



Halogenated and organophosphorus flame retardants in European aquaculture samples



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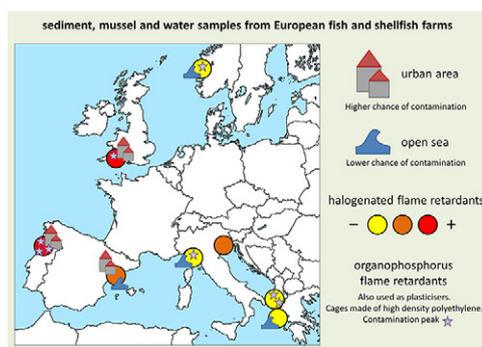
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HIGHLIGHTS

- Flame retardants in European fish and shellfish farms: water, sediment, mussels.
- PBDEs were in 95% of the sediments ($8.6 \pm 23 \text{ ng g}^{-1} \text{ dw}$) and mussels ($<10 \text{ ng g}^{-1} \text{ lw}$).
- DBDPE was at levels lower than BDE-209 in sediment and similar levels in mussels.
- OPFRs levels were much higher; they are plasticisers and plastics are used in farms.
- Farms away from urban shores and river mouths minimize flame retardants input.

GRAPHICAL ABSTRACT



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ABSTRACT

This work monitors flame retardants in sediment, mussel and water samples from European fish farms. Polybrominated diphenyl ethers (PBDEs) were detected in 95% of the sediment and mussel samples with mean levels of $8.60 \pm 22.6 \text{ ng g}^{-1} \text{ dw}$ in sediments and $0.07 \pm 0.18 \text{ ng g}^{-1} \text{ dw}$ in mussels. BDE-209 was the main contributor for the sediments and BDE-47 was found in about 60% of the samples of both matrices. Pentabromoethylbenzene (PBEB) and hexabromobenzene (HBB) were detected in 42% of the sediments, but not in mussels. Decabromodiphenyl ethane (DBDPE) was found in about 55% of the samples of both matrices. The same happened for dechloranes in mussels, but they were detected in 92% of the sediments. *Syn*-DP and *anti*-DP were always the main contributors. Methoxylated PBDEs (MeO-PBDEs) were detected in all mussels and some sediments, mainly 6-MeO-BDE-47 and 2'-MeO-BDE-68. Organophosphorus flame retardants (OPFRs) were found in all matrices with concentrations of $0.04\text{--}92.8 \text{ ng g}^{-1} \text{ dw}$ in sediment, $0.50\text{--}102 \text{ ng g}^{-1} \text{ dw}$ in mussel and $0.43\text{--}867 \text{ ng l}^{-1}$ in water. Only OPFRs were analysed in water samples as halogenated flame

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Seafood
Persistent organic pollutants

retardants and MeO-PBDEs are highly unlikely to be detected in water due to their physicochemical properties. Flame retardants have no application in fish farming so results should reflect the impact of human activity on the farm locations. A large majority of the most contaminated samples were collected from sampling spots that were at urban shores or in enclosed water bodies not completely open to the sea.

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1. Introduction

Environmental contaminants in seafood are a hot topic for the scientific community and different research projects study seafood contamination or the presence of these compounds in the water surrounding aquaculture systems. This work monitors flame retardants (FRs) in samples from European fish and shellfish farms.

FRs are compounds that applied to plastics, electronic devices, furniture, vehicles, etc. increase the fire resistance of these materials (Alaee et al., 2003). The most used FRs are polybrominated diphenyl ethers (PBDEs). Typically, PBDEs are produced at three levels of bromination, including Penta-BDE, Octa-BDE and Deca-BDE, and are classified according to their average bromine content. Because these compounds are simply blended into polymers instead of covalently bonded, they are constantly released from materials (Alaee et al., 2003). Thus, PBDEs have been found in all kind of environmental matrices like sediment, sludge and water (Gorga et al., 2013; Guerra et al., 2010; Sánchez-Avila et al., 2011), and in biological matrices like seafood (Aznar-Alemaný et al., 2017; Lacorte et al., 2010).

PBDEs are considered persistent organic pollutants (POPs) by the Stockholm Convention on Persistent Organic Pollutants (Convention, 2008). Criteria for POPs are for compounds to persist in the environment, to accumulate in food chains, to have potential for long-range transportation and to have toxic effects on the environment and humans. PBDEs can affect hormonal regulation and thyroid, liver and neuronal activity (Branchi et al., 2003; Costa and Giordano, 2011; Mikula and Svobodova, 2006). For these reasons, before the inclusion of Penta-BDE and Octa-BDE in the Stockholm Convention in 2011, their sale was already banned in the European Union (EU) in 2004 under Directive, 2003/11/EC (in concentrations higher than 0.1% by mass) (Directive, 2003). Deca-BDE is currently in the Stockholm Convention as well since summer 2017 and it has been banned in the EU since 2008 by the European Court of Justice, case C-14/06 (Judgment, 2008). On the other hand, in 2010 Deca-BDE was also added to the REACH regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals) (REACH, 2006), which provides a legislative framework for chemicals manufacture and use in Europe. Additionally, the EU Marine Strategy Framework Directive (MSFD) (Directive 2008/56/CE) establishes requirements to achieve a good environmental status of the marine environment by 2020 (MSFD, 2008). One of the MSFD eleven descriptors focuses on meeting the existing regulations on contaminants in seafood. The MSFD also highlights some compounds with no regulated limits that should be monitored, including PBDEs (the 8 congeners in this study), and monitoring decabromodiphenyl ethane (DBDPE) and hexabromobenzene (HBB) is recommended too (Swartembroux et al., 2010).

DBDPE and HBB, along with pentabromoethylbenzene (PBEB), are emerging flame retardants (EFRs) now used as alternatives to PBDEs. Since their production increased for three decades, they have been included in monitoring programs (Covaci et al., 2011). To meet the REACH regulation requirements, these substitutes should be safer for the environment and human health than PBDEs.

Dechloranes—including Dechlorane Plus (DP) and Dec 602, Dec 603 and Dec 604—are also EFRs (Zhu et al., 2014). Their behaviour and occurrence in the environment have become a topic of interest in the last decade. They have been found in several environmental matrices, including, but not limited to, sediment, sludge, water and seafood (Aznar-Alemaný et al., 2017; Hong et al., 2010; Houde et al., 2014; Sverko et al., 2007; Torre et al., 2010).

Apart from halogenated flame retardants (HFRs), there are also organophosphorus flame retardants (OPFRs). They accounted for 20% of the FR use in 2006 in Europe — twice as much as brominated FRs — and have been increasingly applied after the ban on PBDEs (Van der Veen and de Boer, 2012). However, inorganic FRs were preferred, accounting for the remaining 70%. Like PBDEs, OPFRs leak from materials and can access environmental matrices through deposition, washout, infiltration, etc. (Andresen et al., 2004; Schreder and La Guardia, 2014). Additionally, OPFRs are used as plasticisers; hence they can be released from the tones of plastic present in seas and oceans. OPFRs have been found in sediments, fish and water (Chung and Ding, 2009; Gao et al., 2014; Giulivo et al., 2016). OPFRs can have toxic reproductive, systemic and endocrine and carcinogenic effects (Hou et al., 2016; Van der Veen and de Boer, 2012).

On top of anthropogenic compounds, methoxylated PBDEs (MeO-PBDEs) are produced by red algae or sponges and, therefore, occur naturally in the marine environment (Vetter et al., 2002). As the organisms that produce MeO-PBDEs live in the sea, these compounds cause concern only in sea waters. Their concentrations are similar to PBDEs' or even higher at further points away from the shore (Vetter et al., 2002). They have been found in cetaceans or seafood (Alonso et al., 2014; Aznar-Alemaný et al., 2017; Losada et al., 2009) around the world.

This work studied the occurrence of PBDEs, HBB, DBDPE, PBEB, MeO-PBDEs, dechloranes and OPFRs in sediment, mussels and water from different European fish and shellfish farming sites. As these compounds have no application in aquaculture activities, this is a study of the environmental contamination at the location of said farms.

2. Materials and methods

2.1. Sampling

Sediment ($n = 24$), mussel ($n = 17$) and water ($n = 27$) samples were collected in summer 2016 from fish farms and shellfish farms from Albania, Greece, Italy, Norway, Portugal, Spain and the United Kingdom (UK). See Table 1 and Fig. 1 for details.

None of the Albanian, Greek or Italian sampling spots were close to urban areas. The Albanian location was in a lagoon connected to the Ionian Sea and the Greek location was in the open Ionian Sea. The Italian fish farm in the northern Tyrrhenian Sea was 1.5 km off-shore and away from two small towns (about 40,000 inhabitants combined). In that location, mussels were collected at the boundary of the farm and between the cages, while sediment and water samples were collected either inside or outside the farm. The set of samples from the Italian shellfish farm were collected from Sacca di Goro, at the Po River Delta, which is separated from the Adriatic Sea by a sandy barrier. The Norwegian fish farm was about 200 m from urbanisation and close to a road, while the shellfish farm was about 1 km away. The Portuguese sampling spot was the closest to urban areas, in fact, quite surrounded by them. Portuguese mussels were collected from natural banks in the area of the shellfish farm. The Spanish sampling spot was by the shore close to both urban and agricultural land. Spanish mussels were unroot cultivated mussels. The three sampling spots in the UK were at the mouth of river Exe after it broadens. Two spots were right next to the opening to the sea; the third one was slightly further up at an urban shore. Mussels from the UK were collected from that third spot.

As OPFRs are also used as plasticisers, plastic was avoided in the sampling using aluminium or glass containers instead in order to

Table 1
Sampling data.

Country	Farm	Water samples	Coordinates	Sediment samples	Water (%) in sediments ^a	Mussel samples	Water (%) in mussels ^a	Fat (%) in mussels ^a
Albania	Shellfish farm	ALwS1	39°45'18.71"N 20°02'27.72"E	ALsS1	42.6	ALmS1	85.6	1.23
Albania	Shellfish farm	ALwS2	39°45'17.26"N 20°02'08.59"E	ALsS2	53.6	ALmS2	88.4	1.11
Albania	Shellfish farm	ALwS3	39°45'22.74"N 20°02'02.08"E	ALsS3	43.8	ALmS3	88.7	1.09
Greece	Fish farm	GRwF1	39°40'16.9"N 20°04'23.3"E	GRsF1	59.6	–	–	–
Greece	Fish farm	GRwF2	39°40'14.9"N 20°04'21.7"E	GRsF2	51.2	–	–	–
Greece	Fish farm	GRwF3	39°40'12.4"N 20°04'16.6"E	GRsF3	44.4	–	–	–
Italy	Fish farm	ITwF1	Northern Tyrrhenian Sea	ITsF1	41.2	ITmF1	92.5	0.32
Italy	Fish farm	ITwF2	Northern Tyrrhenian Sea	ITsF2	35.1	ITmF2	90.5	0.53
Italy	Fish farm	ITwF3	Northern Tyrrhenian Sea	ITsF3	39.8	ITmF3	90.7	0.62
Italy	Shellfish farm	ITwS1	44°47'43.25"N 12°17'40.72"E	ITsS1	28.7	ITmS1	81.2	1.46
Italy	Shellfish farm	ITwS2	44°47'47.99"N 12°19'22.32"E	ITsS2	42.9	ITmS2	80.2	2.35
Italy	Shellfish farm	ITwS3	44°48'45.64"N 12°18'40.08"E	ITsS3	51.9	ITmS3	75.1	2.95
Norway	Fish farm	NOwF1	60°30'43.9"N 4°55'48.4"E	–	–	–	–	–
Norway	Fish farm	NOwF2	60°30'58.9"N 4°55'30.0"E	NOsF2	40.7	–	–	–
Norway	Fish farm	NOwF3	60°31'15.5"N 4°55'08.0"E	NOsF3	36.9	–	–	–
Norway	Shellfish farm	NOwS1	60°31'03.7"N 4°54'15.7"E	–	–	NOmS1	88.3	0.83
Norway	Shellfish farm	NOwS2	60°31'02.8"N 4°53'59.5"E	–	–	–	–	–
Norway	Shellfish farm	NOwS3	60°30'59.0"N 4°53'31.0"E	NOsS3	62.6	–	–	–
Portugal	Shellfish farm	PTwS1	40°38'40.5"N 8°43'59.3"W	PTsS1	26.6	PTmS1	84.9	0.83
Portugal	Shellfish farm	PTwS2	40°38'37.0"N 8°43'55.8"W	PTsS2	29.6	PTmS2	86.0	1.11
Portugal	Shellfish farm	PTwS3	40°38'22.1"N 8°43'46.0"W	PTsS3	15.5	PTmS3	86.2	1.13
Spain	Shellfish farm	SPwS1	40°37'18.5"N 0°36'32.8"E	SPsS1	39.7	SPmS1	87.5	0.89
Spain	Shellfish farm	SPwS2	40°37'16.4"N 0°37'18.6"E	SPsS2	25.6	SPmS2	84.0	1.20
Spain	Shellfish farm	SPwS3	40°37'31.8"N 0°39'26.9"E	SPsS3	38.0	SPmS3	89.4	0.78
United Kingdom	Shellfish farm	UKwS1	50°36'45.29"N 3°25'45.39"W	UKsS1	16.6	–	–	–
United Kingdom	Shellfish farm	UKwS2	50°36'38.63"N 3°25'57.85"W	UKsS2	27.7	–	–	–
United Kingdom	Shellfish farm	UKwS3	50°37'46.74"N 3°26'52.19"W	UKsS3	42.2	UKmS3	86.4	0.59

^a Referenced to wet weight.

avoid contamination with those compounds. The samples were sent frozen to the analytical laboratories by courier delivery and stored in a freezer at -24°C . Sediments and mussels were freeze-dried at the analytical laboratory. Several whole mussels had been collected from each point and the edible content was combined and homogenised after freeze-drying.

Water content ranged from 15.5–62.6% for sediment samples and from 75.1–92.5% for mussel samples. Lipid content in mussel samples referenced to wet weight (ww) was between 0.32 and 2.95%. See Table 1 for details.

2.2. Standards and reagents

HBB, DBDPE, PBEB and the standard mixture of MeO-PBDEs (5-MeO-BDE-47, 6-MeO-BDE-47, 4'-MeO-BDE-49, 2'-MeO-BDE-68, 5'-MeO-BDE-99, 5'-MeO-BDE-100, 4'-MeO-BDE-101 and 4'-MeO-BDE-103) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Native and ^{13}C -labelled standards mixtures of PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209), *syn*-DP and *anti*-DP isomers and ^{13}C -*syn*-DP were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Dec 602 (95%), Dec 603 (98%) and Dec 604 (98%) were purchased from Toronto Research Chemical Inc. (Toronto, ON, Canada). As for OPFRs, tris(2-butoxyethyl)phosphate (TBOEP), tris(chloroethyl)-phosphate (TCEP) and tris(chloroisopropyl)-phosphate (TCIPP) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 2-ethylhexyldiphenyl phosphate (EHDP) was purchased from AccuStandard (New Haven, CT, USA). Tributyl phosphate (TBP), triphenyl phosphate (TPHP) and tris(1,3-dichloro-2-propyl)phosphate (TDCPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Internal standards d_{15} -TDCPP, d_{27} -TBP, d_{12} -TCEP and $^{13}\text{C}_2$ -TBOEP were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada) and d_{15} -TPHP was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Labelled compounds were used as internal standards. Alumina (0.063–0.2 mm) and copper (b63 μm) were obtained from Merck (Darmstadt, Germany). Acetone, dichloromethane (DCM), hexane, methanol, toluene, water and

sulphuric acid were purchased from Merck (Darmstadt, Germany). Acetonitrile and ethyl acetate, both LC-MS grade, were obtained from Rathburn Chemicals Ltd. (Walkerburn, UK). Methanol and ultrapure water Optima™, LC-MS Grade, were purchased from Fisher Scientific (Loughborough, UK). Al-N cartridges were provided by Biotage (Uppsala, Sweden).

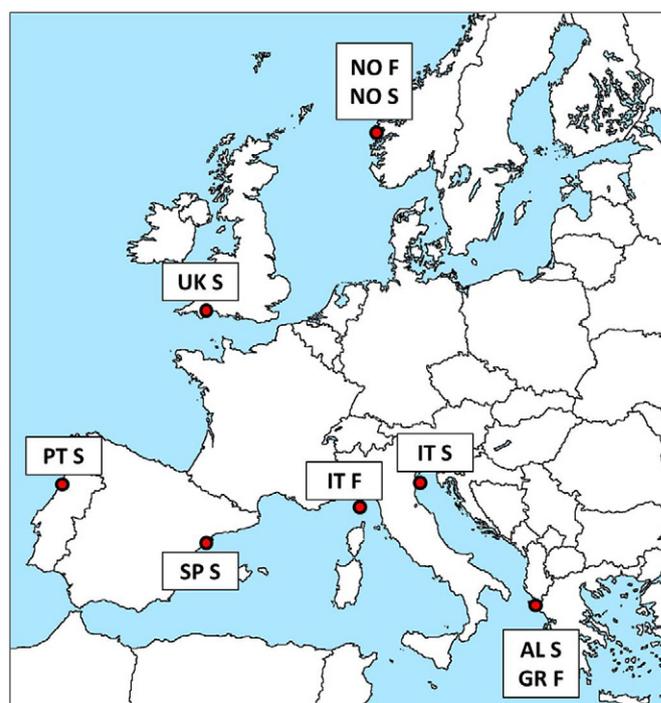


Fig. 1. Sampling locations with codes: F = fish farm, S = shellfish farm, AL = Albania, GR = Greece, IT = Italy, NO = Norway, PT = Portugal, SP = Spain, UK = United Kingdom.

2.3. Sample preparation

The extraction of HFRs and MeO-PBDEs from mussels was carried out using a previously optimized method (de la Cal et al., 2003; Labandeira et al., 2007). Freeze-dried mussel (1.5 g) was spiked with ¹³C-PBDEs and ¹³C-syn-DP. Pressurized liquid extraction (PLE) was used using hexane:DCM (1:1). After extraction, the lipid content was determined gravimetrically. The extract was re-dissolved in hexane and fat was removed with concentrated sulphuric acid. Afterwards, the organic phase underwent a solid phase extraction (SPE) using neutral alumina cartridges (5 g) eluted with hexane:DCM (1:2). Extracts were reconstituted in 40 µl of toluene for the instrumental analysis.

The extraction of HFRs and MeO-PBDEs from sediments was performed by PLE (Barón et al., 2014). Freeze-dried sediment (1.5 g) was spiked with ¹³C-PBDEs and ¹³C-syn-DP. Sample was grown with alumina and copper (1:2:2) and loaded into an extraction cell previously filled with alumina (6 g). PLE conditions were the same as for mussel samples. Extracts were reconstituted in 40 µl of toluene for the instrumental analysis.

The extraction of OPFRs from mussels was carried out by ultrasound assisted extraction according to an existing method (Giulivo et al., 2016). Freeze-dried mussel (0.5 g) was extracted by sonication with hexane:acetone (1:1) twice. The combined extract was reconstituted in 5 ml of hexane:methanol (1:3). The solution was centrifuged and an aliquot of 200 µl was used for the instrumental analysis. Purification was performed on-line at the beginning of the instrumental analysis. Labelled OPFRs standards were added prior to analysis by turbulent flow chromatography coupled to LC-MS/MS (TFC-LC-MS/MS), correcting the possible fluctuations of the analytical instrument.

The extraction of OPFRs from sediments was carried out by ultrasound assisted extraction. Freeze-dried sediment (2.0 g) was spiked with the labelled OPFRs and extracted by sonication with 10 ml of DCM:acetonitrile (1:1) for 10 min three times. The extracts were centrifuged for 5 min at 2500 rpm to collect the supernatants and combined. Elemental sulphur was removed by adding 1 g of activated copper powder. The extract was blown down to 200 µl, 30 ml of water was added and the extracts were purified by SPE on HLB cartridges according to the water extraction protocol. The eluate was blown down to near dryness and reconstituted in 300 µl of ethyl acetate for the instrumental analysis.

The extraction of OPFRs from water samples was carried out with an Oasis HLB 200 mg column. Sample was defrosted overnight and spiked with the labelled OPFRs mixture. Unfiltered sample (700 ml) was loaded onto a pre-conditioned (5 ml methanol) and pre-equilibrated (5 ml ultrapure water) Oasis HLB 200 mg column. Flow rate was maintained at 5–10 ml min⁻¹ using a vacuum manifold and a vacuum pump. After loading, the sorbent was washed with 5 ml of ultrapure water and dried under vacuum for 30 min. Elution was performed by sequential percolation of (1) 3 ml ethyl acetate, (2) 3 ml of ethyl acetate:DCM (1:1) and (3) 3 ml of DCM. The eluate was blown down to near dryness and reconstituted in 300 µl of ethyl acetate for the instrumental analysis.

2.4. Instrumental analysis

PBDEs, MeO-PBDEs, HBB, DBDPE and PBEB were analysed with an Agilent 7890A gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer. Chromatographic separation was carried out with a DB-5ms column (15 m × 0.25 mm × 0.1 µm of film thickness). The instrumental conditions and elution program were based on our previous works (Eljarrat et al., 2002; Eljarrat et al., 2007). For the spectrometric determination (Barón et al., 2014), electronic ionization (EI) at 300 °C was used. For the analysis of dechloranes (Barón et al., 2012), the chromatographic separation was carried out with another DB-5 ms column. Negative ion chemical ionization (NICI) at 175 °C was used, with methane as ionization gas. BDE-209 and DBDPE were analysed with the same chromatographic conditions as PBDEs and an

Agilent 5975A mass spectrometer because of better sensibility. For the spectrometric determination (Eljarrat et al., 2004), NICI at 250 °C was used. For all HFRs, except for BDE-209 and DBDPE, and MeO-PBDEs selective reaction monitoring (SRM) mode was used with two transitions monitored for each one. For BDE-209 and DBDPE selected ion monitoring (SIM) was used with one ion monitored for each one. Recoveries for individual compounds ranged 51–109% and relative standard deviations (RSDs) were 1.1–38%. Limits of quantification (LOQs) and limits of detection (LODs) were, respectively, 0.008–20.8 ng g⁻¹ lipid weight (lw) and 0.002–6.24 ng g⁻¹ lw for mussel samples and 0.001–4.78 ng g⁻¹ dry weight (dw) and 0.0003–0.96 ng g⁻¹ dw for sediments.

For OPFRs in mussels, online sample purification and LC-MS analysis was performed with a Thermo Scientific TurboFlow™ system according to previous work (Giulivo et al., 2016). Cyclone™-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm) columns were used in combination for purification. Chromatographic separation was achieved with an analytical column Purosphere Star RP-18 (125 mm × 0.2 mm). Mobile phase was a gradient of water (0.1% formic acid) and methanol (0.1% formic acid) at 0.75 ml min⁻¹. Spectrometric analysis was performed with a triple quadrupole with a heated-electrospray ionization source. LC flow rate was 5 µl min⁻¹, ion transfer tube temperature was 320 °C and vaporizer temperature was 50 °C. SRM mode was used. Recoveries for individual compounds ranged 47–98% and RSDs were 2.4–16%. LOQs and LODs were, respectively, 0.97–24.8 ng g⁻¹ lipid weight (lw) and 0.19–19.3 ng g⁻¹ lw.

OPFRs in sediment and water samples were analysed using an Agilent Technologies 7890A GC system coupled to an Agilent 5975 series Mass Selective Detector. Chromatographic separation was carried out with a DB-5ms column, 1 µl injected splitless, using helium as carrier gas. The temperature gradient started at 110 °C for 2 min and increased 5 °C min⁻¹ to a final 250 °C that were held for 4 min. For the spectrometric determination, EI at 230 °C and selected ion monitoring (SIM) mode were used. Recoveries for individual compounds ranged 28–165% and RSDs were 6.0–23% and 55% for EHDP. LOQs and LODs were, respectively, 0.12–9.98 ng g⁻¹ dw and 0.04–2.99 ng g⁻¹ dw for sediment and 0.52–69.0 ng l⁻¹ and 0.13–20.7 ng l⁻¹ for water.

2.5. Statistical analysis

A *t*-test was used for the statistical analysis taking *p* < 0.05 as the criterion for statistical difference. In box plots figures, outliers (×) were calculated as values above Q3 + 1.5 IQR and below Q1 - 1.5 IQR (Q3 = third quartile, IQR = interquartile range, Q1 = first quartile). For all means and standard deviations (SD), concentrations below LOQ were given the LOD value and concentrations below LOD were considered to be 10% of the LOD.

3. Results and discussion

3.1. Contaminant levels

See Table 2 for concentrations of FRs in sediment samples. PBDEs were detected in 96% of the sediments with total concentrations up to 36.6 ng g⁻¹ dw. Only the UK sample situated in an urbanised estuary downstream of the city of Exeter (urban area of over 250,000 inhabitants) showed 106 ng g⁻¹ dw of PBDEs. BDE-209 was the main contributor to the profiles, with BDE-47, BDE-99 and BDE-100 almost always below their LOQs. BDE-183 was detected in only one sample and below its LOQ. The alternative PBEB and HBB were detected in 42% of the samples, including Greek, Portuguese, Spanish and British sediments, but always below their LOQs. On the other hand, DBDPE was found in 63% of the samples, never in Norwegian sediment and only in one Greek sample. Despite it being the substitute of BDE-209, a logically expected shift in concentrations has not happened yet. Concentrations of DBDPE went up to 4.18 ng g⁻¹ dw. Taking all sediments as a group and excluding the outlier values, the quantity of DBDPE (0.15 ± 0.23 ng g⁻¹ dw) was lower than the quantity of BDE-209 (1.24 ±

Table 2
Results for sediments (ng g⁻¹ dw) and mussels (ng g⁻¹ lw).

Sediments	ng g ⁻¹ dw	PBDEs	PBEB	HBB	DBDPE	MeO-PBDEs ^a	Decs ^b	OPFRs
ALsS	Mean	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOQ	1.99
n = 3	SD							2.07
GRsF	Mean	<LOQ	<LOQ	<LOQ	1.40	<LOQ	0.04	24.4
n = 3	SD				2.41		0.08	29.7
ITsF	Mean	9.68	<LOD	<LOD	0.12	<LOD	0.41	12.5
n = 3	SD	10.1			0.09		0.27	11.8
ITsS	Mean	18.1	<LOD	<LOD	1.05	<LOD	0.70	8.50
n = 3	SD	18.3			0.97		0.70	12.9
NOsF	Mean	0.44	<LOD	<LOD	<LOD	<LOD	0.18	39.3
n = 2	SD	0.24					0.06	3.26
NOsS	n = 1	1.08	<LOD	<LOD	<LOD	<LOD	0.83	25.8
PTsS	Mean	1.06	<LOQ	<LOQ	0.68	<LOQ	0.23	58.2
n = 3	SD	0.55			0.51		0.20	33.0
SPsS	Mean	1.79	<LOQ	<LOQ	0.23	<LOQ	1.05	9.55
n = 3	SD	0.29			0.12		0.98	14.0
UKsS	Mean	37.9	<LOQ	<LOQ	0.34	<LOD	0.27	15.2
n = 3	SD	59.3			0.45		0.14	22.1
Freq. of det. (%)		96	42	42	63	33	92	100
LOD ^c		0.01	0.03	0.03	0.01	0.06	0.0003	0.04
LOQ		0.02	0.09	0.09	0.02	0.20	0.001	0.12
Mussels	ng g ⁻¹ lw	PBDEs	PBEB	HBB	DBDPE	MeO-PBDEs ^d	Decs ^b	OPFRs ^e
ALmS	Mean	<LOQ	<LOD	<LOD	2.52	<LOQ	0.35	808
n = 3	SD				4.35		0.60	235
ITmF	Mean	<LOQ	<LOD	<LOD	1.05	<LOQ	21.5	1380
n = 3	SD				1.75		13.3	541
ITmS	Mean	4.78	<LOD	<LOD	<LOQ	<LOQ	<LOD	534
n = 3	SD	3.73						119
NOmS	n = 1	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	2.74
PTmS	Mean	1.85	<LOD	<LOD	0.83	<LOQ	1.28	723
n = 3	SD	3.13			0.83		2.22	72.7
SPmS	Mean	<LOQ	<LOD	<LOD	<LOD	<LOQ	0.59	11.1
n = 3	SD						1.03	7.04
UKmS	n = 1	5.42	<LOD	<LOD	<LOD	<LOQ	<LOQ	11.8
Freq. of det. (%)		94	0	0	53	100	53	100
LOD ^c		0.04	0.18	0.20	0.08	0.43	0.002	0.19
LOQ		0.12	0.61	0.67	0.26	1.42	0.008	1.03

^a 5-MBDE-99 and 4-MBDE-101 were not detected in any sediment (<0.53 and <0.45 ng g⁻¹ dw).

^b Dec604 was not detected in any sediment (<0.001 ng g⁻¹ dw) or mussels (<0.007 ng g⁻¹ lw).

^c LOD and LOQ of the compound with the lowest values of the group for PBDEs, MeO-PBDEs, Decs and OPFRs.

^d 5-MBDE-47, 4-MBDE-49, 5-MBDE-99 and 4-MBDE-101 were not detected in any mussels (<0.43, <2.13, <3.75 and <3.19 ng g⁻¹ lw).

^e TCIPP, EHDP were not detected in any sample (<1.48 and <0.53 ng g⁻¹ lw).

2.04 ng g⁻¹ dw) according to the *t*-test ($t = 2.39$, $df = 38$, $p < 0.05$). Dechloranes occurred as frequently as PBDEs, in 92% of the sediments, but always below 2 ng g⁻¹ dw, in concentrations similar to DBDPE. *Syn*-DP and *anti*-DP were the main contributors, found in 79% and 75% of the samples, respectively. OPFRs were found in all samples in concentrations up to 92.8 ng g⁻¹ dw. However, with a few exceptions, concentrations of OPFRs were higher than those of PBDEs. Grouping all sediments and excluding the outliers, sediments contained more OPFRs (17.5 ± 18.6 ng g⁻¹ dw) than PBDEs ($t = 4.14$, $df = 41$, $p < 0.01$). This difference was also observed in sediments from three rivers from the south of Europe sampled between 2014 and 2015 (Giulivo et al., 2017). TPHP, EHDP and TCIPP were the most occurring OPFRs, in 92%, 83% and 54% of the samples, most likely owing to their higher hydrophobicity (log K_{ow} of 4.59, 5.73 and 2.59, respectively). The same three compounds accounted for an average of 31%, 19% and 30% of the total OPFRs contamination in each sample. TBOEP could not be analysed in sediment as it had low recovery and high RSD. Finally, the naturally produced MeO-PBDEs were detected in just 33% of the samples, including Greek, Portuguese and Spanish sediments.

See Table 2 for concentrations of FRs in mussel samples. PBDEs were detected in 94% of the mussels mostly below the LOQ and always below 10 ng g⁻¹ lw. BDE-28, BDE-47 and BDE-100 were the most occurring congeners: in 65%, 59% and 53% of the samples. Contrary to what was

seen for sediments, BDE-209 was only detected in two of the 17 samples (12%). BDE-99 and BDE-138 were also detected in one and two samples, respectively, but below their LOQs. The alternative PBEB and HBB were not detected in mussels. On the other hand, DBDPE and dechloranes were found in 53% of the samples, also below their LOQs or LODs and below 10 ng g⁻¹ lw. The exception was dechloranes in two mussel samples from the Italian fish farm: 25.4 and 32.4 ng g⁻¹ lw. Therefore, levels of PBDEs, DBDPE and dechloranes in mussels were similar (all *t*-tests, $df = 25$ or 26 , $p > 0.1$). As for sediments, *syn*-DP (in 41% of the samples) and *anti*-DP (in 29%) were the main contributors. OPFRs were found in all samples in concentrations ranging from 6.67 to 2005 ng g⁻¹ lw, which was clearly higher than any of the other compounds. The recent study mentioned in the previous paragraph analysed fish from the same rivers with levels up to 650 ng g⁻¹ lw (Giulivo et al., 2017). Our results were not too dissimilar taking into account that our biota samples were not fish but mussels. The frequency of detection of individual OPFRs in mussels opposed the findings in sediment, as the least occurring compounds were TPHP (in 41% of the samples) and TCIPP and EHDP, which were not detected in any sample. TBP accounted for an average of 94% of the total OPFRs contamination in Greek, Italian and Portuguese individual samples, but it was not detected in mussels from Norway, Spain or the UK. Similar results were obtained in a previous study (Brandtsma et al., 2015) where despite high OPFR sediment concentrations, the inferior

levels of the trophic food web showed very limited OPFR contamination. The authors concluded that in contrast to PBDEs, OPFRs showed limited affinity for lipids, which might explain their relative low levels in lipid-rich bivalves such as mussels. Finally, MeO-PBDEs were detected in 100% of the samples, basically due to the presence of 6-MeO-BDE-47 (in 94%) and 2'-MeO-BDE-68 (in 88%).

See Fig. 2 for concentrations of OPFRs in water samples. As HFRs and MeO-PBDEs have higher octanol-water partition coefficient ($\log K_{ow} = 6$ to 14) than OPFRs ($\log K_{ow} = -9$ to 9) and much lower water solubility (10^{-8} to 10^{-2} mg l⁻¹ versus 10^{-1} to 10^3 mg l⁻¹ at 25 °C), it is highly unlikely to detect the halogenated compounds in water and only OPFRs were analysed in water samples (Alaee et al., 2003; Covaci et al., 2007, 2011; Hou et al., 2016; Sverko et al., 2011; Van der Veen and de Boer, 2012). OPFRs were found in 100% of the samples with concentrations of 0.43 to 867 ng l⁻¹. Most samples were below 92 ng l⁻¹, but samples from the UK and Portugal showed outstandingly high results. This correlates with the sampling spots that were the closest to urban areas. One sample from Albania, which was located in a lagoon, and two of the Portuguese samples owe their high levels to TBOEP, and the samples from the UK owe them to TCIPP. TCEP was found in 100% of the samples (5.26 ± 7.49 ng l⁻¹) and TPHP in 74% of them (1.77 ± 2.22 ng l⁻¹).

Regarding the environmental relevance of these levels, the Directive 2013/39/EU regarding priority substances in the field of water policy set environmental quality standards (EQS) for some compounds of concern (PSWP, 2013). As HFRs are unlikely to be found in water, the Directive set an EQS limit for PBDEs in biota (sum of congeners 28, 47, 99, 100, 153 and 154) with a value of 0.0085 ng g⁻¹ ww. PBDEs were found in 94% of the mussel samples at 0.002–0.21 ng g⁻¹ ww (mean 0.03 ± 0.05 ng g⁻¹ ww). Over two thirds of the samples (71%) exceeded the limit, 29% with quantifiable concentrations and 42% with values of individual PBDEs below their LOQs but above LODs greater than the EQS limits. The remaining 29% of the samples could not be assigned over or below the limit as they are placed in non-quantifiable ranges that include the EQS value. Even though EQS limits were designed to protect the environment and not humans, the 0.0085 ng g⁻¹ ww value was set applying large safety factors to protect human consumers and it was based on observed effects of BDE-99 on rats. Thus, some authors question the suitability of this value (Jürgens et al., 2013) since even the Sub-Group on Review of the Priority Substances List (under Working Group E of the Common Implementation Strategy for the Water Framework Directive) calculated 44.4 ng g⁻¹ ww to be a safe limit to protect wildlife predators (EC European Commission, 2011). This alternative EQS is much higher than the concentrations of PBDEs in the mussels sampled for the present study.

3.2. Differences between locations

As previously stated, FRs have no application in aquaculture so levels of contamination in the sampled spots should reflect the human activity surrounding the locations. Therefore, our results did not show the

impact of the farms, but the environmental contamination in the area. This might indicate the suitability of a location to host a fish farm.

While PBEB, HBB and MeO-PBDEs were always below their LOQs or even their LODs; PBDEs, DBDPE, dechloranes and OPFRs were quantifiable in most samples. However, PBDEs, DBDPE and dechloranes showed concentrations below 10 ng g⁻¹ lw (<2 ng g⁻¹ dw) in mussels and mostly below 10 ng g⁻¹ dw for sediments with three Italian sediments (including both locations) and one British sediment above that. On the other hand, OPFRs contamination was much higher for all matrices: 17.5 ± 18.6 ng g⁻¹ dw for sediments, 827 ± 769 ng g⁻¹ lw (64.3 ± 65.1 ng g⁻¹ dw) for mussels and 18.9 ± 24.1 ng l⁻¹ for water.

As aforementioned, a large majority of the most contaminated samples were collected from sampling spots that were the closest to urban areas or in somewhat enclosed water bodies not in the open sea. OPFRs have been previously identified at µg/l in wastewaters, with limited removal efficiencies through treatment plants of about 50% on average and nearing zero for the chlorinated OPFRs (Marklund et al., 2005; O'Brien et al., 2015). Our results suggest an influence of wastewater discharges in samples from Portugal, the UK and Norway. The Portuguese sediment and water samples had the highest contamination of OPFRs (58.2 ± 33.0 ng g⁻¹ dw and 547 ± 437 ng l⁻¹). The Portuguese mussels also had some of the greatest values for that matrix (723 ± 72.7 ng g⁻¹ lw). Notice that the Portuguese sampling spot was quite surrounded by urban areas. The sampling area in the UK was situated in the estuary of the river Exe, and a pattern of decreasing water concentrations towards the mouth of the estuary, along the salinity gradient, was observed. The water samples from those spots were also high in OPFRs (275 ± 34.9 ng l⁻¹) and the mussel sample from the urban shore showed outstanding levels of PBDEs (106 ng g⁻¹ lw). The Norwegian sediments showed greater quantity of OPFRs about 200 m from urban areas and next to a road between two pieces of land (37.0 and 41.6 ng g⁻¹ dw) than 1 km away at sea (25.8 ng g⁻¹ dw). Finally, the Italian samples from the shellfish farm tended to show concentration values around or above the median in all matrices when compounds were detected. This could be due to the fact of the sampling spot being at the Po Delta and separated from the Adriatic Sea by a sandy barrier, which might diminish the dilution effect that sampling spots at high sea enjoy.

As seen in Fig. 3, the levels of OPFRs were higher than other compounds regardless of sampling location. It is important to note that PBDEs levels have been decreasing for over a decade and the ban should reinforce this trend (ECCC Environment and Climate Change Canada, 2016). Moreover, FRs—either banned or emerging— have no application in fish farms but OPFRs could be present as plasticisers in all sorts of plastic instruments and materials used in the farms. It is important to note that cages are commonly made of high density polyethylene, which is plastic. That would explain that Greek and Italian sediments, Albanian and Italian mussels and Albanian and Italian water samples from shellfish farms show concentrations of OPFRs comparable to the Spanish samples, which is closer to the shore. This direct input of OPFRs to mussels would add to the input from environmental levels in

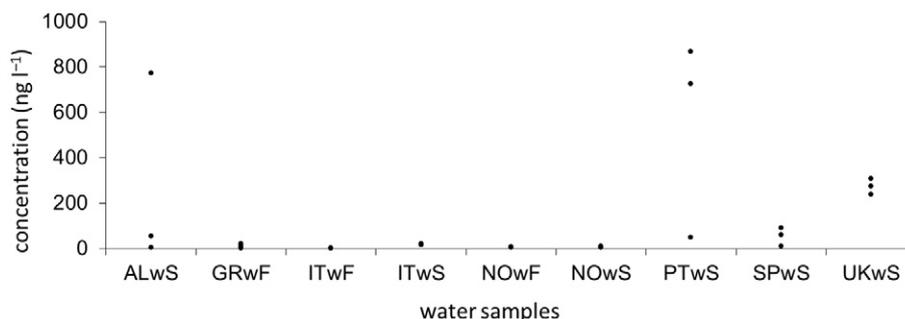


Fig. 2. Total OPFRs concentration in the water samples.

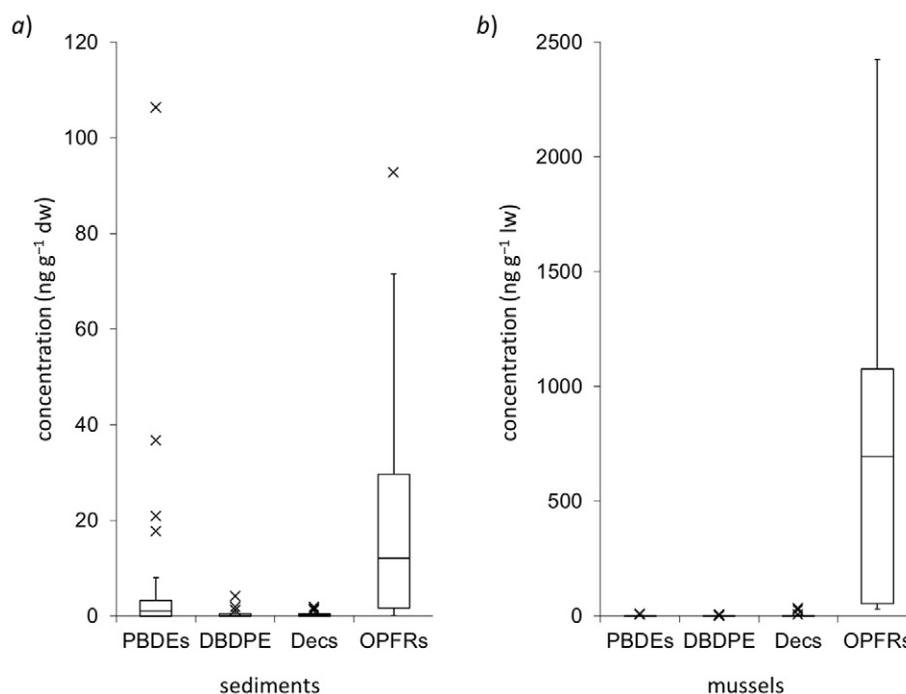


Fig. 3. Concentrations of FRs in sediments and mussels. Outliers shown (x).

sediments and water, which can be easily seen in the Portuguese samples, with high levels in sediment and water samples that translate into high levels in mussels.

3.3. Levels compared to literature

Looking at literature for OPFRs levels in water samples, individual concentrations of the compounds in European rivers and in effluents of water treatment plants have been found to reach the order of magnitude of 10^3 ng l⁻¹ and even 10^4 ng l⁻¹ in some occasions (Andresen et al., 2004; Bacaloni et al., 2007; Bester, 2005; Green et al., 2008; Regnery and Püttmann, 2010; Rodríguez et al., 2006). In the present study, only the samples from the UK showed comparable levels of TCIPP (208–252 ng l⁻¹) and the Greek and Portuguese samples had high levels of TBOEP (up to 834 ng l⁻¹), while the rest of compounds were in the lowest range, below 70 ng l⁻¹ and mostly below 20 ng l⁻¹. The levels and relative compound distribution of OPFRs in the UK samples were consistent with those previously reported in another coastal area of the south coast of England, TCIPP being the dominant compound (Aminot et al., 2017). It is important to note that most literature data correspond to river samples and our British and Portuguese samples were collected from the mouth of a river before opening to the sea. Most of the other samples were taken from locations at sea, further away from the urban influence and with a possible dilution effect.

Looking at literature for FRs levels in sediments, samples from the Baltic Sea from 2012 (Ruczyńska et al., 2016) and two different sets of sediments from the North Sea from 2012 to 2013 (Sühling et al., 2015; Sühling et al., 2016) reached 1.84 ng g⁻¹ dw of PBDEs, excluding BDE-209. BDE-209 concentrations were 0.61–0.67 ng g⁻¹ dw and 2.43–7.21 ng g⁻¹ dw in the Baltic Sea and the North Sea, respectively. Our Norwegian sediments from the North Sea reached 0.70 ng g⁻¹ dw (excluding BDE-209) and went up to 1.65 ng g⁻¹ dw for BDE-209. For Italian sediments from the Po Delta collected in 2014, BDE-209 accounted for >95% of the contamination in all samples (1.63–42.4 ng g⁻¹ dw), followed by BDE-47 and BDE-99 (below 0.05 ng g⁻¹ dw) (Casatta et al., 2016). Our sediments from the same location contained up to

36.6 ng g⁻¹ dw of BDE-209. Additionally, BDE-47 and BDE-99 were the only other congeners that had some values above their LOQs in our whole set of European sediments. In the same samples from the North Sea concentrations were up to 0.03 ng g⁻¹ dw of HBB in almost 50% of the samples, up to 0.53 ng g⁻¹ dw of PBEB in 34% of the samples, and below their LOD for MeO-PBDEs (0.02 ng g⁻¹ dw) (Sühling et al., 2015; Sühling et al., 2016). *Syn*-DP and *anti*-DP were also detected in almost all those samples with an added concentration up to 1.16 ng g⁻¹ dw. Our sediments were in the same range, as 42% of the samples had 0.03–0.09 ng g⁻¹ dw of HBB, the same numbers applied to PBEB, 25% of the samples had 0.1–0.5 ng g⁻¹ dw of MeO-PBDEs and dechloranes concentrations went up to 1.94 ng g⁻¹ dw. Regarding OPFRs, sediments from Norway showed concentrations of TPHP, TCEP, TCIPP, TDCPP from under 0.15 ng g⁻¹ dw to 288–24,000 ng g⁻¹ dw (Green et al., 2008; Leonards et al., 2011), clearly above our maximums of 1.36–34.1 ng g⁻¹ dw for Norwegian sediments and even any of the other sediment samples in the present study.

Looking at literature for FRs levels in mussels, PBDEs were comparable to findings in western Mediterranean samples from 2007 to 2012 (Pardo et al., 2014), 2012 (Parera et al., 2013), and 2014–2015 (Aznar-Aleman et al., 2017) and Atlantic samples from 2011 (Villaverde-de-Saa et al., 2013), with means up to 0.83 ng g⁻¹ ww; up to 0.11 ng g⁻¹ ww in this work. However mussels from the Netherlands were reported to contain 2.09–12.4 ng g⁻¹ ww of PBDEs (Van Ael et al., 2012). On the other hand, while HBB and PBEB have been reported in concentrations up to 2.9 ng g⁻¹ lw in Italian mussels (Poma et al., 2014) and mostly below 9 ng g⁻¹ lw for HBB and below 5 ng g⁻¹ lw for PBEB in samples from all Europe (Aznar-Aleman et al., 2017), the present study did not detect any of them (<0.20 ng g⁻¹ lw). Conversely, none of these two studies nor a third Spanish one (Villaverde-de-Saa et al., 2013) detected DBDPE, but the present work found a mean concentration of 0.79 ± 1.92 ng g⁻¹ lw (all samples included). As for dechloranes, they were not detected in the Spanish shore of the Atlantic (Villaverde-de-Saa et al., 2013) and ranged from 0.22–44.2 ng g⁻¹ lw in European commercial samples (Aznar-Aleman et al., 2017), whereas our results were mostly below their LOQs (<0.008 ng g⁻¹ lw) and always below 8 ng g⁻¹ lw. Although our results for MeO-PBDEs in mussels were

lower than other studies, in which a few results reached 305 or 420 ng g⁻¹ lw, the dominant congeners were always 2'-MeO-BDE-68, 6-MeO-BDE-47 (Aznar-Alemaný et al., 2017; Löfstrand et al., 2011). Regarding OPFRs, mussels from Norway and Sweden showed concentrations of individual OPFRs from under 0.2 to 110 ng g⁻¹ lw in general and up to 1300 ng g⁻¹ lw for TCIPP (Leonards et al., 2011; Sundkvist et al., 2010). Conversely, only TDCPP could be quantified in our Norwegian mussel sample and only TPHP and TBP reached such high concentrations in other samples, up to 149 and 2005 ng g⁻¹ lw, respectively.

4. Conclusions

PBDEs were detected in 95% of the sediment (8.60 ± 22.6 ng g⁻¹ dw) and mussel (<10 ng g⁻¹ lw) samples. DBDPE, dechloranes and MeO-PBDEs were detected in both sediments and mussels. OPFRs were found in all matrices with concentrations of 0.04–92.8 ng g⁻¹ dw in sediment, 6.67–2005 ng g⁻¹ lw (0.50–102 ng g⁻¹ dw) in mussel and 0.43–867 ng l⁻¹ in water.

Regarding environment and seafood safety, over two thirds of the mussel samples exceeded the EQS limit for PBDEs in biota. The remaining samples were placed in non-quantifiable ranges that included the EQS value. Since the official EQS limit was set with the consumers in mind, exceeding the limit might be a cause of concern. On the other hand, previous risk assessment of European seafood considered mussels with PBDEs concentrations ranging from 6.1–141 ng g⁻¹ lw to be safe (Aznar-Alemaný et al., 2017). Therefore, farmed mussels containing fewer than 10 ng g⁻¹ lw of total PBDEs should not pose a threat to aquaculture or the consumers. On the other hand, the proposed alternative EQS focusing on wildlife instead of human consumers is three orders of magnitude higher than the concentrations of PBDEs in the mussels sampled for the present study. Moreover, there is a 4.5% annual decrease trend of PBDEs levels over the last decade (ECCC. Environment and Climate Change Canada, 2016) hinting at an improvement. Judging by the official EQS value, the levels found are harmful for the environment. However, the suggested alternative EQS exceeds by far our results, making the levels clearly safe. While there are no regulated limits for the EFRs, they were found in fewer samples and with only DBDPE and dechloranes above their LOQs. Therefore, the EFRs do not seem to threaten European aquaculture so far.

As FRs have no application in aquaculture, levels of contamination in the sampled spots could not be attributed to the aquaculture activity. They should reflect the impact of human activity on the locations instead. A large majority of the most contaminated samples – e.g. Portuguese, British – were collected from sampling spots that were at urban shores or in enclosed water bodies not completely open to the sea. This suggested that proximity to urban shores increased the chance of contamination due to proximity to its source. On the other hand, the farms seemed to benefit from the dilution effect of being on the open sea. Therefore, fish farms would be better placed on the open sea avoiding urban shores and river mouths in order to minimize the FRs input from human activity. OPFRs were found at higher concentrations than other compounds. OPFRs could be present as plasticisers in all sorts of plastic instruments and materials used in the farms. Cages are commonly made of high density polyethylene, which is plastic. That would explain that some samples collected at open sea show higher OPFRs levels when other compounds do not show this increase. This input of OPFRs could be reduced by selecting alternative materials.

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