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## Abstract

Nowadays, environmental pollution by microplastics (< 5mm; MP) is a major issue. MP are contaminating marine organisms consumed by humans. This work studied MP contamination in two bivalve species of commercial interest: blue mussel (*Mytilus edulis*) and common cockle (*Cerastoderma edule*) sampled on the Channel coastlines (France). In parallel, 13 plastic additives and 27 hydrophobic organic compounds (HOC) were quantified in bivalves flesh using SBSE-TD-GS-MS/MS to explore a possible relationship between their concentrations and MP contamination levels. MP were extracted using a 10% potassium hydroxide digestion method then identified by µ-Raman spectroscopy. The proportion of contaminated bivalves by MP ranged from 34 to 58%. Blue mussels and common cockles exhibited 0.76 ± 0.40 and 2.46 ± 1.16 MP/individual and between 0.15 ± 0.06 and 0.74 ± 0.35 MP/g of tissue wet weight. Some HOC and plastic additives were detected in bivalves. However, no significant Pearson or Spearman correlation was found between MP loads and plastic additives or HOC concentrations in bivalve tissues for the two species.



## Keywords

microplastic, mussel, cockle, plastic additive, hydrophobic organic compounds


## Capsule

Microplastic contamination and pollutant levels of commercially important bivalves in France.

## 1. Introduction

Nowadays, plastic is a commonly used material with numerous benefits for everyday human life (Thompson et al., 2009). To meet the growing demand, plastic production increased exponentially since the 1950's from 2 million metric tons produced in 1950 to 381 million metric tons in 2015 (Geyer et al., 2017). Simultaneously, plastics tend to accumulate in natural environments due to their durability, resistance and trash mismanagement (Barnes, 2002; Horton et al., 2017).

Small plastic particles, called microplastics (MP; <5 mm) (Arthur et al., 2009) are ubiquitous in the marine environment (Li et al., 2016b) and are found in beach sediment (Claessens et al., 2011; Dekiff et al., 2014) and in the water column (Collignon et al., 2012; Desforges et al., 2014; Lattin et al., 2004). Coastal environments are also subjected to MP pollution. Indeed, as these environments are interfaces between land and sea, pollution can either originate from terrestrial or marine origins. Terrestrial activities are responsible on average for 80% of plastic load in the Oceans (Andrady, 2011) but with high variability along the coasts (Filella, 2015). Several studies found MP in coastal environments (Li et al., 2016b; Naidoo et al., 2015; Nel and Froneman, 2015; Ng and Obbard, 2006) with the highest levels up to 16,272 MP/$m^3$ in coastal waters around Geoje Island, South Korea (Song et al., 2014) and up to 8,720 MP/kg (dry weight) of beach sediment in Wanning, China (Qiu et al., 2015).

Numerous marine species are known to ingest MP (Lusher, 2015) including coastal species harvested or cultivated for human consumption. Due to their commercial interest and the fact that the whole animal is eaten by consumers, contaminations of bivalves are a major subject of concern for food safety and human health. Their feeding mode directly exposes bivalves to pollutants such as MP present in their surrounding environment. Consequently bivalves, especially mussels (*Mytilus* spp.), are commonly used as a sentinel organism to monitor anthropogenic pollution in marine coastal environments (Goldberg, 1975; Li et al., 2019).

Indeed, ingestion of MP has been demonstrated *in situ* in numerous bivalves species including mussels (*Mytilus edulis* and *Mytilus galloprovincialis*), oysters (*Crassostrea gigas*) or clams (*Venerupis philippinarum*) (Davidson and Dudas, 2016; Phuong et al., 2018; Van Cauwenberghe and Janssen, 2014; Vandermeersch et al., 2015). For example Van Cauwenberghe and Janssen (2014) reported up to 0.36 MP/g of tissue wet weight (ww) in mussels collected on German coasts whereas Phuong et al., (2018) reported 0.23 MP/g of tissue ww in mussels sampled on the French Atlantic coast. In laboratory experiments, uptake of MP resulted in different side effects on bivalves physiology (Browne et al., 2008; Cole and Galloway, 2015; Paul-Pont et al., 2016; Sussarellu et al., 2016; von Moos et al., 2012; Wegner et al., 2012). For example, exposition of oysters (*C. gigas*) to polystyrene microspheres modified their feeding capacity and affected reproductive outputs (Sussarellu et al., 2016). Apart from the physical injuries caused by MP, their ingestion could also be associated with the release of hydrophobic organic compounds (HOC) or plastic additives (Hermabessiere et al., 2017). Some studies proposed the use of several chemicals as proxies of plastic ingestion including polychlorinated biphenyls (PCB) (Teuten et al., 2009), di(2-ethylexyl) phthalate (DEHP) (Fossi et al., 2014, 2012) or polybrominated diphenyl ethers (PBDE) (Tanaka et al., 2013).

In the present study, two species of bivalves, the blue mussel (*M. edulis*) and the common cockle (*Cerastoderma edule*) were chosen to study MP contamination. These species live in different habitats and are both commonly found on French coasts. Cockles live at the interface between sediment and water where higher contamination is expected (Besseling et al., 2014) whereas mussels live in the water column on rocks or on lines and piling in aquaculture. In addition, both species are commercially important seafood products. France is one of the top producers of mussels (*Mytilus* spp.) in Europe with 47,394 tons produced in 2016 (FAO, 2018) and 1,890 tons of cockle were produced in France in 2016 (FAO, 2018). Globally, in Europe,

mussels are one of the most consumed seafood products with an apparent consumption of 1.33 kg/capita in 2015 (European Commission, 2018).

The aims of this work were (i) to quantify MP content in two common commercial bivalves species, the blue mussel and the common cockle, sampled along the Channel coast of France, (ii) to quantify HOC and plastic additives in the bivalves tissues and (iii) to explore relationships between MP loads in bivalves and HOC and plastic additive tissue concentrations.

# 2. Material and methods

## 2.1. Sampling

Sampling sites were located along the Channel coasts (Fig. 1) which exhibit the most important tide system in Europe associated with strong currents (Salomon and Breton, 1993). The Baie des Veys (BdV) (Fig. 1A) is an estuarine bay under the influence of two rivers: the Taute and the Vire with a total mean discharge of 53 $m^3$/s. The intertidal part of the bay supports intensive oyster farming with around 10,500 tons (Grangeré et al., 2009). The BdV also supports mussel farming and professional fishing of *C. edule*. Moreover, this bay could also be influenced by the Seine flow depending on meteorological conditions (Ellien et al., 2004). The Baie d'Authie (BA) (Fig. 1B) is a small estuary influenced by the river Authie which has a mean flow of 9 $m^3$/s mainly influenced by agricultural activities (Billon et al., 2001; Gillet et al., 2008). Finally, Le Portel (LP) (Fig. 1B) beach is not influenced by any river but is located in an area under the influence of 116,000 inhabitants in 2014 (INSEE, 2015). Overall, BdV and BA are small estuaries with small influence of human activities whereas LP is located in a relatively high populated area.

Mussels (n=50) were collected at LP (50°42'30.02"N, 1°33'34.43"E) on Oct 29$^{th}$, 2015. Mussels (n=50) and cockles (n=50) were then sampled at the BdV (49°22'23.4"N, 1°06'40.0"W) on Nov 1$^{st}$, 2015. Finally, cockles (n=50) were sampled at the BA

(50°22'17.22"N, 1°35'4.8"E) on Nov 15th, 2015. In total, 100 mussels and 100 cockles were collected. After field sampling, the shell was cleaned in the laboratory with filtered bidistilled water and length was measured for all individuals. Then bivalves were shelled and their soft tissue wet weights (ww) were recorded. Samples were wrapped in a piece of paper then in aluminum foil and stored at -20°C before subsequent analysis. Atmospheric blank was performed during opening and weighting (see 2.2.1).

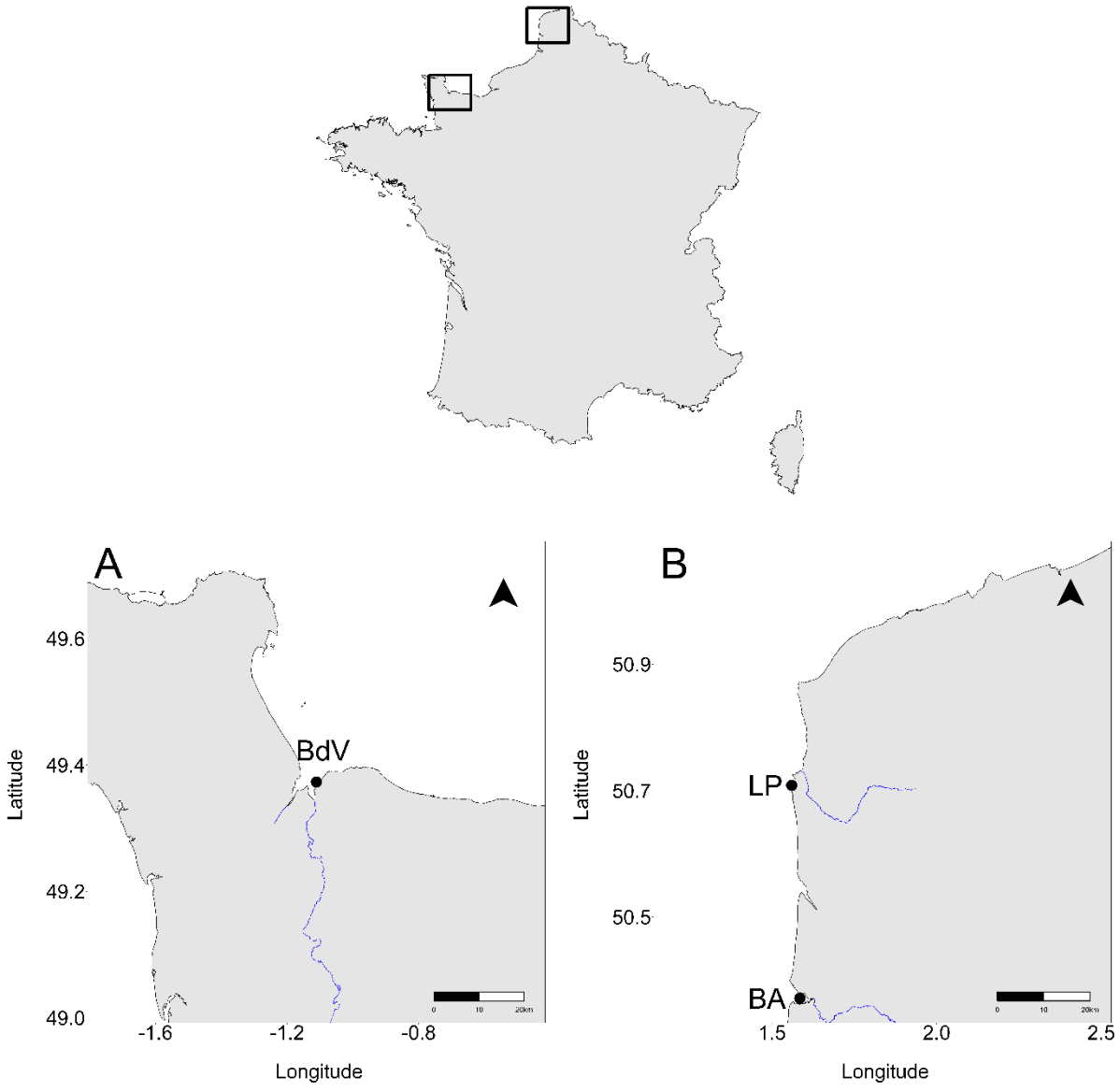



**Fig 1: Blue mussel and common cockle sampling locations along the French coasts of the Channel in Normandy (A) and in Hauts-de-France (B). BA: Baie d'Authie, BdV: Baie des Veys and LP: Le Portel.**

## 2.2. Microplastics analyses

### 2.2.1. Prevention of procedural contamination

To avoid overestimation of the MP concentration in bivalves due to airborne, container, and tool contamination, preventative measures were applied. All used materials were made of glass. All solutions: distilled water, 70% (v/v) ethanol, 10% potassium hydroxide (KOH) were filtered through a 90 mm diameter GF/A 1.6 µm glass fiber filters (Whatman, Velizy-Villacoublay, France) until no particle was found on the filter. Moreover, all glassware, tools and bench surfaces were rinsed with filtered distilled water, filtered 70% ethanol then by filtered distilled water before being used. Glass fiber filters were verified under a stereomicroscope to ensure the absence of particle before being used.

Atmospheric blanks were performed at every step of the work: dissection, digestion and filtration using glass petri dishes open to the environment during procedures. Furthermore, for each digestion batch, a procedural blank made of only 10% KOH followed the same treatment as the bivalve samples. Digestion and filtration were performed in a fume hood specifically dedicated to MP analyses, with a switched-off aspiration system, to prevent contamination with airborne particles from the ambient air. Finally, operators did not wear gloves and synthetic clothing to limit contamination due to fixed airborne particles and they wore lab coats made of cotton.

The used µ-Raman spectroscopy method did not allow the polymer identification of fibers because these particles are too thin (Käppler et al., 2016). Thus, fibers were only counted. In addition, in order to account for airborne contamination, whenever a fiber was found in a blank (atmospheric or procedural), it was subtracted from the final result if a fiber of the same type (*i.e.* color and shape) was found in the sample. Results for fiber counts without subtraction of blank are available in Supplementary Table 1. Some particles were classified as pigment

containing particles as no confirmation of polymeric composition could be made. Together with fibers, pigment containing particles were not considered in MP contamination results.

### 2.2.2. Tissue digestion and filtration

Digestion of mussels and cockles were performed according to Dehaut et al., (2016). After thawing, individuals were placed in 300 mL Erlenmeyer flasks then 10% KOH was added. For bivalves from LP and BA, 100-250 mL of 10% (w/w) KOH were added (ChimiePlus, Saint Paul de Varax, France) and then samples were placed 24 h in an incubator (Binder BD 240, Tuttlingen, Germany) set at 60 ± 1°C with agitation set at 200-300 rpm (IKA KS250, Staufen, Germany or 2mag MIXDrive 6 HT, Munich, Germany). For bivalves sampled at BdV, a solution of 10% KOH (w/v) was prepared using KOH pellets (Sigma-Aldrich, Saint-Quentin-Fallavier, France) and distilled water. Bivalves were put in 250 mL of prepared 10% KOH then placed on an agitation plate (IKA RT15, Staufen, Germany) set at 300 rpm and 60 ± 1°C for 24 h. After digestion, all samples were filtered on clean 90 mm diameter GF/A 1.6 µm glass fiber filters (Whatman,Velizy-Villacoublay, France) using a vacuum system. Filters were then placed in closed Petri dishes until subsequent analysis. Procedural blank was performed at the same time as manipulating mussel and cockle soft tissue (see 2.2.1).

Except for mussels from LP due to a handling issue, two pools of 100 mL, of bivalve digestates by species and location, obtained with 10 mL belonging to 10 different individuals were prepared and conserved at -20°C until further analyses of HOC and plastic additives.

### 2.2.3. Visual sorting and µ-Raman analysis

Filters were observed under a Zeiss Stemi 508 stereomicroscope (Zeiss, Marly le Roi, France). Particles resembling MP (MP-like) were counted and characterized according to their shape (fragment, fiber, bead, foam or pellet) and color (Lusher et al., 2017). Colors were attributed by a unique operator in order to allow comparisons. MP-like were then isolated for subsequent µ-Raman spectroscopy analysis. Pictures of MP-like were taken and sizes were measured in

pixel using GIMP 2 software (2.8.16). The maximum length in µm of MP-like particles was calculated using a scale bar. No particle smaller than 15 µm was observed on filters. Particles were thus categorized depending on their maximum size according to the following class sizes: 15-50, 50-100, 100-500 and >500 µm.

µ-Raman analyses were conducted according to Frère et al., (2016). Briefly, all MP-like were analyzed with a LabRam HR800 (HORIBA Scientific, Villeneuve d'Ascq, France) using laser wavelength set at 785 nm (Laser diode, Oxxius, Lannion, France) or 514 nm (Ar Laser, Melles Griot, Bensheim, Germany). A laser wavelength of 785 nm was first attempted and if identification was not conclusive, acquisition with laser wavelength set at 514 nm was carried out. Experimental conditions for µ-Raman analyses - integration time, accumulation, laser power and wavelength - were set to limit fluorescence and increase the spectral quality of the analyzed particles. Particles identifications were performed by comparing acquired spectra to reference spectra to home-made database including the following reference polymers: Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Polypropylene (PP), Polystyrene (PS), Unplasticized Polyninyl Chloride (uPVC), Polyethylene Terephthalate (PET), Polyamide-6 (PA-6), Polyamide-12 (PA-12), Polytetrafluoroethylene (PTFE), Polymethylmethacrylate (PMMA), Acrylonitrile-Butadiene-Styrene (ABS), Polyurethane (PUR) acquired from GoodFellow (Lille, France). Then, downstream, chemometric analyses were carried out in order to obtain a better identification for previously unidentified particles (Batzan et al., 2018) (Supplementary Table 2). Identification was established based on the similarity percentage (minimum value of 70%) between particles and reference spectra. In addition, spectra with no identification in the home-made database were compared to spectra described by Socrates (2004).

## 2.3. Analyses of hydrophobic organic compounds and plastic additives

### 2.3.1. Target chemicals

Five groups of chemicals were analyzed including 15 polycyclic aromatic hydrocarbons (PAH), 6 polychlorinated biphenyls (PCB), 6 organochloride pesticides (OCP), 6 phthalates and 7 PBDE (Table 1).

**Table 1: List of the analyzed chemicals in digestates of cockles from Baie d'Authie (BA), cockles and mussels from Baie des Veys (BdV).**

| Chemical family | Target chemical or congener | PubChem ID |
|---|---|---|
| PAH | Naphtalene | 931 |
| | Benzothiophene | 7221 |
| | Biphenyl | 7095 |
| | Acenaphtylene | 9161 |
| | Acenaphtene | 6734 |
| | Fluorene | 6853 |
| | Dibenzothiophene | 3023 |
| | Benzo(g,h,i)perylene | 9117 |
| | Phenanthrene | 995 |
| | Anthracene | 8418 |
| | Fluoranthene | 9154 |
| | Pyrene | 31423 |
| | Benzo(a)anthracene | 5954 |
| | Chrysene | 9171 |
| | Benzo(b+k)fluoranthene | 9158 |
| PCB | PCB-7 | 36399 |
| | PCB-28 | 23448 |
| | PCB-52 | 37248 |
| | PCB-105 | 36188 |
| | PCB-156 | 38019 |
| | PCB-169 | 23448 |
| OCP | Hexachlorobenzene | 8370 |
| | Aldrin | 101611446 |
| | Isodrin | 10066 |
| | Dieldrin | 3048 |
| | 2,4-DDE [a] | 246598 |
| | Endrin | 12358480 |
| PBDE | BDE-28 | 154083 |
| | BDE-47 | 95170 |
| | BDE-99 | 36159 |
| | BDE-100 | 154083 |
| | BDE-153 | 155166 |
| | BDE-154 | 15509898 |
| | BDE-183 | 15509899 |

| | | |
|---|---|---|
| Phthalate | BBP [b] | 2347 |
| | DEHP [c] | 8343 |
| | DEP [d] | 6781 |
| | DMP [e] | 8554 |
| | DnBP [f] | 3026 |
| | DEHA [g] | 7641 |

[a] 2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethene
[b] butyl benzyl phthalate
[c] di(2-ethylexyl) phthalate
[d] diethyl phthalate
[e] dimethyl phthalate
[f] di-n-butyl phthalate
[g] Diethylhexyl adipate

205

206 **2.3.2. Chemical analyses**

207 Analytes were directly extracted then analyzed from the digestate pool using stir bar sorptive
208 extraction-thermal desorption-gas chromatography-tandem mass spectrometry (SBSE-TD-
209 GC–MS/MS) (Lacroix et al., 2014). Briefly, a polydimethylsiloxane stir bar (Twister 20
210 mm×0.5 mm, Gerstel, Mülheim an der Ruhr, Germany) was placed in the 100 mL digestate and
211 extractions were carried out on a magnetic laboratory agitator (MIX15, Munich, Germany) set
212 at 700 rpm for 16 h in the dark at room temperature. After the extraction step, stir-bars were
213 retrieved, rinsed with Evian® water and placed on a gas chromatography system Agilent 7890A
214 coupled with an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies,
215 Little Falls, USA) and equipped with a Thermal Desorption Unit (TDU) combined with a
216 Cooled Injection System (CIS) (Gerstel, Mülheim an der Ruhr, Germany). Thermodesorption
217 was performed at 280°C for 6 min and samples where then cryofocused in the CIS at -10°C.
218 Injection in the GC-MS/MS system was carried out in splitless mode and the CIS was heated
219 to 300°C at 12°C/s. The GC temperature program was set as follows: 70°C for 0.5 min, then
220 increase to 150°C at 20°C/min and finally increase to 300°C at 7°C/min, maintained for 5 min.
221 A Rxi-5MS (30 m, 0.25 mm, 25 µm thickness) (Restek, Lisses, France) capillary column was
222 used. Helium was used as a carrier gas with a constant flow rate of 1 mL/min.

Limits of quantification (LOQ) were calculated according to Shrivastava and Gupta (2011) using the calibrations curves method. Limits of detection (LOD) were calculated by dividing the LOQ by 3.3. Calibration curves were drawn using mussel tissues digested in 10% KOH for 3 h at 80°C added with standard chemicals. Analytes were quantified relatively to deuterated compounds using a calibration curve ranging from 0.01 ng to 10 ng. For PAH, PCB and OCP quantification naphthalene d8, biphenyl d10, phenanthrene d10, pyrene d10, chrysene d12, benzo(a)pyrene d12, benzo(g,h,i)perylene d12 were used as standards. For the plastic additives, phthalates and PBDE, di (2-ethylhexyl) phthalate d4 and BDE 77 were respectively used as standards. All standards were obtained from LGC Standard (Wesel, Germany) and Interchim (Montluçon, France).

## 2.4. Statistical analyses

All statistical analyses were performed using R Statistical Software version 3.4.0 (R Core Team, 2015). As hypothesis of residuals normality, tested using Shapiro-Wilk test, and homoscedasticity, tested on regression residues, were not verified, non-parametric Kruskal-Wallis tests were performed instead of ANOVA. When significant differences were highlighted, a post-hoc test using the Fisher's least significant difference (LSD) criterion and Bonferroni correction was applied using the *agricolae* package (1.2-7) (De Mendiburu, 2014). Microplastic color, sizes classes and polymer composition were compared using Chi-Square test. To perform Chi-square test, data were summed to meet application requirements (Cochran, 1952). Relationships between MP or anthropogenic particle (AP) load and HOC or plastic additive concentration in bivalves were assessed using correlation test (Pearson or Spearman) with the corrplot package (0.84) (Wei et al., 2017). Differences were considered significant when p-value was below 0.05.

Results are expressed as a mean ± 2 standard error (S.E.), representing the 95% confidence interval (95% CI). Contamination results were expressed as percentage of contaminated

individuals, mean particles/individuals and mean particles/g of tissue ww. Results were given for contamination by MP, fiber, pigment containing particles (PCP) and all categories (MP+fiber+PCP).

# 3. Results and discussion

## 3.1. Biometric parameters

Mussels from LP and BdV measured 47.3 ± 1.2 mm and 47.9 ± 1.5 mm and weighted 3.5 ± 0.5 g (soft tissue wet weight) and 5.7 ± 0.6 g, respectively. Cockles from BA and BdV measured 35.2 ± 0.4 mm and 27.3 ± 0.5 mm and weighted 3.2 ± 0.1 g and 3.0 ± 0.2 g, respectively. On average, cockles and mussels measured legal market sizes: 27 mm and 30 mm for cockles for professional and recreational fishing respectively and 40 mm for mussels for recreational and professional fishing (LegiFrance, 2018; Préfecture de Haute Normandie, 2015a, 2015b; Préfecture de Normandie, 2016).

## 3.2. Microplastics in mussels and cockles

Overall, 1636 particles were visually isolated and sorted from the 200 sampled bivalves of BA, LP and BdV. Among them, 324 were fibers varying from 2.4 to 50.2% of particles according to sites and species. A total of 1312 particles (80%) were analyzed with µ-Raman spectroscopy and identified as MP, PCP, natural particles, or unidentified (Table 2). The identified particles correspond to MP (5 to 32.8%), PCP (0 to 2.5%), and natural particles (6.6 to 21.5%) (Table 2). Unidentified particles with µ-Raman account for 27 to 60.9% of analyzed particles (Table 2). Absence of identification was due to absence of peak in particles spectra, saturated signal due to high fluorescence or mismatch with databases. Overall, natural particles (6.6 to 21.5%) were mainly composed of minerals (exclusively quartz), organic and inorganic carbon corresponding to sand or shell particles (Table 2). The majority of PCP contained a blue

pigment known as the phthalocyanine blue 15 (PB15). As this pigment is used in the plastic industry, it can be attributable to plastic (Lewis, 2005). Such PCP were also found in mussels sampled in Germany (Van Cauwenberghe and Janssen, 2014). Nevertheless, these could not be rigorously considered as MP since these PCP could also be paint particles, as demonstrated by Imhof et al. (2016).

**Table 2. Particles analyzed by µ-Raman spectroscopy and fibers visually isolated and sorted from mussels and cockles.**

| Species | Sampling site [1] | Fiber [3] | Unidentified particles [4] | Identified [4] | | | Total |
|---|---|---|---|---|---|---|---|
| | | | | Natural particles [5] | Microplastics | PCP[6] | |
| Mussel | LP [2] | 77 | 465 | 164 | 38 | 19 | 763 |
| | | *10.1%* | *60.9%* | *21.5%* | *5.0%* | *2.5%* | *100%* |
| | BdV [2] | 121 | 65 | 16 | 39 | 0 | 241 |
| | | *50.2%* | *27.0%* | *6.6%* | *16.2%* | *0%* | *100%* |
| Cockle | BA [2] | 9 | 169 | 65 | 123 | 9 | 375 |
| | | *2.4%* | *45.1%* | *17.3%* | *32.8%* | *2.4%* | *100%* |
| | BdV [2] | 117 | 73 | 39 | 28 | 0 | 257 |
| | | *45.5%* | *28.4%* | *15.2%* | *10.9%* | *0%* | *100%* |

[1] LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

[2] Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

[3] Not analyzed by µ-Raman

[4] Analyzed by µ-Raman

[5] Natural particle included minerals, organic and inorganic carbon.

[6] Pigment containing particles

For mussels sampled at LP, MP were identified in 17 mussels (34%) whereas 23 mussels (46%) from BdV were contaminated by MP. Concerning cockles from BA, 29 (58%) individuals were contaminated by MP whereas 21 cockles (42%) from BdV were contaminated (Table 3). The presence of MP in cockles and mussels from the four sites varied from 0.56 ± 0.22 MP/individual, namely 0.19 ± 0.08 MP/g of tissue wet weight (ww) for cockle from BdV to 2.46 ± 1.16 MP/individual, namely 0.74 ± 0.35 MP/g of tissue ww for cockle from BA (Table 3). MP contamination in mussels and cockles was significantly different according to location and species (Kruskal-Wallis, $p<0.05$). Cockles sampled at BA were more contaminated by MP (Post-hoc after Kruskal-Wallis, $p<0.05$) in comparison with mussels sampled at LP and cockles

sampled at BdV. These differences were both observed for results expressed as MP/individual and MP/g of tissue ww (Table 3). Higher contamination by MP in cockles sampled at BA could be due to their position in the water column. Indeed, as suggested by Besseling et al. (2014), MP concentration in sediment is expected to be higher in comparison with the water column. However, as cockles from BdV are less contaminated by MP in comparison with cockles from BA, plastic local sources may, in the present study, be a major source of contamination. However, to date, no study has been carried out to describe MP contamination at these sampling sites; consequently, it is difficult to clearly relate MP loads to the presence of MP in water or sediment of sites where bivalves were collected.

**Table 3. Microplastics (MP), fibers, pigmented particles and total (MP+fiber+pigment) contamination of mussels and cockles sampled at Le Portel (LP), Baie d'Authie (BA) and Baie des Veys (BdV).**

| | Mussel | | Cockle | |
|---|---|---|---|---|
| | **LP** | **BdV** | **BA** | **BdV** |
| **% of contaminated individual by MP [1]** | 34 % | 46% | 58 % | 42 % |
| **MP/individual [1, 2, 3]** | 0.76 ± 0.40 [a] | 0.78 ± 0.30 [a b] | 2.46 ± 1.16 [b] | 0.56 ± 0.22 [a] |
| **MP/g of tissue ww [1, 2, 3]** | 0.25 ± 0.16 [a] | 0.15 ± 0.06 [a b] | 0.74 ± 0.35 [b] | 0.19 ± 0.08 [a] |
| **% of contaminated individual by fiber** | 40 % | 80 % | 16 % | 80 % |
| **Fiber/individual [2, 3]** | 1.54 ± 1.2 [a] | 2.42 ± 0.55 [b] | 0.18 ± 0.12 [c] | 2.34 ± 0.77 [b] |
| **Fiber/g of tissue ww [2, 3]** | 0.49 ± 0.42 [a] | 0.44 ± 0.1 [b] | 0.06 ± 0.04 [c] | 0.82 ± 0.28 [b] |
| **% of contaminated individual by PCP [1, 4]** | 24 % | 0 % | 8 % | 0 % |
| **PCP/individual [1, 2, 3, 4]** | 0.38 ± 0.23 [a] | 0 [b] | 0.18 ± 0.2 [b] | 0 [b] |
| **PCP/g of tissue ww [1, 2, 3, 4]** | 0.12 ± 0.07 [a] | 0 [b] | 0.06 ± 0.06 [b] | 0 [b] |
| **% of contaminated individual by all categories** | 68 % | 86 % | 72 % | 86 % |
| **Total/individual [2, 3]** | 2.68 ± 1.33 [a] | 3.20 ± 0.60 [b] | 2.82 ± 1.14 [a b] | 2.90 ± 0.77 [a b] |
| **Total/g of tissue ww [2, 3]** | 0.86 ± 0.47 | 0.59 ± 0.12 | 0.86 ± 0.34 | 1.02 ± 0.28 |

[1] Particles identification as MP and pigmented particles were obtained after µ-Raman spectroscopy.

[2] Results expressed as the mean ± 2 S.E (95% confidence interval).

[3] Superscript letters correspond to significant differences (per lines) after a Kruskal Wallis post-hoc test using the Fisher's least significant difference and Bonferroni correction ($p<0.05$).

[4] Pigment containing particles

In the present study, quantities of MP, *stricto sensu*, recorded for the bivalves along French coasts of the Channel were in accordance with studies from other European coasts (Table 4) where contamination in bivalve varied from 0.04 ± 0.09 MP/g of tissue ww in Mediterranean blue mussels (*M. galloprovincialis*) sampled at Erbo Delta (Spain) to 4.44 MP/g of tissue ww for blue mussels (*M. edulis*) sampled at Oban (Scotland) (Table 4). In this work and others carried out in Europe, MP contamination appeared to be more influenced by location than by species even though the number of investigated species is limited. However, MP contamination in the two bivalves species is lower than contaminations recorded in bivalves sampled along Chinese coasts (Li et al., 2016a; Li et al., 2015) (Table 4). Mussels along the Chinese coasts were contaminated by 0.9 to 4.6 MP/g (Li et al., 2016a). These differences are likely due to MP contamination level of the studied sites. Indeed, Chinese environments were reported to be highly contaminated by MP and other plastic debris (Cai et al., 2018).

311 **Table 4. Overview of microplastic contaminations in bivalves.**

| Species | Individuals | MP/individual | Isolation procedure | Filter pore size (in µm) | Polymer chemical identification | Sampling location | Reference |
|---|---|---|---|---|---|---|---|
| *Mytilus galloprovincialis* | 100 | 0.34 ± 0.33 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Tagus estuary, Portugal | [5] |
| *Mytilus galloprovincialis* | 100 | 0.05 ± 0.11 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Po estuary, Italy | [5] |
| *Mytilus galloprovincialis* | 25 | 0.15 ± 0.33 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Fangar Bay, Spain | [5] |
| *Mytilus galloprovincialis* [a] | 25 | 0.25 ± 0.26 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Coro, Italy | [5] |
| *Mytilus galloprovincialis* [a] | 100 | 0.04 ± 0.09 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Ebro delta, Spain | [5] |
| *Mytilus galloprovincialis* [a] | 18 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Mytilus galloprovincialis* [b] | 80 | 1.9 ± 0.2 | $H_2O_2$ | 1.2 | FTIR | Ionian Sea, Greece | [14] |
| *Mytilus edulis* | 25 | 0.06 ± 0.13 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Saint Brieuc bay, France | [5] |
| *Mytilus edulis* | 25 | 0.32 ± 0.22 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | Inschot, Netherlands | [5] |
| *Mytilus edulis* | 36 | 0.36 ± 0.07 p/g | $HNO_3$ | 5 | Raman | North sea, Germany | [3] |
| *Mytilus edulis* | - | 0.2 ± 0.3 p/g | $HNO_3$ | 5 | Raman [c] | North sea | [4] |
| *Mytilus edulis* | - | 0.37 p/g | $HNO_3$:$HClO_4$ | 13 | Hot Needle | North sea, Belgium | [1] |
| *Mytilus edulis* | 390 | 1.5 – 7.6 (0.9 – 4.6 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [6] |
| *Mytilus edulis* | - | 1.05 – 4.44 p/g | Enzyme | 52 | FTIR | Oban, Scotland | [9] |
| *Mytilus edulis* | 120 | 0.60 ± 0.56 (0.23 ± 0.20 p/g) | KOH | 12 | FTIR | Atlantic coast, France | [10] |
| *Mytilus edulis* | 162 | 1.1 – 6.4 (0.7 – 2.9 p/g) | $H_2O_2$ | 5 | FTIR | United Kingdom coast | [11] |
| *Mytilus trosssulus* | 450 | 0.04 ± 0.19 (0.26 ± 1.3 p/g) | Enzyme | 20 | FTIR | Baltic sea | [13] |
| *Crassostrea gigas* | 11 | 0.47 ± 0.16 p/g | $HNO_3$ | 5 | Raman | Atlantic ocean | [3] |
| *Crassostrea gigas* [a] | 12 | 0.6 ± 0.9 (2.10 ± 1.71 p/g) | KOH | - | None | Pacific ocean | [2] |
| *Crassostrea gigas* | 60 | 2.10 ± 1.71 (0.18 ± 0.16 p/g) | KOH | 12 | FTIR | Atlantic coast, France | [10] |
| *Saccostrea cucullata* | 330 | 1.7 – 4.0 (1.5 – 7.2 p/g) | KOH | 20 | FTIR | Pearl River Estuary, China | [12] |
| *Venerupis philippinarum* | 27 | 8.4 ± 8.5 (0.9 ± 0.9 p/g) | $HNO_3$ | 1.2 | None | British Columbia, Canada | [7] |
| *Venerupis philippinarum* | 27 | 11.3 ± 6.6 (1.7 ± 1.2 p/g) | $HNO_3$ | 1.2 | None | British Columbia, Canada | [7] |
| *Venerupis philippinarum* [a] | 24 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Scapharca subcrenata* [a] | 6 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Tegillarca granosa* [a] | 18 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Patinopecten yessoensis* [a] | 6 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Alectryonella plicatula* [a] | 18 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Sinonovacula constricta* [a] | 6 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Meretrix lusoria* | 18 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |
| *Cyclina sinensis* | 30 | 4.3 – 57.2 (2.1 – 10.5 p/g) | $H_2O_2$ | 5 | FTIR | Chinese coasts | [8] |

[a] Individuals sampled on local market; [b] Research of MP in gills and digestive gland; [c] Fibers were no considered as MP
"-" : No data; p/g: Particle/g of tissue wet weight; FTIR: Fourier Transform Infra-Red

References : [1] De Witte et al. (2014); [2] Rochman et al. (2015); [3] Van Cauwenberghe and Janssen (2014); [4] Van Cauwenberghe et al. (2015); [5] Vandermeersch et al. (2015); [6] Li et al. (2016); [7] Davidson and Dudas (2016); [8] Li et al. (2015); [9] Courtene-Jones et al. (2017); [10] Phuong et al. (2018); [11] Li et al. (2018b); [12] Li et al. (2018a); [13] Railo et al. (2018); [14] Digka et al. (2018)

312

313 It is important to be aware that considering all recorded fibers as plastic particles could
314 overestimate the contamination. Indeed, fibers identification with Raman or FTIR spectroscopy
315 is an issue due to fibers being too thin (Cho et al., 2019; Käppler et al., 2016). According to
316 Hermsen et al. (2017), studies with the lowest MP contamination levels in fish are those using
317 clean air conditions, high quality assurance criteria and polymer identification. Moreover, Dris
318 et al. (2016) demonstrated that fiber identification is important. Indeed, only 29% of analyzed
319 fibers could be considered as plastics while others fibers were mostly made of cotton (Dris et
320 al., 2016). Equivalent results were recently found in mussels sampled in the United Kingdom
321 with some fibers (≈10%) identified as natural particles (Li et al., 2018b) and with 89% of fibers
322 being identified as natural particles or unidentified in bivalves sampled on fishery markets in
323 South Korea (Cho et al., 2019). More recently, Stanton et al. (2019) found that natural fibers
324 represent 93.8% of the total fibers found in freshwater and atmospheric fallout samples. All
325 these studies highlight that plastic fibers are not always dominating samples and as recently
326 recommended (Käppler et al., 2018; Remy et al., 2015), fibers identification should be
327 performed to allow their inclusion in MP pollution counts, only if they are made of plastic.
328 However, to date, in some studies on MP contamination in bivalves, fibers were accurately
329 identified as plastic particles (Courtene-Jones et al., 2017; Murray and Cowie, 2011; Phuong et
330 al., 2018) but in other studies fibers or others particles were not verified using identification
331 techniques (Davidson and Dudas, 2016; De Witte et al., 2014; Santana et al., 2016) (Table 4)
332 possibly leading to an overestimation of MP contamination. Some studies (Avio et al., 2015;
333 Davison and Asch, 2011; Foekema et al., 2013; Ory et al., 2017; Santana et al., 2016; Van
334 Cauwenberghe et al., 2015) did not included fibers in their final MP *stricto sensu* counts, in
335 order to estimate accurate MP contamination levels in seafood products.

336 In addition to MP contamination variability according to locations, methodological approaches
337 can also be sources of heterogeneity and variability of the results found in the literature (Table

4). Indeed, to date, there is no harmonized protocol for the extraction and characterization of MP from seafood products, despite the call from different institutions (Directive Strategy Framework, 2017; OSPAR, 2016). Differences of methods used to determine MP contamination in bivalves included different chemicals used to digest organism tissue which may lead to substantial degradation of some polymers (Dehaut et al., 2016), different types of filters (pore size and composition), identification of particles (visual vs chemical) which may lead to false positive or false negative results (Lenz et al., 2015), inclusion of fibers in the results and management of atmospheric contaminations. In this work, KOH 10% was used as its suitably was shown for bivalve digestion without degrading multiple plastic polymers (Dehaut et al., 2016; Kühn et al., 2017) whereas some acids ($HNO_3$ and $HClO_4$) discolored and degraded some polymers (Dehaut et al., 2016) leading to possible underestimation of MP in organisms. In addition, after chemical digestion, filtrations at 1.6 µm porosity were realized in order to recover a broad size of MP even if the smallest particle found in the present work measured 15 µm. Then, particles resembling MP were chemically identified using µ-Raman spectroscopy. Such chemical identification step is essential to accurately estimate MP contamination in the environment and the biota (Dehaut et al., in press; Hermsen et al., 2018). Some guidelines were recently suggested to improve and harmonize protocols used to study MP contamination in seafood products (Dehaut et al., in press).

Fibers were found in all samples (Table 3). Mussels and cockles from BdV were significantly more contaminated (Post-hoc after Kruskal-Wallis, $p<0.05$) with respectively 2.42 ± 0.55 fiber/individual, namely 0.44 ± 0.10 fiber/g of tissue ww and 2.34 ± 0.77 fiber/individual, namely 0.82 ± 0.28 fiber/g of tissue ww, in comparison with mussels and cockles sampled at LP and BA (Table 3). Fibers and MP were found in bivalves from all sampling sites whereas PCP were only found in bivalves sampled at LP and BA (Table 3). PCP were found in 12 mussels (24%) from LP with 0.38 ± 0.23 pigment/individual (0.12 ± 0.07 pigment/g of tissue

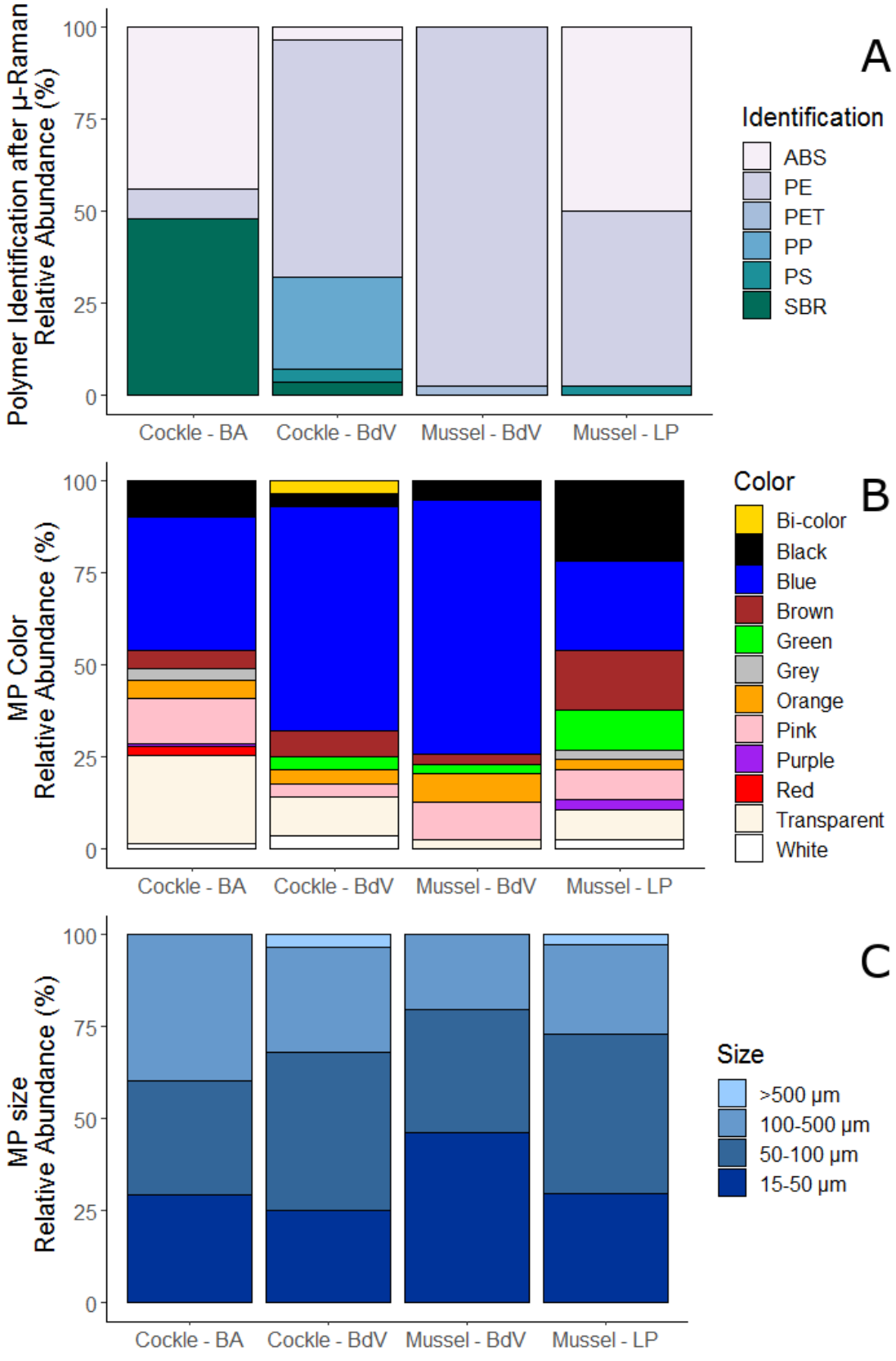
ww) and were found in 4 cockles (8%) from BA with 0.18 ± 0.2 pigment/individual (0.06 ± 0.06 pigment/g of tissue ww). For mussels, all categories of particles, MP, fibers and PCP, were found in 34 individuals (68%) from LP and 43 mussels (86%) from BdV (Table 3). In BdV, 43 cockles (86%) were contaminated while all types of particles were found in 36 cockles (72%) from BA (Table 3). Levels of MP, fiber and PCP contamination per individual was significantly higher in mussels sampled at BdV (3.20 ± 0.60) in comparison with mussels sampled at LP (2.68 ± 1.33) (Post-hoc after Kruskal-Wallis, $p<0.05$). Fibers were the dominant particles in mussels and cockles sampled from BdV (Table 2). The Baie des Veys is influenced by two rivers with a total mean discharge of 53 $m^3/s$ (Grangeré et al., 2009) and could also be influenced by the Seine depending on meteorological conditions (Ellien et al., 2004). As particles found in the Seine river are mainly in form of fibers (Dris et al., 2015), the Seine discharge could contaminate mussels and cockles of the BdV with fibers. In the present study, when PCP and fibers were included in MP counts, corresponding to all particles, contamination levels for mussels and cockles were much higher (Table 3) but stayed between the observed contamination levels found for bivalves sampled along the European coast (Table 4). Presenting data with and without PCP and fibers allowed comparisons between all available studies including those executed with and without chemically identified particles.

Among the 228 MP found in mussels and cockles, five different polymers and one copolymer were identified using µ-Raman spectroscopy: PE, PP, PS, ABS, PET and styrene butadiene rubber (SBR) copolymer. PE, ABS and SBR were the most common polymers found in the cockles and mussels sampled with respectively 36.8%, 32.5% and 26.3% of all identified MP. Each remaining polymer represented less than 5% of the MP found in the bivalves. Proportions of polymers found in bivalves according to species and sampling sites were significantly different (Chi-square, $p<0.001$) (Fig. 2A). Indeed, PE was mainly found in mussels sampled at BdV and LP and in cockles sampled at BdV whereas ABS and SBR were mainly found in

388 bivalves sampled in BA and LP. Moreover, PP was only found in cockles sampled at BdV (Fig.
389 2A). PE is common in samples which is in accordance with the available literature as PE is one
390 of the most common polymers found in the marine environment (Frère et al., 2017; Rezania et
391 al., 2018) and it is the most abundant polymer product worldwide (Geyer et al., 2017;
392 PlasticsEurope, 2018). Polymers found in cockles sampled at BA were more contaminated with
393 SBR in comparison with other sites (Fig. 2A). SBR is mainly used to make tires (Hao et al.,
394 2001; Wagner et al., 2018) which could be a source of MP in marine environment (Rochman,
395 2018). Moreover, this plastic polymer is also used in road materials (Sundt et al., 2014). As a
396 highway is present above the Authie River, tire and road material could be the source of this
397 polymer found in cockles although particles identified as SBR were not all black. Additionally,
398 positions of common cockle and mussel in the water column could influenced MP exposure in
399 those species. Indeed, ABS density is greater than seawater (Tarrazó-Serrano et al., 2018) and
400 will tend to sink to the bottom explaining the large proportion of this polymer contaminating
401 cockles sampled at BA (Fig 2A). However, cockles from BdV (Fig 2A) are contaminated by
402 PE, PP and PS although their densities are lighter than seawater (Andrady and Rajapakse,
403 2017). Nevertheless, MP colonization by bacteria and MP incorporation into marine aggregates
404 may increase their sinking rate (Galloway et al., 2017) and explain PE, PP and PS contamination
405 of cockles in BdV. Furthermore, cockles live in intertidal sand flats where floating MP may be
406 deposited at low tide.
407 MP colors varied significantly according to sampling sites and species (Chi-square, $p<0.001$)
408 (Fig. 2B). For all sampling sites and species, the blue color dominated in all samples with 24%
409 and 69% of MP found in mussels sampled at LP and BdV and 36% and 61% in cockles sampled
410 at BA and BdV (Fig. 2B). Other colors representing important proportions were black,
411 transparent, pink, brown and green (Fig. 2B). In another study conducted on the Atlantic coast
412 of France, mussels and oysters were mainly contaminated by grey, black, green and red MP but

were less contaminated by blue MP (Phuong et al., 2018). Here differences in polymers and particles colors found in bivalves could be related to differences in sources of plastic in the studied sites that remain to be ascertain in environmental studies requiring further significant analytical development.

No significant difference was found for MP size classes according to species and sampling sites (Chi-square, p=0.17) (Fig. 2C). For all the 228 identified MP, size classes were represented as follow: 31.7% 15-50 µm, 34.8% 50-100 µm, 32.6% 100-500 µm and 0.9% >500 µm with the smallest MP measuring 15 µm. A majority of the MP measured less than 100 µm (66.5%). It is in agreement with a recent study using a slightly different protocol (10% KOH digestion step followed by a density separation step; 5 µm pore size cellulose filters) and size cut-off (5-5000 µm), which demonstrated that 83% of MP found in mussels sampled on the French Atlantic coast measured between 20 and 100 µm (Phuong et al., 2018). The presence of smaller MP in both shellfish species reflects the fact that bivalves are filter feeders. Indeed, mussels ingest preferentially particles measuring 7-35 µm (Strohmeier et al., 2012) and cockles ingest particles measuring 7-11 µm (Iglesias et al., 1992). In addition, plastic particle numbers tend to increase with decreasing particle sizes (Erni-Cassola et al., 2017).




429

430 **Fig 2: Relative abundance of microplastics (MP) according to polymer identification (A), color (B) size classes (C) found**
431 **in mussels and cockles sampled at Le Portel (LP), Baie d'Authie (BA) and Baie des Veys (BdV). Microplastics were**
432 **identified as polyethylene (PE), polypropylene (PP), polystyrene (PS), acrylonitrile butadiene styrene (ABS), styrene**
433 **butadiene rubber (SBR) and polyethylene terephthalate (PET).**

### 3.3. Hydrophobic organic compounds and plastic additives concentrations in mussels and cockles

The second objective of this study was to combine particular and chemical analyses to test if chemical pollutants can be a proxy of plastic contamination by comparing particles and chemical analyses.

PAH were detected in mussels and cockles from BdV at 5.48 ± 3.09 ng/g and 0.06 ± 0.02 ng/g, respectively (Fig. 3). The most abundant compound was phenanthrene (24-32% of $\Sigma_{PAH}$). PAH concentrations are far below levels found in mussels from the Bay of Brest (France) (639 ± 73 ng/g and 492 ± 44 ng/g) and Barcelona (Spain) (273 – 405 µg/kg dry weight) (Lacroix et al., 2017; Porte et al., 2001). However, PAH concentrations are in the range of those found in mussels and clams sampled in Milan market (Italy): not detected (n.d) - 13.95 ng/g and n.d - 4.35 ng/g (Chiesa et al., 2018).

PCB were only found in mussels sampled at BdV with 1.00 ± 0.59 ng/g, PCB 105 being the most concentrated congener (Fig. 3). PCB concentration in mussels from BdV were below concentrations found in mussels sampled at Milan market (Italy) (n.d – 49.2 ng/g) (Chiesa et al., 2018) or sampled at Le Conquet (France) (10.46 ng/g dry weight) (Bodin et al., 2007). Moreover, the PCB concentration found in mussels from the BdV, a non-polluted area, are far below concentrations recorded in mussels (538.45 ng/g dry weight) sampled at Antifer, Bay of Seine (France) which is a highly polluted estuary (Bodin et al., 2007).

Organochloride pesticides (OCP) were measured in mussels from BdV and cockles from BA at concentrations of 0.23 ± 0.12 ng/g and 0.04 ± 0.08 ng/g respectively (Fig. 3). These levels are below the concentrations found in mussels (max: 7.58 ng/g dry weight) from the Adriatic Sea (Kožul et al., 2009).

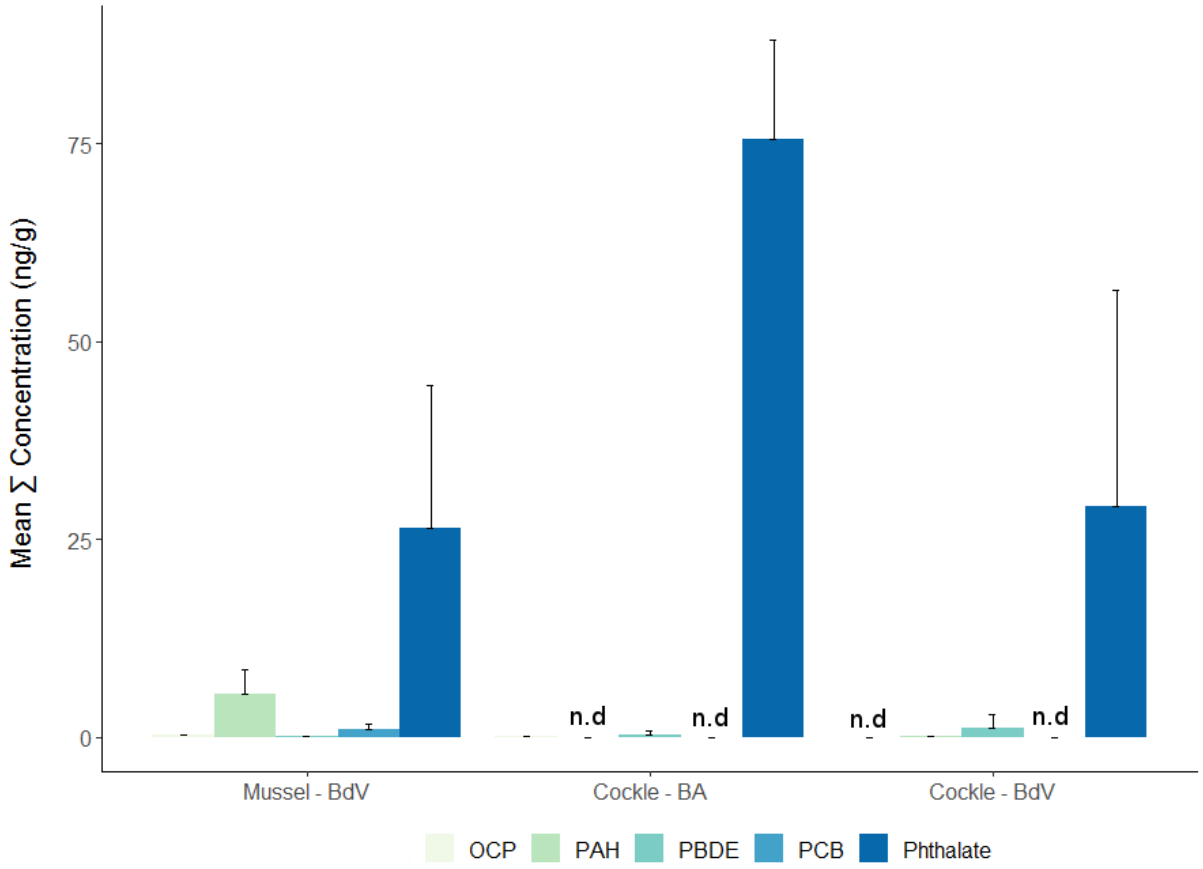



**Fig 3: Average concentration (ng/g) of ΣPAH, ΣOCP, ΣPBDE, ΣPCB and ΣPhthalate (+ confidence interval 95%) in mussels sampled at BdV (n=2) and cockles sampled at BA and BdV. n.d: values below limit of detection. BA: Baie d'Authie; BdV: Baie des Veys.**

Phthalates were the most concentrated pollutants in digestates for all samples (Fig. 3). Average $\Sigma_{Phthalate}$ were respectively 26.36 ± 18.16 ng/g, 75.53 ± 12.49 ng/g and 29.18 ± 27.23 ng/g for mussels sampled at BdV and cockles samples at BA and BdV with DEHA or DMP being the most concentrated phthalates for all samples (50-98% of $\Sigma_{Phthalate}$). These results are in accordance with studies on the contamination of mussels and oysters by phthalates at False creek, Vancouver, Canada. In the study by Mackintosh et al. (2004), mean $\Sigma_{Phthalate}$ (including DEP, diisobutyl phthalate (DiBP), DnBP, DEHP, di-n-octyl phthalate (DnOP) and dionylphthalate (DNP)) were 17.27 ng/g and 16.78 ng/g for mussels and oysters respectively. However, as phthalates studied by Mackintosh et al. (2004) are not the same, comparisons have to be made carefully. In another study, Blair et al. (2009) found 585 ng/g wet weight for the monobutyl phthalate (MnBP) in mussel tissue.

Finally, PBDE were detected in both species from all the sampling locations with 0.07 ± 0.05 ng/g, 0.23 ± 0.45 ng/g and 1.16 ± 1.71 ng/g for mussels from BdV and cockles from BA and BdV, respectively (Fig. 3). These are below concentrations found in mussels from the coast of Spain (0.229 ng/g) and France (2.71 – 9.88 ng/g) (Bellas et al., 2014; Johansson et al., 2006). at relatively low levels compared with commonly reported levels in coastal and estuarine areas.

Overall, the contaminant levels found in soft tissue of bivalves are low and may not represent a danger for seafood consumption. Indeed, for PCB, the regulatory threshold is fixed at 75 ng/g in Europe (European Commission, 2011). For PAH, the regulatory threshold is fixed at 50 ng/g for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (European Commission, 2014). No regulatory threshold exists in Europe for plastic additives in seafood products.

Two hypotheses were tested by measuring HOC, plastic additives and MP contents in mussels and cockles: (i) whether the HOC and plastic additive contamination could be used as indicators of MP contamination (as suggested in Fossi et al. (2012)); (ii) whether highly contaminated areas in terms of dissolved chemicals also correspond to MP hotspots. However, no correlation was found between MP contamination and HOC or plastic additive concentrations possibly because of the low number or concentrations of MP and chemicals found in the sampled bivalves. Previous studies have demonstrated that HOC or plastic additives could be used as indicators for MP contamination in marine mammals, birds or fish (Fossi et al., 2014, 2012; Rochman et al., 2014; Tanaka et al., 2013). However, some other studies demonstrated that MP are not a vector of HOC to marine organisms (Gouin et al., 2011; Kwon et al., 2017). Plastic additives are not commonly studied despite the fact that plastics are sources of these chemicals in the environment and for marine organisms (Hermabessiere et al., 2017). Indeed, Jang et al. (2016) demonstrated that mussels (*Mytilus galloprovincialis*) living on expanded polystyrene (ePS) buoys concentrated more hexabromocyclodecane (HBCD) than mussels living on other

497 substrates. As HBCD is a plastic additive present in ePS buoys, Jang et al. (2016) suggested
498 that transfer occurs when mussels ingest ePS particles. To date, this is the only reported
499 relationship between MP contamination and plastic additive concentration in bivalve.

500 ## 4. Conclusion

501 This study is the first to describe MP contamination in commercially important bivalves from
502 the French Channel coast and the first to evaluate the microplastic contamination of the cockle
503 *Cerastoderma edule*. The present work contributes to the assessment of MP contamination in
504 bivalves used as seafood and highlights some important points. Blue mussels and common
505 cockles sampled from the French Channel coastlines exhibited between 0.76 ± 0.40 and 2.46 ±
506 1.16 MP/individual and between 0.15 ± 0.06 and 0.74 ± 0.35 MP/g of tissue wet weight. As
507 demonstrated in the present study, formal identification for MP studies is mandatory and has to
508 be performed for all studies on MP pollution to ensure correct estimations. Indeed, without
509 proper identification, MP contamination could be overestimated. Beyond the fact that formal
510 MP identification is mandatory to properly assess MP pollution, MP characteristics measured
511 by spectroscopy (shape, polymers) also provided some clues on MP sources and fates in the
512 environment. For instance, in the present study particles identification provided evidence that
513 plastic pollution in BA is different from a close site (LP) and that in a same site (BdV), bivalves
514 ingest different plastic polymer depending on their habitat meaning that plastic pollution is
515 different in the water column. In this work, no relationship between MP contamination of
516 bivalves and the concentration of HOC or plastic additive could be shown probably due to the
517 low number of MP and chemicals found in the bivalves soft tissues.

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## Declaration of interest

None.

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# Microplastic contamination and pollutant levels in mussels and cockles collected along the English Channel coasts

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## Supplemental Table 1: Particles analyzed by µ-Raman spectroscopy and fibers (blank not subtracted) visually sorted from mussels and cockles.

| Species | Sampling site [1] | Fiber [3] | Unidentified particles [4] | Identified [4] | | | Total |
|---|---|---|---|---|---|---|---|
| | | | | Natural particles [5] | Microplastics | Pigments | |
| Mussel | LP [2] | 239 | 465 | 164 | 38 | 19 | 925 |
| | | *25.8%* | *50.3%* | *17.7%* | *4.1%* | *2.1%* | *100%* |
| | BdV [2] | 184 | 65 | 16 | 39 | 0 | 304 |
| | | *60.5%* | *21.4%* | *5.3%* | *12.8%* | *0%* | *100%* |
| Cockle | BA [2] | 188 | 169 | 65 | 123 | 9 | 554 |
| | | *33.9%* | *30.5%* | *11.7%* | *22.2%* | *1.6%* | *100%* |
| | BdV [2] | 156 | 73 | 39 | 28 | 0 | 296 |
| | | *52.7%* | *24.7%* | *13.2%* | *9.5%* | *0%* | *100%* |

[1] LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

[2] Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

[3] Not analyzed by µ-Raman

[4] Analyzed by µ-Raman

[5] Natural particle included minerals, organic and inorganic carbon.

## Supplemental Table 2: Particles analyzed by µ-Raman spectroscopy and fibers (blank not subtracted) visually sorted from mussels and cockles. Results are expressed before chemometrics treatment.

| Species | Sampling site [1] | Fiber [3] | Unidentified particles [4] | Identified [4] | | | Total |
|---|---|---|---|---|---|---|---|
| | | | | Natural particles [5] | Microplastics | Pigments | |
| Mussel | LP [2] | 239 | 513 | 140 | 2 | 31 | 925 |
| | | *25.8%* | *55.5%* | *15.1%* | *0.2%* | *3.4%* | *100%* |
| | BdV [2] | 184 | 84 | 15 | 0 | 21 | 304 |
| | | *60.5%* | *27.6%* | *4.9%* | *0.0%* | *6.9%* | *100%* |
| Cockle | BA [2] | 188 | 284 | 67 | 1 | 14 | 554 |
| | | *33.9%* | *51.3%* | *12.1%* | *0.2%* | *2.5%* | *100%* |
| | BdV [2] | 156 | 95 | 29 | 2 | 14 | 296 |
| | | *52.7%* | *32.1%* | *9.8%* | *0.7%* | *4.7%* | *100%* |

[1] LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

[2] Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

[3] Not analyzed by µ-Raman

[4] Analyzed by µ-Raman

[5] Natural particle included minerals, organic and inorganic carbon.