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Fleurine Akoueson, Ika Paul-Pont, Kévin Tallec, Arnaud Huvet, Périne Doyen, et al.. Additives in polypropylene and polylactic acid food packaging: Chemical analysis and bioassays provide complementary tools for risk assessment. *Science of the Total Environment*, 2023, 857 (Part 2), pp.159318. 10.1016/j.scitotenv.2022.159318 . anses-03811053

HAL Id: anses-03811053

<https://anses.hal.science/anses-03811053v1>

Submitted on 25 Oct 2022

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1 **Additives in polypropylene and polylactic acid food packaging: chemical** 2 **analysis and bioassays provide complementary tools for risk assessment**

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12 **Abstract**

13 Plastic food packaging represents 40% of the plastic production worldwide and belongs to the
14 10 most commonly found items in aquatic environments. They are characterized by high
15 additives contents with more than 4000 formulations available on the market. Thus they can
16 release their constitutive chemicals (i.e. additives) into the surrounding environment,
17 contributing to chemical pollution in aquatic systems and to contamination of marine organism
18 up to the point of questioning the health of the consumer. In this context, the chemical and
19 toxicological profiles of two types of polypropylene (PP) and polylactic acid (PLA) food
20 packaging were investigated, using *in vitro* bioassays and target gas chromatography mass
21 spectrometry analyses. Plastic additives quantification was performed both on the raw
22 materials, and on the material leachates after 5 days of lixiviation in filtered natural seawater.
23 The results showed that all samples (raw materials and leachates) contained additive
24 compounds (e.g. phthalates plasticizers, phosphorous flame retardants, antioxidants and UV-
25 stabilizers). Differences in the number and concentration of additives between polymers and
26 suppliers were also pointed out, indicating that the chemical signature cannot be generalized

27 to a polymer and is rather product dependent. Nevertheless, no significant toxic effects was
28 observed upon exposure to the leachates in two short-term bioassays targeting baseline
29 toxicity (Microtox® test) and Pacific oyster *Crassostrea gigas* fertilization success and embryo-
30 larval development. Overall, this study demonstrates that both petrochemical and bio-based
31 food containers contain harmful additives and that it is not possible to predict material toxicity
32 solely based on chemical analysis. Additionally, it highlights the complexity to assess and
33 comprehend the additive content of plastic packaging due to the variability of their composition,
34 suggesting that more transparency in polymer formulations is required to properly address the
35 risk associated with such materials during their use and end of life.

36 **Keywords**

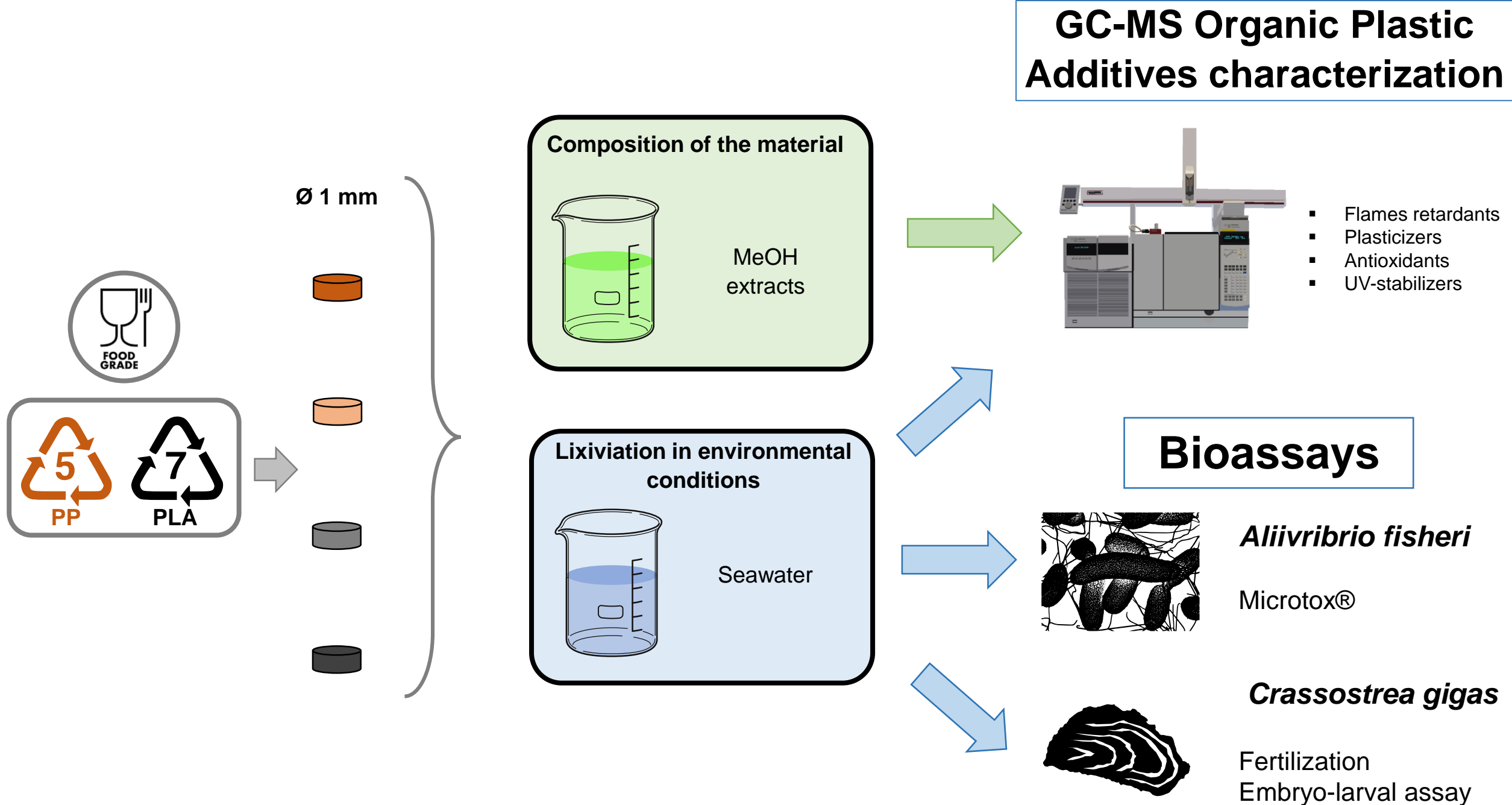
37 Food containers, plastic, additives, leachates, bioassays

38 **Highlights**

39

- 40 • Petro- and bio- based plastics packaging contains organic plastic additives
- 41 • Leaching of organic plastic additives was observed with all the tested protocols
- 42 • Differences in chemical signature between polymers and suppliers were observed
- 43 • Leachates from PP and PLA food containers did not show any *in vitro* toxicity

44



45 **1. Introduction**

46 Plastic debris is the major fraction of solid waste pollution in the marine environment. It is
47 estimated that 75% of all marine litter is plastic (Napper and Thompson, 2020). Among this
48 plastic pollution, approximately 50% of the items are food packaging materials (de Kock et al.,
49 2020; Gerigny et al., 2018; OSPAR et al., 2010), of which polypropylene (PP) is one of the
50 most employed resin. Its production accounts for 80-90% of the global plastic demand
51 (PlasticEurope, 2021; Zimmermann et al., 2019), along with expanded polystyrenes (EPS) and
52 polyethylene terephthalate (PET) (Iñiguez et al., 2016; Zhou et al., 2011). More than 4000
53 chemicals are known to be in the composition of plastic packaging (Groh et al., 2019), including
54 additives which are intentionally used to improve the properties of the material. A few review
55 papers have highlighted the most common groups of additives in plastics, such as plasticizers,
56 flames retardants, antioxidants and stabilizers (Fred-Ahmadu et al., 2020; Hahladakis et al.,
57 2018). Many of the additives (e.g. bisphenols, phthalates, nonylphenols) are known to be
58 hazardous, even at low concentrations, posing a risk for marine organisms (Hahladakis et al.,
59 2018; Oehlmann et al., 2009) as a main driver of plastic toxicity (Beiras et al., 2021). The
60 majority of plastic additives are not chemically bound to the plastic polymers and have the
61 potential to leach out from the plastic to the surrounding environment (Andrady, 2011;
62 Koelmans et al., 2014), causing various types of damages to organisms (e.g., embryo
63 development, immobility, physical activity, mortality, endocrine disruption, gene mutation). The
64 ecotoxicity of some plastic leachates has been characterized on diverse aquatic organisms
65 such as copepods, barnacles, oysters, mussels, urchins, lugworms, fish and photosynthetic
66 bacteria (Gardon et al., 2020; Hamlin et al., 2015; Huang et al., 2021; Oliviero et al., 2019;
67 Tallec et al., 2022; Tetu et al., 2019), without, however, identifying the compound(s)
68 responsible for the observed toxicity. Just one single study (Tian et al., 2021) managed this
69 demonstrating the link between the high concentration of a rubber additive subproduct and
70 acute toxicity events in coho salmon.

71 The use of petro-based food packaging being controversial, bio-based plastics are more and
72 more promoted as an alternative to conventional plastics, with a production volume which is
73 expected to increase in the future (EuropeanBioplastics, 2021; Geueke et al., 2014). The
74 polylactic acid (PLA) is nowadays the most produced bio-based plastic, especially for food
75 container (FC) products (Ncube et al., 2020). Either way, whether the materials are derived
76 from a natural or petrochemical resource, they are both produced to fulfill the same function
77 and are therefore similarly formulated. There are now some studies that gave first indications
78 of the toxicity of bio-based and biodegradable products and that also demonstrated that bio-
79 based materials are not necessarily safer than conventional plastics (Chagas et al., 2021a;
80 Chagas et al., 2021b; de Oliveira et al., 2021; Klein et al., 2021; Lambert and Wagner, 2017;
81 Malafaia et al., 2021; Zimmermann et al., 2020a).

82 Moreover, concerns have arisen concerning the safety of FCs (Groh and Muncke, 2017)
83 especially in regards to the migration of a wide variety of chemicals for which there is a lack of
84 hazard information (Muncke, 2011). Therefore, it is important to explore the threats of complex
85 and diverse chemical mixtures emitted by plastic products. However, although non-target
86 screening analyses have previously been applied on plastics leachates, most of the chemicals
87 remained unidentified (Muncke, 2011; Zimmermann et al., 2021). As a result, a better and
88 more specified understanding of the composition of plastics is required to relate their chemical
89 content and their toxicity.

90 The migration of compounds that are allowed to be included in FCs are only tested in regards
91 to food contact application. However, plastic FCs are widely found in the environment and are
92 therefore present as microplastics (MPs). Hence, it's important to question the impacts of these
93 particles once in the environment. Thus, this study aims to assess and compare the chemical
94 additive contents and the ecotoxicity of the chemicals leaching from plastic marketed FCs
95 made of PP and PLA. Target chemical analyses were carried out by a stir bar sorptive
96 extraction (SBSE) followed by a thermal desorption gas chromatography–mass spectrometry
97 in tandem (TD-GC-MS/MS) to characterize chemicals present in the plastics and the ones

98 released in sea water leachates. In addition, we also assessed the leachates ecotoxicity
99 through sensitive short-term bioassays: (i) the base line toxicity with the Microtox[®] test on the
100 bioluminescent *Aliivibrio fischeri* bacteria, chosen as a rapid assay which is reproducible, cost
101 effective and more sensitive than other end points for nonspecific toxicity (Neale et al., 2012),
102 and (ii), two bivalve sensitive endpoints (His et al., 1999), fertilization and embryo-larval
103 developmental success of the Pacific oyster *Crassostrea gigas*. This specie was targeted as a
104 key organism for coastal ecosystems and because of its ecological and economical roles
105 (FAO, 2020).

106 **2. Materials and methods**

107 **2.1. Plastic sample selection and production**

108 In this study, four samples of food packaging items, available on the French market, made of
109 polypropylene (PP) and polylactic acid (PLA) were used. Two items were selected per polymer
110 resin, produced by two different suppliers, tagged A and B.

111 Small punches, i.e. cylinders with a diameter of 1 mm, were cut into the food packaging items
112 using biopsy punches from Farla-Medical (Antwerpen, Belgium). Homogeneity of punches was
113 assessed by measuring the thickness and the volume of n=20 punches per polymer type using
114 an Olympus SZX16 stereomicroscope (France) equipped with a UC90 camera and treated
115 using OLYMPYS CellSens Dimension 3.2 software. Data are presented as a mean value \pm
116 standard deviation (s.d).

117 **2.2. Extraction of potential additives**

118 To avoid sample contamination, all glassware was burnt for 6h at 500°C in a Nabertherm
119 LT40/11/B410 muffle furnace (Lilienthal, Germany) prior to the experiments. Additionally, the
120 preparation of the material's leachates was conducted under a laminar flow hood.

121 **2.2.1. Extraction of additives from the punch surface (methanol extracts)**

122 200 ± 0.51 mg of plastic punches were weighed for each plastic (n=4). 10 mL of methanol
123 (MeOH) (Sigma-Aldrich Co., Saint-Quentin-Fallavier, France) were added to the punches in
124 order to obtain a concentration of 20 mg.mL⁻¹. The mixtures were placed on an orbital shaker
125 at 100 rpm for 24h, in dark conditions at room temperature (20 °C). A control experiment, i.e.
126 MeOH treatment without any punch was also carried out following the same conditions. At the
127 end of the extractions, the plastics were removed from the MeOH and the extracts were
128 transferred into clean glass bottles and immediately submitted to chemical analysis (part 2.3).

129 **2.2.2. Preparation of leachates in seawater**

130 Leachates were prepared adding 2g of FC punch samples into 1L of filtered natural seawater
131 (FSW) for each product. The FSW used for the leachate preparation was collected in Harellet
132 (France) autoclaved and filtered on Whatman™ 0.22 µm Millipore filters (Maidstone, United
133 Kingdom). Each leachate was placed on an orbital shaker at 100 rpm, allowing the plastic
134 pieces to move freely in the water. Leaching was performed for 24h and 5 days in dark
135 conditions at room temperature (20°C) in order to be in accordance with previous studies
136 mentioning a fast release of organic plastic additives (OPAs) within the first 120h (Gardon et
137 al., 2020; Jang et al., 2021; Paluselli et al., 2019). Nine leaching treatments were prepared:
138 PLA-A (n=2), PLA-B (n=2), PP-A (n=2) and PP-B (n=2) samples at 24h and 5 days, and a
139 control seawater treatment without plastic. At the end of the leaching period, leachates were
140 filtered through Whatman™ 1.6 µm GF/A filters (to remove punches), transferred to clean
141 bottles and used as a stock solution for preparation of the six leachates concentrations levels
142 obtained by serial dilutions: 0.02, 0.2, 2, 20, 200 and 2000 mg.L⁻¹. The middle range
143 concentration 0,2 and 2 mg.L⁻¹ were chosen to be in similar range as to MPs concentration
144 found in the marine environment, respectively medium and worst case scenario (Paul-Pont et
145 al., 2018). All the leachate solutions were conserved at -20 °C during one week prior to the
146 chemical characterization and the toxicity assays. For each treatment, 100 mL of the initial
147 leachate (2000 mg.L⁻¹) were sampled for chemical analysis.

148

2.3. Target chemical analyses

149 The OPAs were quantified in the methanolic extracts (cf. 2.2.1) and in the FSW leachates (cf.
150 2.2.2) in duplicates by SBSE-TD-GC-MS/MS following the methodology described by Lacroix
151 et al. (2014).

152 Regarding the MeOH extracts, 1 mL of the samples solutions were transferred to a clean glass
153 bottle and supplemented by 9 mL of MeOH and 100 mL of FSW. For the seawater leachates,
154 an aliquot of 100 mL was transferred to a clean glass bottle and 10 mL of MeOH was added.
155 For both MeOH extract and seawater leachate, the prepared samples were doped with 10 ng
156 of each deuterated standards, i.e. deuterated phthalates, deuterated polybromodiphényléters
157 (PBDEs), deuterated polycyclic aromatic hydrocarbons (PAHs) and deuterated nonylphenol
158 (NPd8).

159 Gerstel Twister, 20 mm x 0.5 mm polydimethylsiloxane stir bar, (Mülheim an der Ruhr,
160 Germany) were then placed in each sample on a MIX15 magnetic stirrer (Munich, Germany)
161 and stirred at 700 rpm for 16 h of extraction in the dark at room temperature (20 °C). At the
162 end of the extraction time, stir-bars were removed from the solutions, rinsed with distilled water,
163 dried over a blot paper, and directly analyzed by TD-GC-MS/MS.

164 OPAs were analyzed using an Agilent 7890A gas chromatography system coupled to an
165 Agilent 7000 triple quadrupole mass spectrometer (Little Falls, USA). GC-MS/MS device was
166 equipped with a Gerstel thermal desorption unit (TDU) and a MultiPurpose Sampler in order
167 to automatically introduce stir bars into the system. Thermodesorption were carried out at 280
168 °C for 6 min and samples were then cryofocused at -10 °C via a Gerstel cooled injection system
169 (CIS). Analytes were injected in splitless mode into an Agilent HP-5MS GC column (Agilent
170 Technologies) (30 m x 0.25 mm x 0.25 µm) and the CIS was heated to 310 °C at 12 °C.s⁻¹.
171 The detailed analytical condition of the GC temperature program and the MS are presented in
172 Table S1. A stir bar conditioning was performed on each bar prior to re-use in order to eliminate
173 any compounds not completely desorbed.

174 In total, 57 OPA's, i.e. 18 plasticizers, 19 flames retardants, 5 antioxidants and 15 stabilizers,
175 were targeted and quantified (Table S2) based on criteria of use, toxicity, concentration in
176 plastics and feasibility of GC-MS analysis. The quantitative analysis of plastic additives was
177 performed by external calibration using a multiple reaction monitoring (MRM) method divided
178 into 4 groups containing a maximum of 20 transitions (Table S3) with two transitions for each
179 compound. Several levels of calibration (i.e. 0, 0.5, 1, 5, 10, 50, 100, 500 ng.L⁻¹), in duplicate,
180 were prepared. Data analysis was performed using Mass Hunter software from Agilent
181 (10.2.733.8). Analytes were quantified by calculating the target additive/deuterated analyte
182 ratio, and corrected by subtracting the blank (i.e. MeOH or FSW control without FC materials).

183 **2.4. Toxicity assessment on a unicellular organism - Microtox® assay**

184 The Microtox® assay is an acute test measuring the baseline toxicity of a substance based on
185 the decrease or inhibition of the bioluminescence of the bacteria *Aliivibrio fischeri* (Wadhia and
186 Thompson, 2007). Here, this acute test was performed on each FSW leachate at the highest
187 concentration (i.e. 2000 mg.L⁻¹) and a control treatment (FSW with no addition of plastic), if no
188 effect was observed, the lowest concentrations were not tested.

189 The bioluminescence level was measured by Modern Water Ltd Microtox® FX Analyser (New
190 Castle, DE, USA), following the B-Tox Test procedure of the manufacturer's manual. Briefly,
191 the lyophilized *A.fischeri* were rehydrated with 300 µL of the reconstituted solution (RS), the
192 bacteria and the RS were gently mixed with a micropipette and 100 µL were immediately
193 transferred into a clean test glass vial. After 15 min of exposure, 900 µL of working solutions,
194 i.e. either FSW control or leachates, were added into the test vials. Measurements of the
195 luminescence were recorded prior and 15 min after sample addition. The bioluminescence
196 results were automatically compared and corrected with the light output of the control sample,
197 resulting in a relative luminescence inhibition (%). Each assay was performed in triplicate.

198

2.5. Toxicity assessment on an eukaryote organism - Oysters

199

2.5.1. Biological material (animal and gamete collection)

200 Mature Pacific oysters were produced as described in Petton et al. (2015) and held in the
201 Ifremer nursery in Bouin (France). In January 2022, a stock of 120 oysters (36 month old,
202 average weight: 47.6 ± 7.2 g) was transferred from the Ifremer nursery to the Ifremer
203 experimental facilities in Argenton (France) at stage 0 (i.e. the undifferentiated stage) and
204 conditioned for 6 weeks with suitable conditions for germ cell maturation. Briefly, oysters were
205 placed in an experimental raceway, using a flow-through system with 20 μ m-filtered running
206 seawater at 18 ± 1.0 °C and fed with a mixed diet of two microalgae at a daily ration equal to
207 8% dry mass algae/ dry mass oyster. At ripeness (stage 3), oysters were randomly sampled
208 to perform gametes and embryo-larval assays. Oyster sex was determined under an EVOS™
209 XL Core Imaging System microscope (ThermoFisher Scientific Waltham, Massachusetts,
210 USA), $\times 10$ – 20 magnification, on a 50- μ L subsample from the gonad of each individual.
211 Gametes from 3 males and 3 females were collected by stripping the gonad as described by
212 Steele and Mulcahy (1999). This step was repeated in four replicates, with a total of 12 males
213 and 12 females per condition. Sperm and oocytes solutions were then sieved at 60 μ m in order
214 to eliminate debris. Spermatozoa and oocytes were diluted with respectively 100 mL and 1 L
215 of FSW at 20 °C, and left for 1 h prior to use to ensure gamete quality, i.e. spermatozoon
216 mobility and round shape of oocytes, which were checked by microscopy (Tallec et al., 2018).

217

2.5.2. Fertilization success assay

218 After collecting the gametes, their concentrations were assessed by flow cytometry using a
219 EasyCyte Plus cytometer from Guava Merck Millipore (Burlington, Massachusetts, USA).
220 Gametes were placed at the same time in glass vials with a spermatozoa-to-oocyte ratio of
221 100:1 and a final concentration of 1,000 oocytes.mL⁻¹. Vials were filled with the different
222 solutions of leachate to a final volume of 5 mL (4 leachates from FC, 6 concentrations: 0
223 (control FSW), 0.02, 0.2, 2, 20 and 200 mg.L⁻¹, and 4 replicates per condition, leading to 96

224 vials). After 1.5 h of exposition to FC leachates, samples were fixed with a formaldehyde-
225 seawater solution (0.1% final) to estimate the fertilization yield under a Zeiss Axio Observer Z1
226 microscope with $\times 10$ -40 magnification. Per vial, 100 oocytes were observed. An oocyte was
227 considered fertilized when polar bodies or cell divisions were observable. The fertilization yield
228 (%) was estimated as: number of fertilized oocytes / total of oocytes \times 100. (Martinez-Gomez
229 et al., 2017).

230 **2.5.3. Embryo-larval assay**

231 The standardized ISO 17244:2015 assay (ISO, 2015) was used to determine the embryo-
232 toxicity of FC leachates. Fertilization was carried out following the procedure described above,
233 in 4 replicates, with gametes collected from 3 males and 3 females per replicates (total: 12
234 males and 12 females) in 2-L glass beakers filled with 1.5 L of FSW. Once fertilization was
235 achieved with high fertilization yields (>90%) and embryos were at the 2-cell stage (verified
236 using a Zeiss Axio Observer Z1; $\times 10$ -40 magnification), embryos were collected and placed in
237 25 mL of the different leachate treatments (control FSW, 0.02, 0.2, 2, 20, 200 and 2000 mg.L⁻¹)
238 to achieve 60 embryos.mL⁻¹. After 48h of exposure in dark conditions, all samples were fixed
239 with a formaldehyde-seawater solution (0.1% final) to estimate the normal D-larval yield. For
240 each vial, 100 larvae were observed using a Zeiss Axio Observer Z1 microscope, with $\times 10$ -
241 40 magnification. The normal D-larval yield (%) was defined as: number of normal D-larvae \div
242 (number of normal + abnormal D-larvae) \times 100. Abnormal D-larvae were identified based on
243 morphological malformations (Mottier et al., 2013) such as shell, mantle or hinge
244 malformations, developmental arrest during embryogenesis or evidence of larvae death, e.g.
245 D-stage larvae with an empty shell.

246 **2.6. Statistical analysis**

247 Statistical analyses were performed using R-Studio software (1.4.1106) (R Core Team).
248 Concerning the bioassays, i.e. fertilization success, embryo-larval and Microtox® assay, all
249 data expressed in percentages were normalized using $\sin^{-1}(\sqrt{X})$ transformation. Normality

250 and homoscedasticity were verified before carrying out two-way parametric ANOVA to test the
251 differences in variables between factors, i.e. polymers and leachate concentration. When
252 necessary a Tuckey's post hoc test was carried out using the *car* package (3.0-12) (Fox et al.,
253 2022) was used to determine the significant differences between each group. Assuming that
254 one of the hypotheses was not verified, a non-parametric Kruskal-Wallis test was performed.
255 Kruskal Wallis tests were followed by a Nemeyni's post hoc test using *agricolae* (1.3-5) (De
256 Mendiburu, 2021) and *PMCMR* (4.4) (Pohlert, 2021) packages. Mean differences were
257 considered as significant when p-value < 0.05. Data presented onto the figures are not square
258 root transformed. Target chemical analyses were performed on leachates from all products,
259 statistical significance of differences could not be carried out as only n = 2 leachate solution
260 were analyzed per item. Data were compared based on mix – max of these n = 2 values.

261 **3. Results**

262 **3.1. Characterization of FC punch**

263 The thickness (μm) of each sample punches was measured, and the surface areas (mm^2)
264 and masses (μg) were calculated (Table S4).

265 The thickness, surface area and mass for each resin sample were, respectively: $277 \pm 10 \mu\text{m}$,
266 $2.44 \pm 0.01 \text{ mm}^2$ and $272.00 \pm 0.01 \mu\text{g}$ for PLA-A, $353 \pm 18 \mu\text{m}$, $2.68 \pm 0.06 \text{ mm}^2$ and 346.00
267 $\pm 0.02 \mu\text{g}$ for PLA-B, $451 \pm 13 \mu\text{m}$, $2.99 \pm 0.04 \text{ mm}^2$ and $340.00 \pm 0.01 \mu\text{g}$ for PP-A, and 245
268 $\pm 15 \mu\text{m}$, $2.34 \pm 0.05 \text{ mm}^2$ and $185.00 \pm 0.01 \mu\text{g}$ for PP-B.

269 Significant differences in mass were observed between all samples varying from 25 to 46%,
270 except between PLA-B and PP-A (ANOVA followed by Tuckey post Hoc test, p-value < 0.05).
271 Similarly, significant differences were observed in surface area between all samples (ANOVA
272 followed by Tuckey post Hoc test, p-value < 0.05). However, within a polymer the punches
273 metrics were homogeneous (Table S4 and Fig. S1).

3.2. Target OPAs TD-GC-MS/MS analyses into plastic food packaging materials (MeOH extracts)

A total of 21 compounds: 8 plasticizers, 3 phosphorous flame retardants, 5 antioxidants and 5 UV-stabilizers were quantified in all MeOH extracts (Fig. 1D).

The bio-based PLA samples contained the highest number of chemicals: 17 additives were identified in both PLA samples and only 8 to 9 additives were identified in PP samples. PLA and PP samples both contained a majority of plasticizers (respectively 7 compounds out of 17, and 6 compounds out of 8 to 9) and 3 UV-stabilizers. 6 compounds were common to both PP and PLA samples, i.e. plasticizers: Bis-2-Ethylhexyl Adipate (DEHA), Diisooheptyl phthalate (DIHP), Tributyl Acetyl Citrate (ATBC) and Tri(2-ethylhexyl) phosphate (TEHPA), UV stabilizers: UV-328 and UV-327 (Fig. 1A). However PFRs and nonylphenol antioxidants were exclusively identified in PLA extracts and absent from PP extracts.

Overall, the number of OPAs within samples made of the same polymer, A and B, was equivalent: 17 OPAs were identified in both PLA-A and PLA-B, with 16 compounds in common plus one specific for each product (Fig. 1B). Concerning PP samples, 9 and 8 OPAs were detected in PP-A and PP-B respectively, with 8 compounds in common and one specific to PP-A (Fig. 1C).

The detected OPAs were quantified in the $\text{ng}\cdot\text{mg}^{-1}$ range (i.e. between 0.04 to $7.5 \text{ ng}\cdot\text{mg}^{-1}$). For the 6 compounds common to both PP and PLA extracts, all the concentrations were higher in PLA extracts in comparison to PP samples (e.g. x5 for UV-328 and ATBC, x9 for UV-327, x2 for TEHPA and x12 for DEHA). The concentrations were considered higher when the factor was $> x1.5$.

Out of the 16 additives common to all PLA extracts, 7 compounds (Triphenyl Phosphate (TPhP), UV-327, Dicyclohexyl phthalate (DCHP), Nonylphenol Monoethoxylate (NP1OE), 4-Tert-Octylphenol (4-t-OP), 4-Nonylphenol Monoethoxylate (4-NP1OE) and 4-nonylphenol (4-NP)) were measured in higher concentrations (x12 for TPhP and x2 for the other OPAs) in

300 PLA-A extracts than in extracts from PLA-B, and 4 compounds (UV-326, TEHPA, Tris(1,3-
301 Dichloro-2-Propyl)Phosphate (TDCPP) and DEHA) were measured in higher concentrations
302 (x3 for UV-326 and x2 for the other OPAs) in PLA-B extracts compared to PLA-A (Fig. 1D).
303 For OPAs that are common in PP extracts, one compound out of 8 (UV-327) was measured in
304 higher concentrations (x5) in PP-A extracts than in PP-B , and 3 (DEHA, Diisononyl
305 hexahydrophthalate (DINCH) and DIHP) were measured in higher concentrations (all x2) in
306 PP-B extracts compared to PP-A (Fig. 1D).

307 *Figure 1 goes here*

308 **3.3. Target OPAs analyses into plastic food packaging leachates**

309 **3.3.1. Impacts of the lixiviation duration on OPAs release**

310 OPAs have been detected in all the leachate samples. Overall, the number of additives
311 identified is slightly higher in the 5 days (5d) leachates than in the 24h leachates. A leaching
312 time of 5 days permitted to retrieve the Tricresyl phosphate (TCrP), Nonylphenols (NPs),
313 NP1OE, 4-NP1OE and UV-328. For PLA-A, 10 and 11 compounds were identified in the 24h
314 and 5d leachates respectively, with 10 compounds in common and one specific to the 5d
315 leachates (Fig. 2A and 3A). For PLA-B, 8 and 9 compounds were identified in the 24h and 5d
316 leachates respectively, with 8 compounds in common and one specific to the 5d leachates
317 (Fig. 2B and 3B). Concerning PP leachates, 9 and 12 compounds were identified in the 24h
318 and 5d PP-A leachates respectively, with 8 compounds in common, one in the 24h leachates
319 only, and 4 specific to the 5d leachates (Fig. 2C and 3C). Finally, 10 and 12 compounds were
320 identified in the 24h and 5d PP-B leachates respectively, with 9 compounds in common, one
321 in the 24h leachates exclusively, and 3 specific to the 5d leachates (Fig. 2D and 3D).

322 The quantitative results do not show any clear pattern in 24h vs. 5d leachates. Concerning
323 PLA-A leachates, 2 compounds (ATBC and NPs) were present in higher concentration in the
324 24h leachates, as well as two compounds (TDCPP and TCrP) that showed higher
325 concentrations in the 5d leachates (Fig. 2E.1.). Similarly, the analysis of PLA-B leachates

326 showed higher concentration for 3 compounds (ATBC, NPs and NP1OE) in 24h leachates, as
327 well as 3 compounds (Dimethyl phthalate (DMP), Tripropyl phosphate (TPP) and TDCPP) in
328 5d leachates (Fig. 2E.2.).

329 The PP-A 5d leachates presented higher concentrations for 5 compounds out of 13 (DMP,
330 TCrP, NPs, 4-NP and NP1OE), when the PP-A 24h leachate had higher concentrations for
331 only 2 OPAs (ATBC and TPP) (Fig. 2E.3.). The PP-B leachate showed 4 compounds out of 13
332 with a higher concentration in the 24h leachates (DMP, DINCH, TPP and TDCPP). In contrast,
333 the 5d leachate had higher concentrations for 3 compounds (ATBC, 4-NP1OE and UV-327)
334 (Fig. 2E.4.).

335 Considering the results presented above, the 5 days leaching time was chosen for the further
336 chemical and ecotoxicological experiments.

337 **Figure 2 goes here**

338 **3.3.2. OPAs in food packaging's 5 days leachates**

339 In 5d leachates from PP and PLA samples, a total 16 OPAs were detected. PLA leachate
340 samples contained plasticizers (3 compounds out of 9 and 12 for A and B suppliers
341 respectively), phosphorous flames retardants (3 and 2 compounds), antioxidants (3
342 compounds each) and UV-stabilizers (2 and 1 compounds). PP leachates samples contained
343 a majority of plasticizers (4 and 5 compounds out of 12, for A and B suppliers respectively),
344 followed by phosphorous flames retardants and antioxidants (3 and 2 compounds each), and
345 UV-stabilizers (2 to 3 compounds). (Fig. 3C).

346 Beyond that, the number of OPAs between the PP and the PLA leachates was equivalent, with
347 11 and 9 OPAs identified in PLA-A and PLA-B respectively (8 common compounds, 3
348 compounds specific to the supplier A and one specific to the supplier B) (Fig. 3A), and 12
349 OPAs identified in both PP-A and PP-B leachates (9 common OPAs and 3 specific to each
350 suppliers) (Fig. 3B).

351 OPA concentrations in 5d FC leachates ranged between 0.02 and 135.82 ng.L⁻¹. The
352 quantitative results of OPAs do not show any clear patterns between the different leachate
353 samples. Detailed results are presented in Figure 4C, and some tendencies, that illustrate
354 differences between polymer leachates or suppliers, are given below: 2 plasticizers (DEHA
355 and DIHP) were quantified exclusively in PP leachates (at concentrations ranging from 1.55 to
356 52.28 ng.L⁻¹). DINCH was only found in PLA-A at a concentration of 16.66 ng.L⁻¹. ATBC was
357 quantified in PP leachates from the supplier B only (135.8 ng.L⁻¹) at a concentration higher (x3)
358 than in PLA-A and B leachates (46.9 ± 1.42). TCrP was only present in PP-A and PLA-A, both
359 at a concentration of 2.22 ng.L⁻¹. All the leachates contained UV-327 at similar concentrations
360 (0.29 ng.L⁻¹) except in PP-B where this additive was measured at higher (x21) concentration
361 (6.12 ng.L⁻¹) (Fig. 3C).

362 **Figure 3 goes here**

363 **3.3.3. Comparison of the additive contents between raw materials and** 364 **seawater leachates**

365 Some additives were only detected in PLA and PP MeOH extracts (Fig. 1D) but not in their
366 respective leachates (Fig. 3C) (i.e. UV-328, UV-326, TPhP and DEHA for PLA and DINCH and
367 ATBC for PP-A only; Fig S2). Conversely, some additives detected in leachates were absent
368 from MeOH extract (i.e. TDCPP and 4-NP in PP samples, TPP in PP samples and PLA-B,
369 DMP in PLA-B, NPs and NP1OE in PP-A sample) (Fig. S2).

370 **3.4. Evaluation of the ecotoxicity of plastic food packaging leachates**

371 **3.4.1. Baseline toxicity using Microtox[®] assay**

372 No significant effect of leachate exposure was observed on the bioluminescence of the bacteria
373 *Aliivibrio fischeri* (Fig. S3). The results showed less than 10% of bioluminescence inhibition
374 regardless of the material and concentration used.

3.4.2. Early life stages of Pacific oyster

3.4.2.1. Effects of FC leachates on fertilization

No significant differences (ANOVA, p -values > 0.05) were observed on the fertilization yield following the exposure of the oyster gametes to the different concentrations of plastic packaging leachates compared to the control treatment (i.e. FSW) ($86.5 \pm 6.5\%$). Only the highest concentration (i.e. 200 mg.L^{-1}) of PLA-B significantly reduced the fertilization yield in comparison to the FSW control treatment (-12% ; ANOVA followed by Tuckey post Hoc test, p -value < 0.05) (Fig. 4). Overall, the fecundation rate remained high ($>70\%$) regardless of the treatment (except for PLA-B at 200 mg.L^{-1}).

Figure 4 goes here

3.4.2.2. Effects of FC leachates on oyster embryo-larval development

The percentage of normal D shaped larvae in controls was $>80\%$ (Fig. 5). None of the leachate concentrations induced embryo-toxicity (ANOVA or Kruskal-Wallis, p -values > 0.05) compared to the control treatment (mean D-larvae yield = $86 \pm 9.2\%$) (Fig. 5).

Figure 5 goes here

4. Discussion

4.1. Characterization of OPAs and their release from FCs

Material MeOH extracts and leachate analyses provided information on the chemicals associated with plastic packaging and those able to desorb into seawater. Despite some additives that were identified below the detection limit, 22 additives (i.e. phthalates, PFRs, antioxidants and UV-stabilizers) were successfully identified and quantified among the selected compounds. Only 7 of the identified chemicals are included in the permitted starting material of EU No 10/2011 (European Commission, 2011) (i.e. Uvinul 3008; UV 327; UV 326; DEHA; DINCH; Di-allyl phthalate (DAIP); ATBC), and 9 are included in the list established by

400 Oltmanns et al. (2020) compiling 2336 potential emerging toxic chemicals used in FCs, based
401 on a previous EFSA study compiling substances registered under the REACH Regulation (i.e.
402 Uvinul 3008, UV 328, UV 327, TPP, TDCPP, DEHA, DINCH, ATBC and 4-NP) (Fig. 1 and 3).
403 It means that for some of the additives not included in those regulatory lists, the sanitary risks
404 remain unknown since no toxicological or migratory test has been performed.

405 It is noteworthy that in this study, the number of detected phthalates are underestimated since
406 some phthalate compounds such as DEHP, DEP, DBP and DIDP could not be properly
407 characterized because they were ubiquitous contaminants in the laboratory and instruments.
408 Additionally, quantities of some additives, e.g. DMP and ATBC, recorded in the controls (MeOH
409 and seawater without plastic) indicated presence of these compounds in the reagents
410 employed in this experiment or contamination during sample preparation. Such results
411 underline the difficulties and the challenge of studying additive composition of plastic in the
412 laboratory (Zimmermann et al., 2019). Indeed, they are omnipresent (e.g. found in indoor air,
413 solvents, water, experimental apparatus, protection equipment, glassware) and may prevent
414 their studies (Hermabessiere et al., 2020; Ye et al., 2013).

415 The higher additive occurrence and concentration (e.g. TDCPP in PLA-A, DMP in PP-A)
416 observed in 5d leachates in comparison to 24h leachates was the basis for choosing a 5 days
417 leaching time for further experiments. This was in agreement with other studies that used a
418 leaching time of 5 days (CEN, 2002; Tetu et al., 2019), and studies that also demonstrated
419 higher additive concentrations in 5d leachates than in 24h leachates (Gardon et al., 2020). It
420 also permitted a great chemical desorption while avoiding the readsorption of the leached
421 chemicals onto the surface of the plastic particle as noticed by Romera-Castillo et al. (2018).
422 However, it is a complicated task to choose an appropriate leaching duration. Indeed, results
423 published in the literature highlight the dependence of additives' desorption processes on
424 many parameters including the polymer type and the additive. For instance, León et al. (2019)
425 mentioned higher additive desorption rates for PP in comparison to PE. Additionally, the
426 leaching dynamics differ according to the nature of additives. For example, the time needed to

427 reach the desorption equilibrium concentration was estimated to be 3 days for BPA, while it
428 was 80 days for phthalates compounds (Suhrhoff and Scholz-Böttcher, 2016).

429 Our results showed the presence of OPAs such as phosphorous flame retardants (PFRs),
430 antioxidants and UV-stabilizers and with a dominance of plasticizer compounds (Fig. 1 and 3)
431 both in MeOH extracts and leachates. Similar compounds have already been identified and
432 quantified in diverse polymer FC items in the literature. For instance, ATBC and Uvinul 3008
433 (i.e. Octabenzone) were identified in PP samples (Lahimer et al., 2017; Zimmermann et al.,
434 2019) and in plastic-based candy wrappers (Galmán Graíño et al., 2018). Several PAEs,
435 ATBC and DINCH were detected in PVC FC (Carlos et al., 2018), and, Lahimer et al. (2017)
436 identified UV 326 (i.e. Bumetrizole) in PLA samples.

437 Discrepancies in the chemical signature of MeOH extracts and leachates suggest that not all
438 OPAs are leaching or that the concentration of the leachable additives was below the detection
439 limit (Zimmermann et al., 2021). The presence of additives in leachates that were not detected
440 in the MeOH extract (e.g. UV 326, TPhP, Di-n-hexyl phthalate (DHP), DCHP, DAIP and 4-OP)
441 suggests a preferential migration or dissolution into water over methanol (Zimmermann et al.,
442 2019; 2021) .

443 On the one hand, differences in chemical composition and concentration of MeOH extracts
444 between the two types of polymers selected were observed. A greater number of additives and
445 higher concentrations were measured in bio-based PLA MeOH extracts in comparison to PP
446 MeOH extracts, which was also observed in the study of Zimmermann et al. (2019) study.
447 Moreover, the presence of PFRs and nonylphenols antioxidants, exclusively identified in PLA
448 extracts and absent from PP extracts, suggest that the bio-based PLA material contains more
449 hazardous additives than the PP material. On the other hand, the number of additives between
450 PLA and PP leachates was more or less equivalent and were only differentiated by the
451 signatures and concentrations of additives which was not in accordance with other studies. For
452 instance, using high performance liquid chromatography (HPLC) coupled to HR-MS, Klein et
453 al. (2021) detected the highest number of chemicals and intensities in bio-based plastic

454 leachates (PBAT + PLA) in comparison to other polymers including PP. The amount of
455 additives in bio-based samples was even comparable to PVC, known to be a polymer
456 containing larger amounts of plasticizers and stabilizers (Groh et al., 2019; Hahladakis et al.,
457 2018). Gewert et al. (2018) also detected similarly low amounts of OPAs in PP using LC-
458 HRMS, while Bradley and Coulier (2007) identified more chemicals but using a wide variety of
459 analytical techniques. Additionally, Zimmermann et al. (2021) showed that products made of
460 PLA leached relatively few products compared to PP. Evidentially, it is complex to draw
461 conclusions about each type of plastic material, as their recipes and individual properties can
462 be major factors in desorption.

463 Results also pointed out leaching differences between polymers. For instance, DEHA
464 plasticizer, which is present in all the materials' MeOH extract samples (Fig 1D), leached in
465 the SW only for PP samples. This may highlight a difference in leaching properties of additives
466 between the two polymers used in this study, which could be explained by the nature of the
467 polymer (Li et al., 2016) and notably their differences in physicochemical properties (i.e.
468 surface and porosity) (Barrick et al., 2021).

469 The surface, known to significantly affect desorption (Sun et al., 2021; Van de Ven, 1994) ,
470 also differs between the sample resin and between the suppliers, but was considered
471 homogeneous within each replicate of the same FC. However, despite the surfaces' disparities
472 no relationship could be established with among leaching concentrations. As an example, the
473 lower surface area of PP-B ($2.34 \pm 0.05 \text{ mm}^2$), compared to the other PP and PLA samples, is
474 not related to lower quantities of additives.

475 **4.2. Complexity of plastic products' chemical composition**

476 This study highlighted differences in the chemical composition and concentration between
477 manufacturers. Diversity in chemical signatures and high variability of OPAs migration between
478 polymers and suppliers have also been observed in a few studies (Hamlin et al., 2015;
479 Zimmermann et al., 2019 and 2021). Beyond differences in the formulation of each plastic
480 product (Groh et al. 2019), highlighted by different chemical signatures in the MeOH extracts

481 within a polymer type, the release of additives from plastic materials in leachates is also
482 modulated by the permeability of the polymeric matrix, gaps between polymer molecules,
483 physicochemical properties of the additives and properties of the surrounding medium (e.g.
484 salinity, temperature, pH) and time (Kwan and Takada, 2016). It reinforces the challenge to
485 assess the exhaustive chemical composition of plastic materials and leachates by current
486 analytical methodologies (Bolgar et al., 2007; Muncke et al., 2020).

487 Given the diversity of plastic associated chemicals (Groh et al., 2019) the target analysis based
488 on 57 targeted additives (Table S1) is certainly not representative. Several studies have lead
489 a non-target screening of compounds in plastic food packaging, revealing more than 1000
490 chemical features in petro- and bio-based FC materials, including PP and PLA (Zimmermann
491 et al., 2020a; Zimmermann et al., 2020b). Nonetheless, compounds identification with non-
492 target screening approaches are approximate and care should be taken when interpreting the
493 results. Zimmerman et al., (2020b; 2021) and von Eyken et al. (2020) demonstrates that most
494 plastic chemicals remain unknown due to incorrect identification by databases. But this
495 approach can however help to highlight patterns and emerging compounds. Additionally,
496 targeting molecules of interest may help to show the presence of potentially toxic compounds,
497 which will, in combination with ecotoxicological studies, be complementary to gain a global
498 insight of the material risk.

499 Overall, this work provides information on the chemical composition of FC samples made out
500 of PP and PLA, along with the identification of 21 additives in these materials and 16 that
501 leached into SW, in particular phthalates, followed by flame retardants, antioxidants and UV
502 stabilizers. Once released from the polymer matrix into the environment, those molecules can
503 become available for organisms and could cause diverse effects such as endocrine disruption,
504 reproductive, development, mutagenic or behavioral effects (Gunaalan et al., 2020; Muncke,
505 2011).

506

4.3. Bioassays

507 The previous chemical analysis showed the leaching of some additive compounds known to
508 be toxic to marine organisms (e.g. NPs and phthalates) (Hamlin et al., 2015; Hermabessiere
509 et al., 2017; Schrank et al., 2019). However, no effects were observed in the study for any of
510 the carried out bioassay, i.e. *Microtox*[®] base line toxicity test, fertilization and embryo-larval
511 development of *C.gigas* for short leachate exposure times (a few minutes in *Microtox*[®] to 1.5 h
512 for the oyster embryo-larval test).

513 Previous *in vitro* experiments conducted on plastic FC leachates (including PP and PLA)
514 reported baseline toxicity migrating from the products (Szczepanska et al., 2018;
515 Zimmermann et al. 2019; 2020b). Nonetheless, Zimmermann et al. (2020b) pointed out that
516 toxicity was less prevalent in FCs than in plastic not intended to be in contact with food.
517 Disparities of additive numbers and concentrations between manufacturers, as well as
518 variation in base line toxicity depending on the products have also been reported (Klein et al.,
519 2021; Zimmermann et al., 2019). However, the leached additives that were toxic *in vitro*
520 remained mostly unidentified (Zimmermann et al., 2019).

521 Additionally, previous studies have demonstrated toxic impacts of various plastic leachates
522 (not labelled FC) on fertilization or embryo development of diverse aquatic species such as
523 oysters (Gardon et al., 2020; Tallec et al., 2022), mussels (Capolupo et al., 2020; Gandara et
524 al., 2016) and urchins (Oliviero et al., 2019). However, it is important to highlight that most
525 studies conducted their experiments with a worst-case scenario approach, i.e. with high
526 concentrations of plastics (5 to 50 times higher than ours). In addition, some studies enhance
527 migration with a polar solvent (dimethyl sulfoxide, dichloromethane, MeOH (Capolupo et al.,
528 2020; Pannetier et al., 2019)), instead of testing migration using more realistic and softer
529 solvents (e.g. seawater). Although the latter example aims to mimic the desorption of polar
530 organic contaminants, it do not represent the conditions occurring in digestive guts of animals
531 which are characterized by specific pH, digestive enzyme contents, and organic matter
532 (Hermabessiere et al., 2020). Besides, in the case of FC studies, the leaching tests of additives

533 are often perform according to the protocol set by the EU regulation for plastic FCs (i.e. during
534 10 days at 40 °C in the dark) (EuropeanCommission, 2011; Zimmermann et al., 2021) which
535 was not selected in this case as this work aimed at studying the impact of chemical release in
536 the marine environment. Moreover, to the best of our knowledge, no study conducted analyses
537 of plastic FC leachate in regards to their effects on fertilization and embryo toxicity.. Thus, this
538 present study could be a first attempt to evaluate the effects of plastic FC leached chemicals
539 on the gamete fertilization and embryo-larval development of an aquatic species.

540 As no toxic effects were observed, the estimation of the half maximal effective concentration
541 (EC50) (i.e. indicating the concentration of a compound when 50% of its maximal effect is
542 observe, that require a wider range of tested concentrations, , was not possible, or, was higher
543 than 2000 mg/L for all the polymer leachates and for all bioassays performed (i.e. embryo
544 toxicity and Microtox[®]). Furthermore, the absence of toxicity can also be explained by an
545 incomplete lixiviation of additives from the materials due to the low diffusivities of certain
546 additives, like NPs, from certain rigid plastics (Berens, 1997; Koelmans et al., 2014), resulting
547 in a low exposure of the test organisms to OPAs. In addition, in this study, leachates were
548 produced in seawater in the dark. However, different environmental conditions such as water
549 movement, salinity, UV irradiance, and environmental degradation processes, influence the
550 leaching behavior of additives from plastic items. These environmental conditions can also
551 facilitate the release of plastic chemicals and/or generate active compounds and, thus, can
552 affect their toxicity to organisms (Huang et al., 2021; Klein et al., 2021). Likewise, in a human
553 health sanitary safety approach, or in the case of OPAs release in the digestive tract after
554 ingestion of micro particles, different and enhanced mechanisms of lixiviation may occur. For
555 instance, NPs being lipophilic could be expected to more readily migrate into fatty foods over
556 food with lower lipid content or seawater (Hamlin et al., 2015).

557 In any case, it should be kept in mind that “the absence of evidence is not evidence of absence”
558 (Leslie and Depledge, 2020). Even if short-term acute bioassays are useful tools, they neither
559 allow the observation of long term and transgenerational effects, nor the assessment of

560 reproductive disruption effects, that are both widely suspected consequences of plastic
561 additives.

562 **5. Conclusion**

563 The results demonstrate that all the tested products (PP and PLA polymers) contained and
564 released OPAs into seawater under the tested conditions. The chemical content and the
565 leachate composition differed from one polymer to another and, most importantly, variations
566 were found among the same polymer type from one supplier to another. As a result, it was not
567 possible to generalize and attribute a chemical pattern to a specific polymer type since
568 variations were recorded at product level. Evidently, this part highlights the importance of the
569 characterization of the “additivome”, i.e the additive’s content, of the microplastics used for
570 toxicological tests.

571 Even if the results demonstrate that the tested petro- and biobased samples both leached
572 additives compounds, none of the *in vitro* bioassay showed any acute toxicity of the leachates
573 at relevant or high environmental concentrations, with the selected experimental conditions.
574 However, although three different bioassays were tested, it is only possible to draw a
575 conclusion for the perimeter of the conditions tested. As a result, beyond the standard tests
576 applied for food contact packaging which imply that these materials do not transfer compounds
577 to food, results showed that once in the environment the tested FC might not induce acute
578 toxic effects. In future work, modifications of environmental parameters (e.g. temperature,
579 microbial activity, UVs, weathering), organisms tested, and duration of exposure, may provide
580 additional understanding of the toxicology associated with the leachates of these FCs.

581 **6. Acknowledgements and fundings**

582 Fleurine Akoueson is very grateful to the Hauts-de-France Regional Council and ANSES
583 (French Agency for Food, Environmental and Occupational Health & Safety) for financially
584 supporting her PhD scholarship. This work was also funded by the European Union (ERDF),
585 the French government, the Hauts-de-France Regional Council and IFREMER, in the

586 framework of the CPER MARCO. Fleurine Akoueson is grateful for the GDR Polymère et
587 Océans 2050 from CNRS for their financial help. We are grateful to C. Lacroix, R. Jezequel
588 and J.Receveur from the CEDRE (Brest, France) for their technical assistance. We would also
589 like to thank the staff at the Argenton Ifremer station for providing mature experimental oysters.
590 Finally, we would like to thank Nicolina Andersson for her careful English proofread of the
591 manuscript.

592 **7. Authors contribution**

593 **F.Akoueson:** Conceptualization, Investigation, Methodology, Formal analysis, Software,
594 Validation, Visualization, Writing - original draft, review & editing. **K. Tallec:** Methodology,
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597 Conceptualization, Funding acquisition, Supervision, Writing - review & editing. **G. Duflos:**
598 Resources, Conceptualization, Funding acquisition, Supervision, Project administration,
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817

818 9. Figures Captions

819 **Figure 1:** Distribution of the chemical compounds identified by SBSE-TD-GC/MS in MeOH
820 extracts of (A) PLA (A and B), (B) PP (A and B), (C) comparison of PLA and PP samples. (D)
821 Heat map of the chemical compounds quantified in MeOH extracts. (n=2). Values were
822 adjusted according to the chemicals found in the control (MeOH). The white color indicate that
823 the quantitative value of the detected compounds was above the quantitation limit (<LQ). *:
824 additives included in the positive list of the European Commission regulation EU No 10/2011.
825 Δ : additives included in the Emerging toxic chemical list of Olmans et al., 2020.

826 With 4-NP: 4-nonylphenol; 4-NP1OE : 4-Nonylphenol Monoethoxylate; 4-tOP: 4-Tert-
827 Octylphenol; ATBC: Tributyl Acetyl Citrate; DAIP: Di-allyl phthalate; DCHP: Dicyclohexyl
828 phthalate; DEHA: Bis-2-Ethylhexyl Adipate; DHP: Di-n-hexyl phthalate; DIHP: Diisooheptyl
829 phthalate; DINCH: Diisononyl hexahydrophthalate; DMP: Dimethyl phthalate; NPs:
830 Nonylphenols isomer; NP1OE: Nonylphenol Monoethoxylate; TDCPP: Tris(1,3-Dichloro-2-
831 Propyl)Phosphate; TEHPA: Tri(2-ethylhexyl) phosphate; TPhP: Triphenyl Phosphate and TPP:
832 Tripropyl Phosphate.

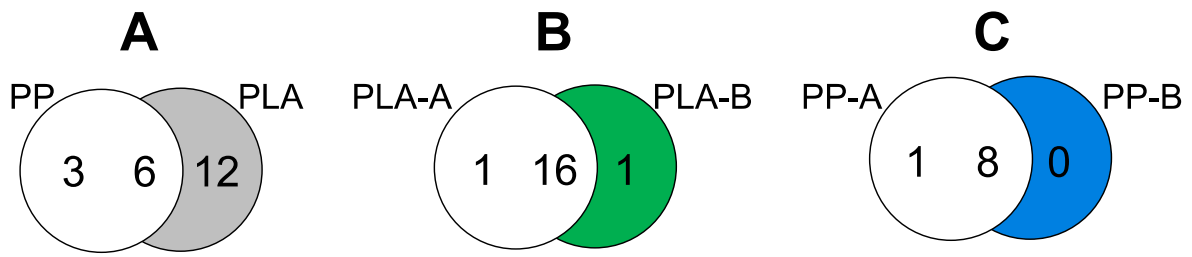
833 **Figure 2:** Distribution of the chemical compounds identified by SBSE-TD-GC/MS in 24h and
834 5 days leachates of (A) PLA A, (B) PLA-B, (C) PP-A and (D) PP-B, at 2000 mg/L. (E) Heat
835 map of the chemical compounds quantified in 24h and 5 days leachates of (E.1.) PLA A, (E.2.)
836 PLA-B, (E.3.) PP-A and (E.4.) PP-B, at 2000 mg/L. (n=2). Values were adjusted according to
837 the chemicals found in the control (seawater). The white color indicate that the quantitative
838 value of the detected compounds was above the quantitation limit (<LQ). *: additives included
839 in the positive list of the European Commission regulation EU No 10/2011. Δ : additives included
840 in the Emerging toxic chemical list of Olmans et al., 2020. *With 4-NP: 4-nonylphenol; 4-NP1OE*
841 *: 4-Nonylphenol Monoethoxylate; ATBC: Tributyl Acetyl Citrate; DEHA: Bis-2-Ethylhexyl*
842 *Adipate; DIHP: Diisooheptyl phthalate; DINCH: Diisononyl hexahydrophthalate; DMP: Dimethyl*
843 *phthalate; NPs: Nonylphenols isomer; NP1OE: Nonylphenol Monoethoxylate; TCrP: Tricresyl*

844 *phosphate; TDCPP: Tris(1,3-Dichloro-2-Propyl)Phosphate; TEHPA: Tri(2-ethylhexyl)*
845 *phosphate and TPP: Tripropyl Phosphate.*

846 **Figure 3:** Distribution of the chemical compounds identified by SBSE-TD-GC/MS in 5 days
847 leachates of (A) PLA (A and B), (B) PP (A and B), at 2000 mg/L. (C) Heat map of the chemical
848 compounds quantified in leachates. (n=2). Values were adjusted according to the chemicals
849 found in the control (seawater). The white color indicate that the quantitative value of the
850 detected compounds was above the quantitation limit (<LQ). “*”: additives included in the
851 positive list of the European Commission regulation EU No 10/2011. “Δ”: additives included in
852 the Emerging toxic chemical list of Olmans et al., 2020. *With 4-NP: 4-nonylphenol; 4-NP1OE :*
853 *4-Nonylphenol Monoethoxylate; ATBC: Tributyl Acetyl Citrate; DEHA: Bis-2-Ethylhexyl*
854 *Adipate; DIHP: Diisooheptyl phthalate; DINCH: Diisononyl hexahydrophthalate; DMP: Dimethyl*
855 *phthalate; NPs: Nonylphenols isomer; NP1OE: Nonylphenol Monoethoxylate; TCrP: Tricresyl*
856 *phosphate; TDCPP: Tris(1,3-Dichloro-2-Propyl)Phosphate; TEHPA: Tri(2-ethylhexyl)*
857 *phosphate and TPP: Tripropyl Phosphate*

858 **Figure 4:** Fertilization yield (%) after exposure (1.5h) of oyster gametes (oocytes +
859 spermatozoa) to leachates of several food containers: PLA-A (yellow), PLA-B (green), PP-A
860 (light blue) and PP-B (blue), at five concentrations: 0.02, 0.2, 2, 20 and 200 mg/L, compared
861 to the FSW control (Red). Homogeneous groups are indicated by the same letter, after
862 statistical tests using ANOVA followed by Tuckey post Hoc test. (n=4)

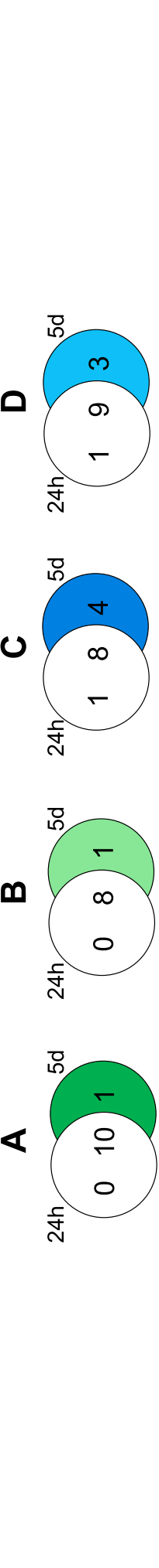
863 **Figure 5:** Normal D-larval yield (%) after exposure (48h) of fertilized oyster oocytes to
864 leachates issued from (A) PLA-A, (B) PLA-B, (C) PP-A and (D) PP-B, food plastic packaging
865 at five concentrations: 0.2, 2, 20, 200 and 2000 mg/L, compared to the FSW control. Values
866 are expresses as mean± 95% confidence interval. Homogeneous groups are indicated by the
867 same letter, after statistical tests using ANOVA or **Kruskal-Wallis** tests. (n=3)

**D**

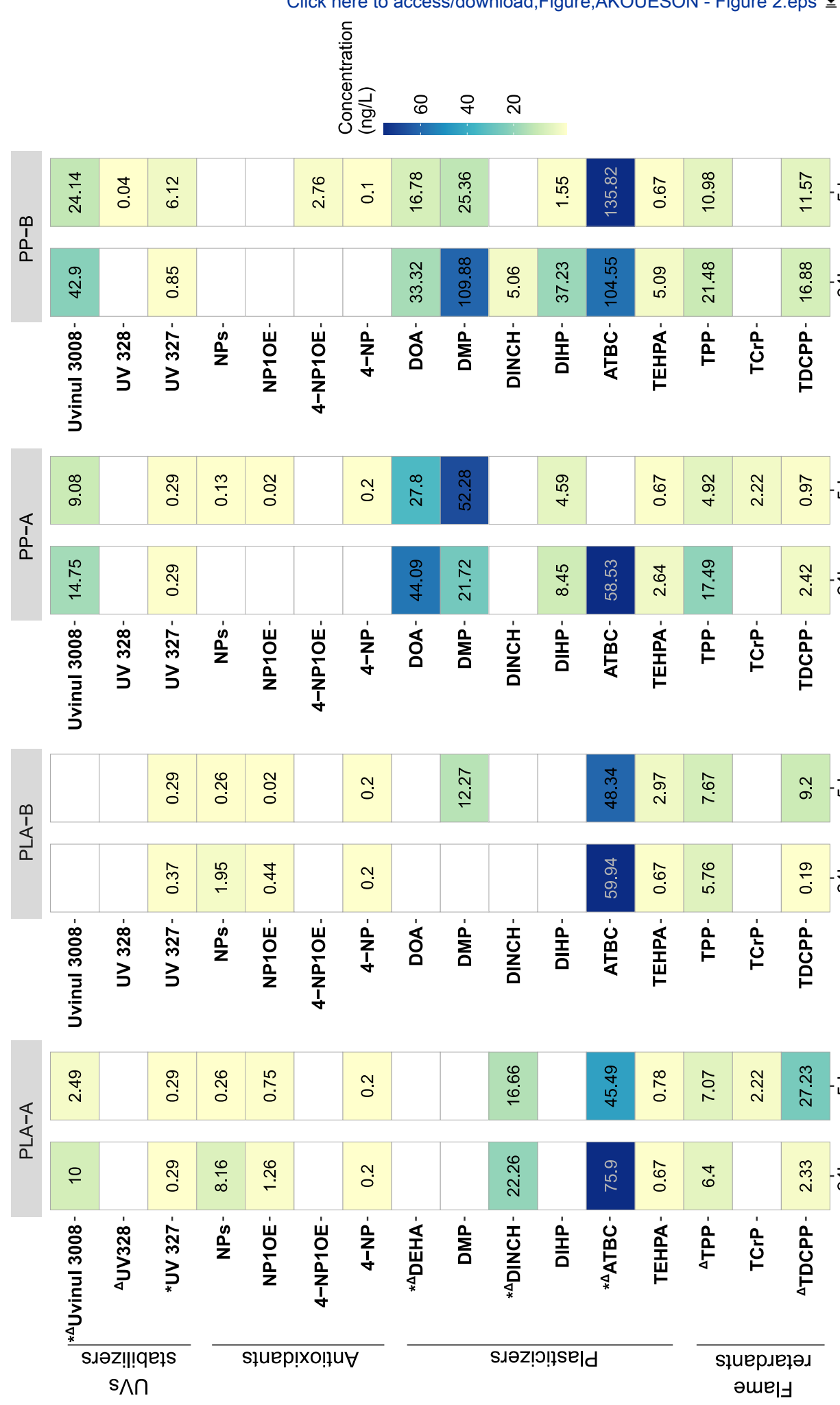
		PLA-A	PLA-B	PP-A	PP-B
Uvs stabilizers	* Δ Uvinul 3008 -			0.2	0.2
	Δ UV328 -	0.19	0.22	0.08	
	*UV 327 -	1.23	0.64	0.17	0.04
	* Δ UV326 -	0.19	0.49		
Antioxidants	NPs -	3.47	3.69		
	NP1OE -	1.61	0.86		
	4-tOP -	2.1	1.27		
	4-NP1OE -	4	1.7		
	4-NP -	0.57	0.32		
	* Δ DEHA -	4.61	7.53	0.3	0.67
	DMP -			1.07	0.77
Plasticizers	* Δ DINCH -			0.25	0.52
	DIHP -	0.22	0.26	0.14	0.26
	DHP -	0.27	0.37		
	DCHP -	0.53	0.21		
	* Δ DAIP -		0.27		
	* Δ ATBC -	3.44	2.33	0.73	0.52
	TEHPA -	0.39	0.65	0.19	0.23
Flame retardants	Δ TPP -	0.17			
	TPhP -	0.12	0.01		
	Δ TDCPP -	0.27	0.45		

Concentration (ng/mg)

Figure 2



E



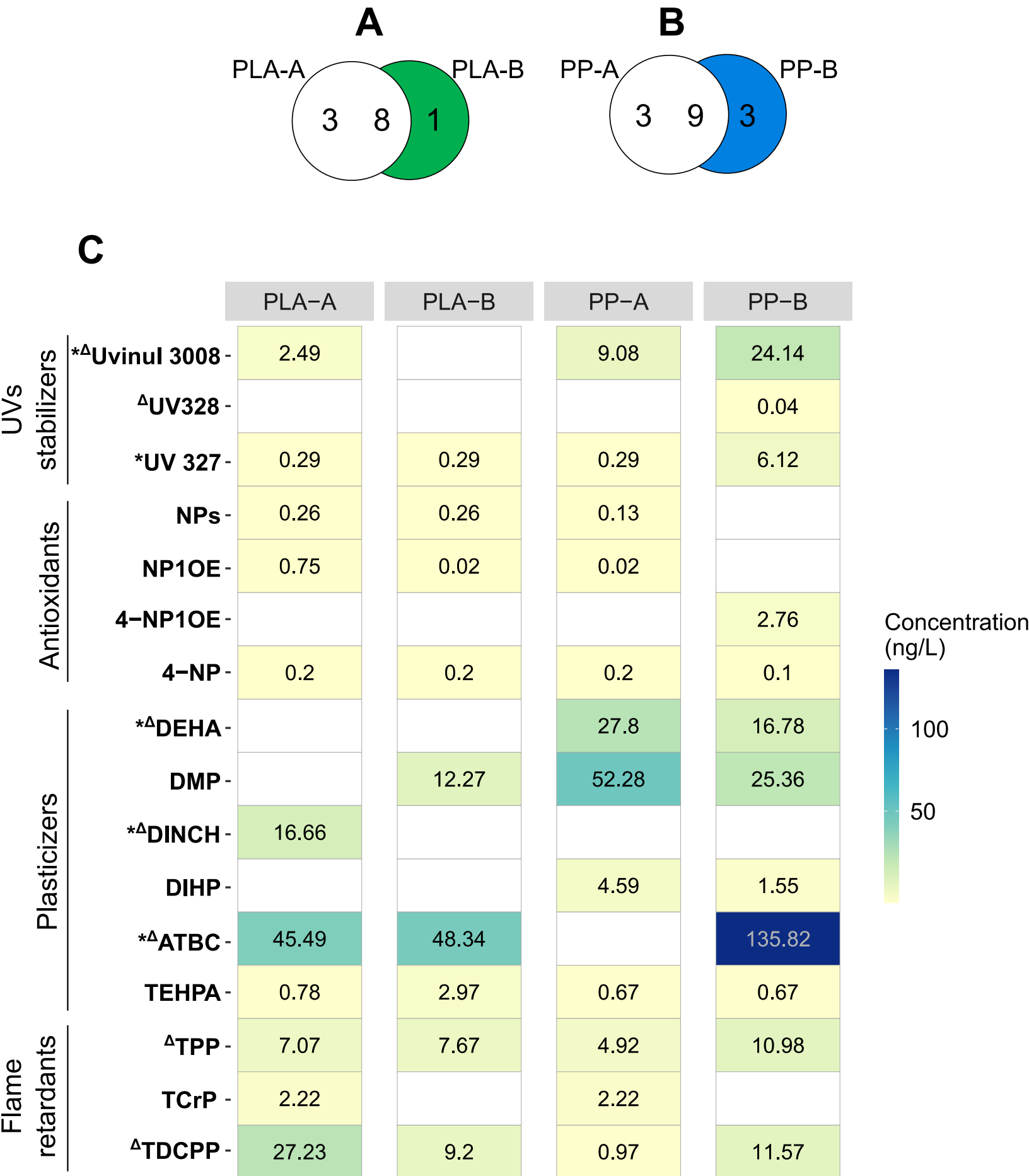


Figure 4

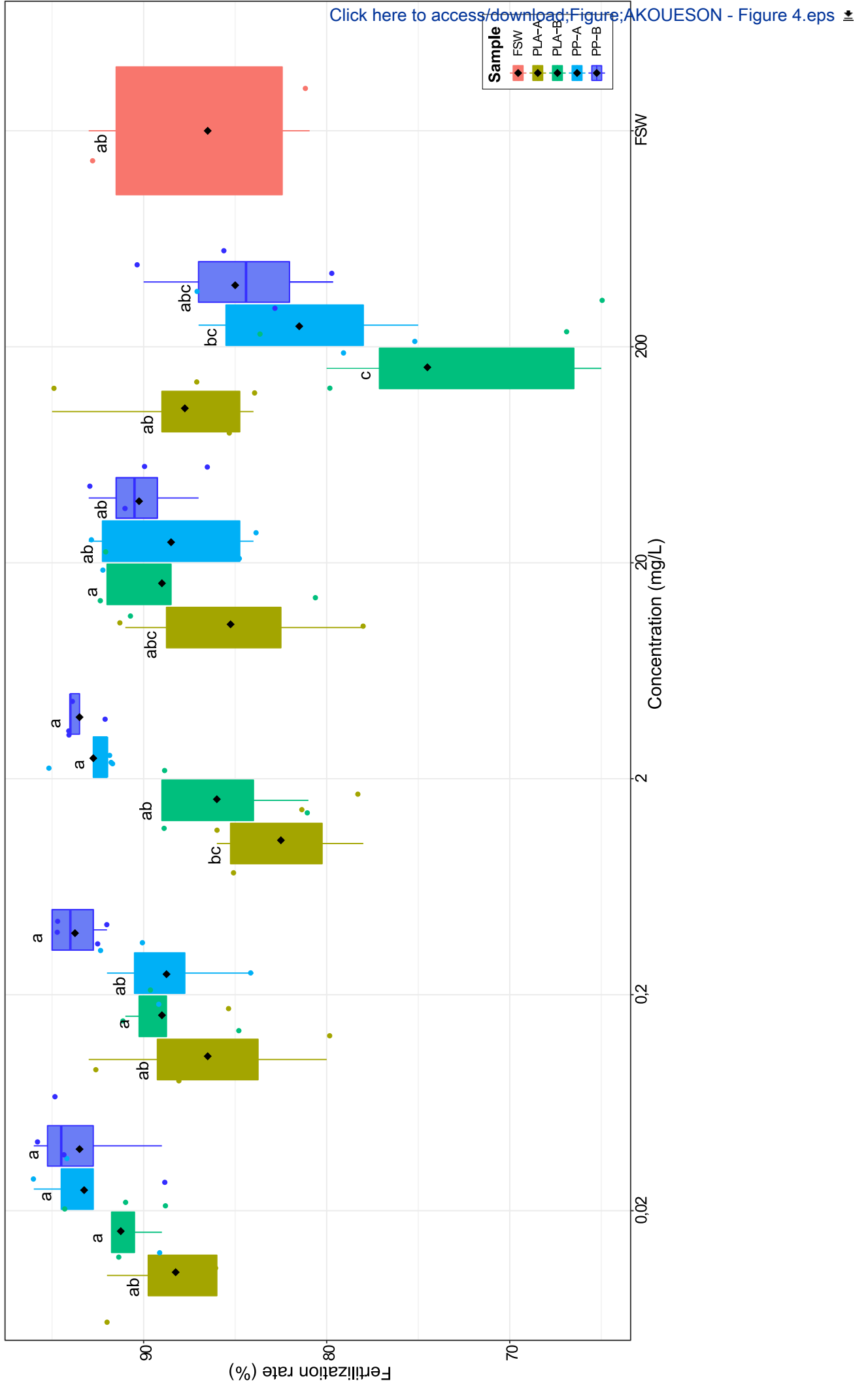
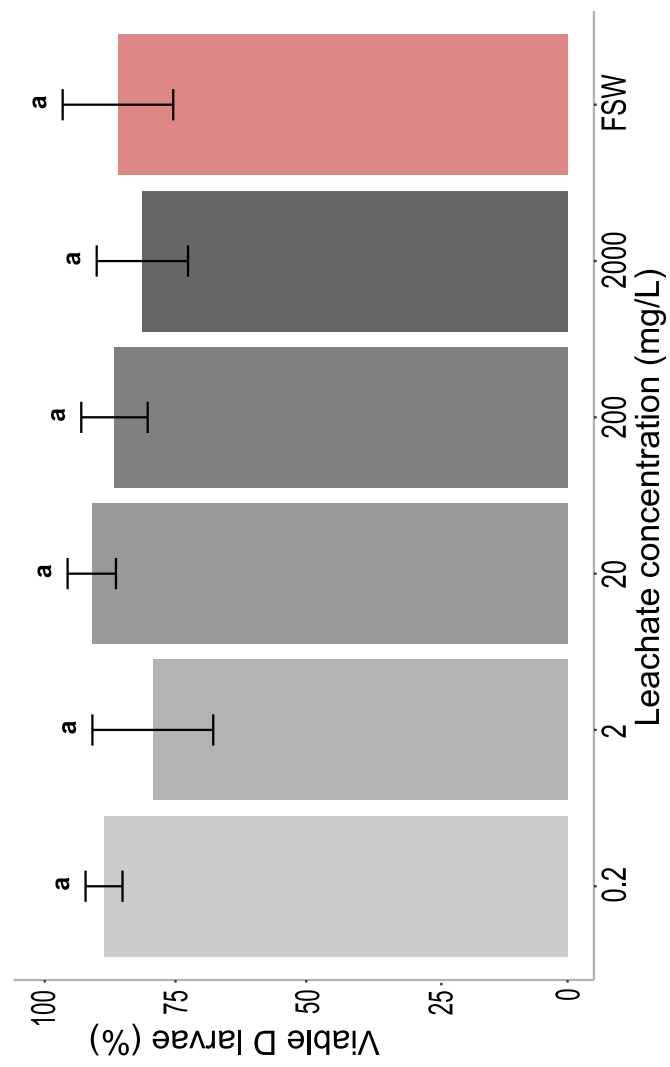
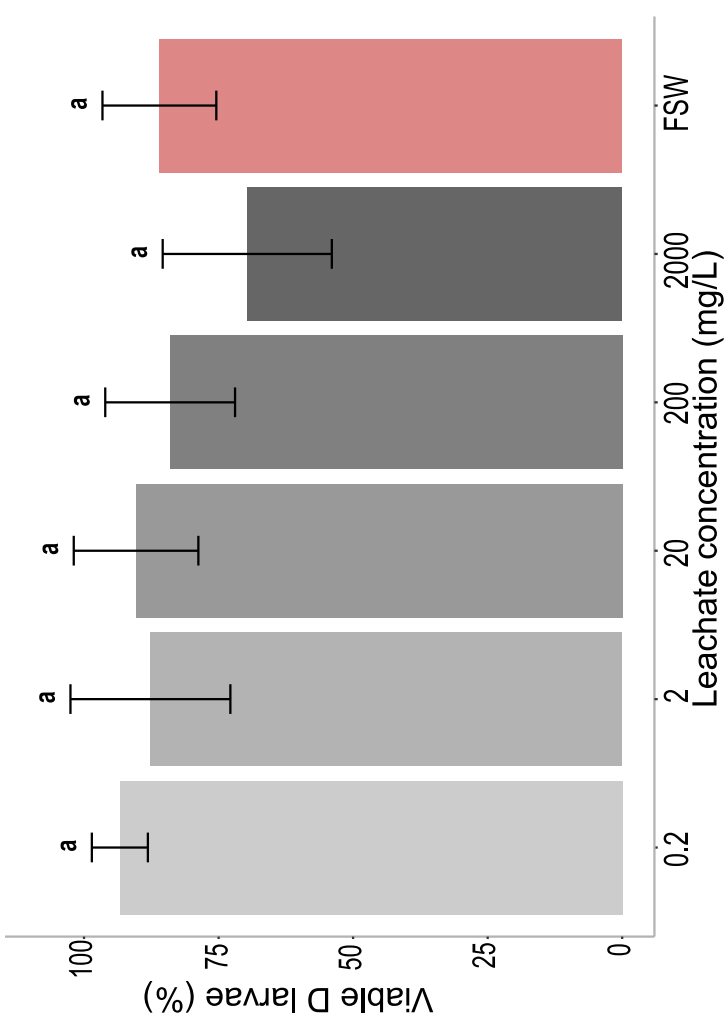


Figure 5

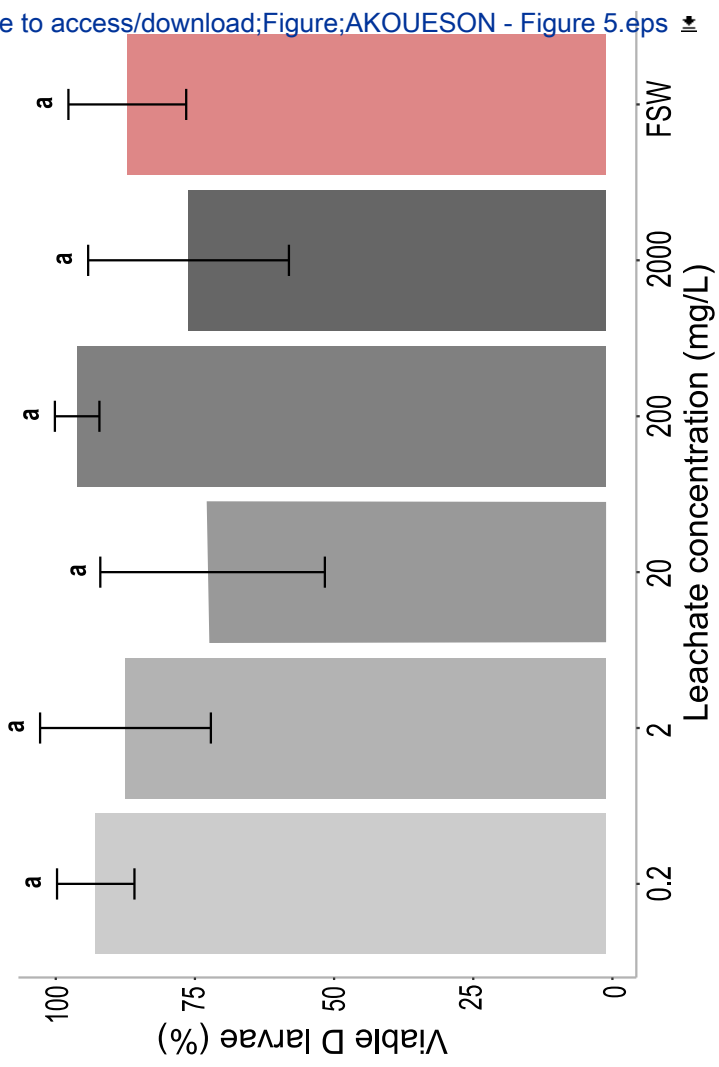
B. PLA-B



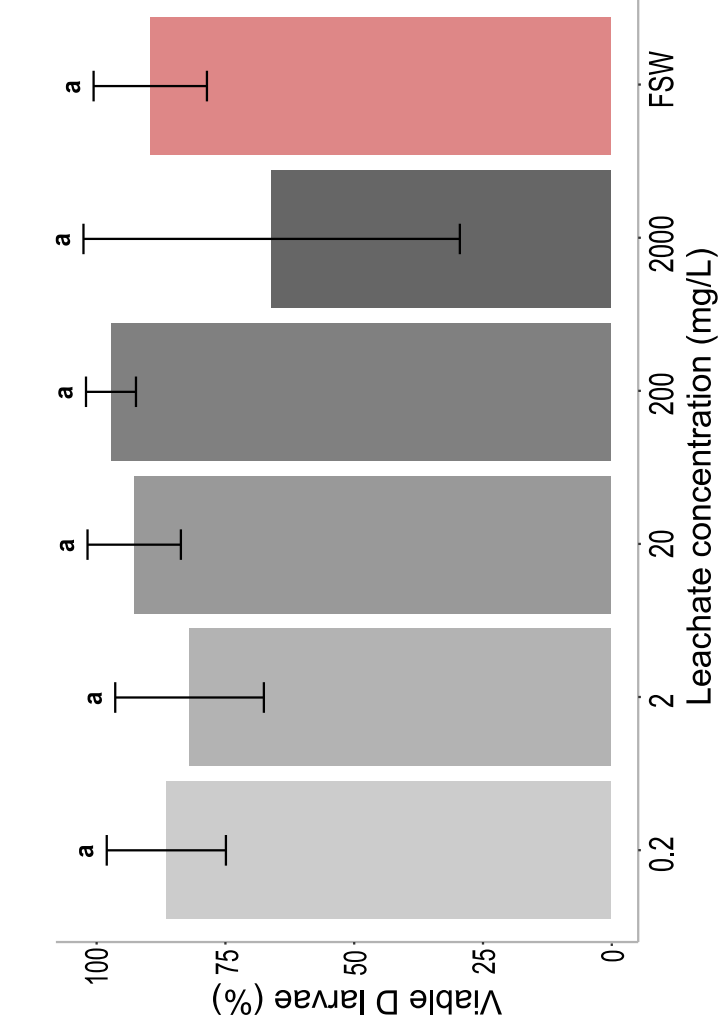
A. PLA-A



D. PP-B



C. PP-A





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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Fleurine Akoueson reports financial support was provided by National Centre for Scientific Research.