated area (see Figure 5), pesticide
 114 concentrations were usually higher inside than outside the marinas, contrary to what was observed
 115 for other marine pollutants (lipophilic biotoxins) in the same sampling locations that did not present
 116 a specific distribution pattern (Bosch-Orea et al., 2017). Total pesticide concentrations in all
 117 seawater samples were below 115 ng/L, with the exceptions of those collected at the Ebro Delta
 118 area inside all marinas and outside marina 18. The latter presented total pesticide concentrations in
 119 the µg/L level. Excluding the Ebro River delta and other marinas with extremely low (11-IN) or
 120 high total pesticide concentrations (14-IN and 15-IN), the pesticide that contributed the most to total
 121 pesticide loads inside the marinas was irgarol (45-99%), which is commonly used as a booster
 122 biocide antifouling agent. In those marinas where irgarol was responsible for 45-75% of the total
 123 pesticide concentrations, the second most abundant pesticide in the mixture was diuron (3-IN, 9-IN,
 124 10-IN) (also a biocide with antifouling applications) or 2,4-D (14-IN).
 125

126
 127 **Figure 5.** Pesticide concentrations in the different investigated provinces along the coastline of
 128 Catalonia. (DIU: diuron, SIMA: simazine, ISO: isoproturon, TER: terbuthylazine, IRG: irgarol,
 129 BEN: bentazone, others: atrazine, azinphos ethyl, diazinon, chlortoluron, fenthion sulfoxide,
 130 mecoprop, metolachlor, and terbutryn).
 131

132 Outside the investigated marinas, the pesticides contributing most to total pesticide
133 concentrations varied among locations, with the most abundant compounds overall: diuron,
134 atrazine, simazine, terbuthylazine, and in a few locations, irgarol.

135 The pesticide contamination pattern in the Ebro Delta was dominated by the presence of
136 bentazone and MCPA in both delta bays (sites 18 and 20), in agreement with previous reports
137 (Köck et al., 2010), and by MCPA and isoproturon in the marina located at the Ebro River mouth
138 (site 19). This pollution pattern is attributed to the intensive agricultural activity that is carried out
139 in the area and, in particular, rice cultivation since MCPA and bentazone herbicides are applied to
140 protect this crop. The elevated pesticide concentrations observed in location 18 could be explained
141 by the direct discharge of agricultural drainage channels into the northern delta bay that is also
142 comparatively smaller in size and depth than the delta southern bay (site 20), and consequently has
143 less dilution capacity, as observed in a previous study (Köck et al., 2010). MCPA and bentazone
144 were found at lower levels in open sea water of the Adriatic Sea (0.07-0.15 ng/L of bentazone and
145 0.03-0.43 ng/L of MCPA) (Loos et al., 2013) and not detected in open sea water in the
146 Mediterranean Sea and the Black Sea (Orlikowska et al., 2015).

147 Concentrations of booster biocides used in antifouling paints detected along the Catalan
148 coast (0.3-20 ng/L for diuron and 0.2-85 ng/L for irgarol) are below those reported for the coast of
149 Izmir, Turkey (92-1779 ng/L of diuron, and 114-998 ng/L of irgarol) (Cavas et al., 2016), the Gulf
150 of Napoli, Italy (1-1380 ng/L of diuron and 0.2-173 ng/L of irgarol) (Di Landa et al., 2009), ports
151 along the Malaysian coast (up to 285 ng/L of diuron) (Ali et al., 2014), ports located along the
152 Tarragona coast fifteen years ago (100-600 ng/L of diuron and 15-370 ng/L of irgarol) (Gimeno et
153 al., 2001), and ports and marinas located at the Canary Islands (2-195 ng/L of diuron and 2-146 of
154 irgarol) (Sánchez-Rodríguez et al., 2011). On the contrary, diuron and irgarol concentrations
155 measured in the present study are above those detected in the upper water layer offshore at the
156 Mediterranean Sea (maximum concentration of 3.6 ng/L of irgarol, and diuron below the method

157 limit of detection in all samples) (Orlikowska et al., 2015), and in open water samples of the
158 Adriatic Sea (0.40-1.96 ng/L of diuron and <0.007-0.13 ng/L for irgarol) (Loos et al., 2013).
159 Finally, a similar contamination profile by irgarol and diuron was reported for harbors and open
160 seawater from the Persian Gulf (Saleh et al., 2014), where diuron and irgarol were found at 29 and
161 63 ng/L, respectively, in harbors and were not detected in open seawater.

162 Contrary to this study, in which organophosphate pesticides were either not detected or
163 presented very low average concentrations (0.2 ng/L of diazinon), these compounds were detected
164 at relative higher average concentrations in Maizuru Bay, Japan (6 ng/L of diazinon and 19 ng/L of
165 fenitrothion) (Harino et al., 2013), and also in the Ebro Delta area in previous studies (10 ng/L of
166 diazinon and 289 ng/L of fenitrothion) (Köck et al., 2010).

167 The triazine herbicides detected in the investigated area (atrazine, simazine, terbuthylazine,
168 and terbutryn) presented in general higher levels (0.63-3.75 ng/L of atrazine, 0.27-24.19 ng/L of
169 simazine, 0.27-59.4 ng/L of terbuthylazine, and 0.06-0.91 ng/L of terbutryn) than those measured in
170 the open sea of the Adriatic Sea (offshore Venice) (0.59-1.49 ng/L of atrazine, 0.74-1.57 ng/L of
171 simazine, 0.77-8.90 ng/L of terbuthylazine and 0.12-0.55 ng/L of terbutryn) (Loos et al., 2013), or
172 the Baltic Sea (maximum concentrations of 2.6 ng/L of atrazine, 3.5 ng/L of simazine, 3.8 ng/L of
173 terbuthylazine, and non detected levels of terbutryn) (Orlikowska et al., 2015), or those recently
174 measured in the western basin of the Mediterranean Sea (terbuthylazine 0.1-0.3 ng/L) (Brumovský
175 et al., 2017). Levels of atrazine measured in the Catalan coastal marine environment were below
176 those reported in open seawater of the Black Sea (maximum concentration of 50.7 ng/L), whereas
177 simazine levels were above those measured in the Black Sea (maximum concentration of 10.9 ng/L
178 of simazine) (Orlikowska et al., 2015).

179 According to the Marine Directive 2008/56/EC, the achievement of a good environmental
180 status of the marine waters in Europe requires that contaminant levels (including pesticides) in the
181 marine environment do not pose a significant risk to aquatic organisms (2010/477/EU) . In

consequence, measurements have to be adopted so that priority substances do not exceed EQS in coastal or territorial waters adjacent to the marine environments. Among the investigated priority substances (irgarol, isoproturon, diuron, simazine, atrazine, terbutryn) only irgarol was present in 70% of the samples collected inside the marinas (26-85 ng/L) above MAC set for this pesticide in Europe (16 ng/L). It was also measured above its MAC in one of the samples collected outside the marina (M12, 29 ng/L). The remaining investigated priority substances were measured at maximum concentrations several orders of magnitude below their corresponding MACs (34 ng/L of terbutryn, 1000 ng/L of isoproturon and simazine, 1800 ng/L of diuron, and 2000 ng/L of atrazine).

4. Conclusions

A reliable (RR of 80-120% and RSD<18% for most compounds and use of surrogate standards for 92% of the analytes), sensitive (LDet at the pg or low ng/L level) and selective analytical method has been proposed and validated to analyze a variety of medium to highly polar pesticides in seawater. The effect of the sample volume extracted and the cartridge wash volume in analyte breakthrough and matrix effect removal was evaluated to maximize method sensitivity. The quantification with the isotope dilution method was valid to compensate for low absolute recoveries, matrix interferences or sensitivity variations during the analysis.

Application of this method to real samples collected inside and outside of marinas located at the Catalan coast revealed the ubiquitous presence of currently allowed pesticides (irgarol and diuron) and EU-banned pesticides (atrazine and terbutryn) in seawater along the coastline. Overall, levels of banned pesticides were much lower than those observed for currently allowed pesticides. This could be a direct consequence of their ban of use several years ago. However, the detection of few of them in seawater is also indicative of their persistence in the environment. Irgarol, one of the priority substances included in EU regulations, was measured above the EU MAC in 70% of the samples collected inside the marinas. Measures have to be adopted to reduce these concentrations

207 below MAC so that the marine environment along the Catalan coast can achieve a good
208 environmental status.

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220 **REFERENCES**

- 221 2002/657/EC Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC
222 concerning the performance of analytical methods and the interpretation of results (Text
223 with EEA relevance) (notified under document number C(2002) 3044)
- 224 2006/118/EC Directive of the European Parliament and of the Council of 12 December 2006 on the
225 protection of groundwater against pollution and deterioration. Official Journal of the
226 European Union L 372/19.
- 227 2008/56/EC Directive of the European Parliament and of the Council of 17 June 2008 establishing a
228 framework for community action in the field of marine environmental policy (Marine
229 Strategy Framework Directive). Official Journal of the European Union L 164/19.
- 230 2009/90/EC Commission Directive of 31 July 2009 laying down, pursuant to Directive 2000/60/EC
231 of the European Parliament and of the Council, technical specifications for chemical
232 analysis and monitoring of water status. Official Journal of the European Union L 201/36
- 233 2010/477/EU Commission Decision of 1 September 2010 on criteria and methodological standards
234 on good environmental status of marine waters (notified under document C(2010) 5956).
235 Official Journal of the European Union L 232/14.
- 236 2013/39/EU Directive of the Parliament and of the Council of 12 August 2013 amending Directives
237 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
238 Official Journal of the European Union L 226/1.
- 239 Ali HR, Arifin MM, Sheikh MA, Shazili NAM, Bakari SS, Bachok Z. Contamination of diuron in
240 coastal waters around Malaysian Peninsular. *Marine Pollution Bulletin* 2014; 85: 287-91.
- 241 Bosch-Orea C, Sanchís J, Farré M, Barceló D. Analysis of lipophilic marine biotoxins by liquid
242 chromatography coupled with high-resolution mass spectrometry in seawater from the
243 Catalan Coast. *Analytical and Bioanalytical Chemistry* 2017; 409: 5451-62.
- 244 Brumovský M, Bečánová J, Kohoutek J, Borghini M, Nizzetto L. Contaminants of emerging
245 concern in the open sea waters of the Western Mediterranean. *Environmental Pollution*
246 2017; 229: 976-83.
- 247 Carafa R, Wollgast J, Canuti E, Ligthart J, Dueri S, Hanke G, et al. Seasonal variations of selected
248 herbicides and related metabolites in water, sediment, seaweed and clams in the Sacca di
249 Goro coastal lagoon (Northern Adriatic). *Chemosphere* 2007; 69: 1625-37.
- 250 Catalá-Icardo M, Lahuerta-Zamora L, Torres-Cartas S, Meseguer-Lloret S. Determination of
251 organothiophosphorus pesticides in water by liquid chromatography and post-column
252 chemiluminescence with cerium(IV). *Journal of Chromatography A* 2014; 1341: 31-40.

253 Cavas L, Donut N, Mert N. Artificial neural network modeling of diuron and irgarol-based HPLC
 254 data and their levels from the seawaters in Izmir, Turkey. *Journal of Liquid Chromatography*
 255 & Related Technologies 2016; 39: 87-95.

256 Di Landa G, Parrella L, Avagliano S, Ansanelli G, Maiello E, Cremisini C. Assessment of the
 257 Potential Ecological Risks Posed by Antifouling Booster Biocides to the Marine Ecosystem
 258 of the Gulf of Napoli (Italy). *Water, Air, and Soil Pollution* 2009; 200: 305-21.

259 Gao T, Du P, Xu Z, Li X. Occurrence of new psychoactive substances in wastewater of major
 260 Chinese cities. *Science of the Total Environment* 2017; 575: 963-9.

261 Gatidou G, Kotrikla A, Thomaidis NS, Lekkas TD. Determination of the antifouling booster
 262 biocides irgarol 1051 and diuron and their metabolites in seawater by high performance
 263 liquid chromatography–diode array detector. *Analytica Chimica Acta* 2005; 528: 89-99.

264 Gimeno RA, Aguilar C, Marcé RM, Borrull F. Monitoring of antifouling agents in water samples
 265 by on-line solid-phase extraction–liquid chromatography–atmospheric pressure chemical
 266 ionization mass spectrometry. *Journal of Chromatography A* 2001; 915: 139-47.

267 Habedank F, Abraham M, Tardel H, Feldhusen F, Schulz-Bull DE. Determination of
 268 organophosphate pesticides in sea and surface water with ultrasound-assisted dispersive
 269 liquid–liquid micro-extraction coupled to GC-MS/MS analysis. *International Journal of*
 270 *Environmental Analytical Chemistry* 2017; 97: 819-30.

271 Harino H, Yatsuzuka E, Yamao C, Ueno M, Ohji M. Current status of organophosphorus
 272 compounds contamination in Maizuru Bay, Japan. *Journal of the Marine Biological*
 273 *Association of the United Kingdom* 2013; 94: 43-9.

274 Huang S, He S, Xu H, Wu P, Jiang R, Zhu F, et al. Monitoring of persistent organic pollutants in
 275 seawater of the Pearl River Estuary with rapid on-site active SPME sampling technique.
 276 *Environmental Pollution* 2015; 200: 149-58.

277 Köck-Schulmeyer M, Olmos M, López de Alda M, Barceló D. Development of a multiresidue
 278 method for analysis of pesticides in sediments based on isotope dilution and liquid
 279 chromatography-electrospray–tandem mass spectrometry. *Journal of Chromatography A*
 280 2013; 1305: 176-87.

281 Köck M, Farré M, Martínez E, Gajda-Schranz K, Ginebreda A, Navarro A, et al. Integrated
 282 ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro
 283 River delta (Spain). *Journal of Hydrology* 2010; 383: 73-82.

284 Konstantinou IK, Albanis TA. Worldwide occurrence and effects of antifouling paint booster
 285 biocides in the aquatic environment: A review. *Environment International* 2004; 30: 235-48.

286 Loos R, Tavazzi S, Paracchini B, Canuti E, Weissteiner C. Analysis of polar organic contaminants
 287 in surface water of the northern Adriatic Sea by solid-phase extraction followed by
 288 ultrahigh-pressure liquid chromatography–QTRAP® MS using a hybrid triple-quadrupole
 289 linear ion trap instrument. *Analytical and Bioanalytical Chemistry* 2013; 405: 5875-85.
 290 Mehdinia A, Einollahi S, Jabbari A. Magnetite nanoparticles surface-modified with a zinc(II)-
 291 carboxylate Schiff base ligand as a sorbent for solid-phase extraction of organochlorine
 292 pesticides from seawater. *Microchimica Acta* 2016a; 183: 2615-22.
 293 Mehdinia A, Rouhani S, Mozaffari S. Microwave-assisted synthesis of reduced graphene oxide
 294 decorated with magnetite and gold nanoparticles, and its application to solid-phase
 295 extraction of organochlorine pesticides. *Microchimica Acta* 2016b; 183: 1177-85.
 296 Mézin LC, Hale RC. Combined effects of humic acids and salinity on solid-phase microextraction
 297 of DDT and chlorpyrifos, an estimator of their bioavailability. *Environmental Toxicology*
 298 *and Chemistry* 2004; 23: 576-82.
 299 Moro L, Pezzotti G, Turemis M, Sanchís J, Farré M, Denaro R, et al. Fast pesticide pre-screening in
 300 marine environment using a green microalgae-based optical bioassay. *Marine Pollution*
 301 *Bulletin* 2018; 129: 212-21.
 302 Orlikowska A, Fisch K, Schulz-Bull DE. Organic polar pollutants in surface waters of inland seas.
 303 *Marine Pollution Bulletin* 2015; 101: 860-6.
 304 Pérez-Carrera E, León VML, Parra AG, González-Mazo E. Simultaneous determination of
 305 pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in seawater and
 306 interstitial marine water samples, using stir bar sorptive extraction–thermal desorption–gas
 307 chromatography–mass spectrometry. *Journal of Chromatography A* 2007; 1170: 82-90.
 308 Pinto MI, Burrows HD, Sontag G, Vale C, Noronha JP. Priority pesticides in sediments of
 309 European coastal lagoons: A review. *Marine Pollution Bulletin* 2016; 112: 6-16.
 310 Rodríguez-González N, Beceiro-González E, González-Castro MJ, Alpendurada MF. On-line solid-
 311 phase extraction method for determination of triazine herbicides and degradation products in
 312 seawater by ultra-pressure liquid chromatography–tandem mass spectrometry. *Journal of*
 313 *Chromatography A* 2016; 1470: 33-41.
 314 Rodríguez-González N, Beceiro-González E, González-Castro MJ, Muniategui-Lorenzo S. An
 315 environmentally friendly method for the determination of Triazine herbicides in estuarine
 316 seawater samples by dispersive liquid–liquid microextraction. *Environmental Science and*
 317 *Pollution Research* 2015; 22: 618-26.

318 Said TO, Okbah MA, Mohamed LA, Othman IM. Detection of persistent OCPs and PCBs
 319 congeners in the near-shore coastal waters of Alexandria, Egypt. *Environmental Monitoring*
 320 *and Assessment* 2015; 187.

321 Saleh A, Sheijooni Fumani N, Molaei S. Microfunnel-supported liquid-phase microextraction:
 322 Application to extraction and determination of Irgarol 1051 and diuron in the Persian Gulf
 323 seawater samples. *Journal of Chromatography A* 2014; 1356: 32-7.

324 Sánchez-Avila J, Quintana J, Ventura F, Tauler R, Duarte CM, Lacorte S. Stir bar sorptive
 325 extraction-thermal desorption-gas chromatography–mass spectrometry: An effective tool for
 326 determining persistent organic pollutants and nonylphenol in coastal waters in compliance
 327 with existing Directives. *Marine Pollution Bulletin* 2010; 60: 103-12.

328 Sánchez-Rodríguez Á, Sosa-Ferrera Z, Santana-del Pino Á, Santana-Rodríguez JJ. Probabilistic risk
 329 assessment of common booster biocides in surface waters of the harbours of Gran Canaria
 330 (Spain). *Marine Pollution Bulletin* 2011; 62: 985-91.

331 Saranjampour P, Vebrosky EN, Armbrust KL. Salinity impacts on water solubility and n-
 332 octanol/water partition coefficients of selected pesticides and oil constituents.
 333 *Environmental Toxicology and Chemistry* 2017; 36: 2274-80.

334 Shams N, Lim HN, Hajian R, Yusof NA, Abdullah J, Sulaiman Y, et al. A promising
 335 electrochemical sensor based on Au nanoparticles decorated reduced graphene oxide for
 336 selective detection of herbicide diuron in natural waters. *Journal of Applied*
 337 *Electrochemistry* 2016; 46: 655-66.

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