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Phthalate release from plastic fragments and degradation in seawater

Paluselli Andrea [1], Fauvelle Vincent [1], Galgani Francois [2], Sempéré Richard [1, *]

[1] Aix-Marseille Univ.; Université de Toulon, CNRS, IRD, Mediterranean Institute of Oceanography (M I O), Marseille, France
[2] IFREMER, Laboratoire Environnement Ressources, Provence Azur Corse (LER/PAC), Ifremer Centre de Méditerranée, ZP de Bregaillon, La Seyne sur Mer, France

* Corresponding author : Richard Sempéré, email address : richard.sempere@mio.osupytheas.fr

Abstract :

Plastic debris in the environment contain plasticizers, such as phthalates (PAEs), that can be released during plastic aging. Here, two common plastic materials, i.e., an insulation layer of electric cables (polyvinylchloride, PVC-cables) and plastic garbage bag (polyethylene, PE-bags), were incubated in natural seawater under laboratory conditions, and the PAE migration to the seawater phase was studied with varying light and bacterial conditions over a 90-day time course. Free PAEs diluted in seawater were also studied for bacterial degradation. Our results showed that, within the first month of incubation, both plastic materials significantly leached out PAEs in surrounding water. We found that di-isobutyl phthalate (DiBP) and di-n-butyl phthalate (DnBP) were the main PAEs released from the PE-bags, with the highest values of 83.4 ± 12.5 and 120.1 ± 18.0 ng g-1 of plastic, respectively. Furthermore, dimethyl phthalate (DMP) and diethyl phthalate (DEP) were the main PAEs released from PVC-cables, with mass fractions as high as 9.5 ± 1.4 and 68.9 ± 10.3 ng g-1, respectively. Additionally, we found that light and bacterial exposure increased the total amount of PAEs released from PVC-cables by a factor of up to 5, whereas they had no influence in the case of PE-bags.

Keywords : Phthalates, seawater, plastic, phthalates, endocrine disruptors, plastic additives

INTRODUCTION

The worldwide production of plastics has increased considerably since the development of synthetic polymers in the middle of the 20th century,[1,2] reaching 335 million tons of plastic produced globally in 2016[2] and giving rise to large emissions and transport of plastic debris[3,4] through rivers, sewage and the atmosphere toward the Ocean.[5] Plastic materials are dispersed by winds and currents, and significant amounts may either sink into the water column,[6,7] incorporate into sediments[8,9] or be assimilated by organisms.[10] Although plastic degradation processes are extremely slow,[5,11] more than 90% of the plastic debris, by numbers, are generally smaller than 5 mm (MP < 5 mm) in aquatic systems.[3,12] These particles find their origins in primary MPs, but most importantly in secondary MPs that are the result of a series of physical, chemical and biological macroplastic degradation processes,[1,13,14,15] which are intensified in coastal environments due to higher seawater dynamics and abrasion induced by sand/coastline.[16] MP may otherwise be assimilated and transferred in the whole marine food web,[10,17-20] including marine mammals.[21,22]

Most plastics contain a number of additives such as phthalic acid esters or phthalates (PAEs) that are used as plastic softeners[23,24] and are considered priority pollutants by the US-EPA, the European Union (EU) and the Chinese water regulations[25] due to their endocrine disruption and carcinogenic properties.[26-30] Importantly, PAEs are not covalently bound to the plastic polymer and are thus likely to leach out of the plastic into the environment or inside an animal's stomach or tissue[1] during abiotic/biotic aging, although little is known regarding these processes. Although PAEs have been detected in aquatic environments,[24,31-36] there is a paucity of data dealing with the preferential pathway driving their introduction in aqueous marine media, the kinetics of their release from various plastic materials and their degradation processes.[37,38] The Mediterranean Sea is a semi-enclosed basin with high solar radiation[39] and high atmospheric inputs,[40,41] a slow turnover time of ~ 80 years[42] and strong urbanization with a large range of industrial activities spread all along the Mediterranean basin,[43] which is greatly affected by marine litter.[3,12,14,31,44-46] Here, we investigated in laboratory i) the potential for commercially available plastic material to release PAEs into the surrounding seawater under varying light exposure, bacterial density and temperature and ii) the biodegradation of 7 common PAEs diluted in Mediterranean coastal seawater.

EXPERIMENTALS

Seawater sampling and pretreatment

For all laboratory experiments, a pool of one hundred liters of seawater was collected in Marseille Bay (NW Mediterranean Sea: 43°16'N; 05°20'E) in June 2015 at a 3 m depth by using a 12-L GO-FLO© (GENERAL OCEANICS) bottle. The bottle was previously rinsed with 1% hydrochloric acid and ultrapure water (Milli-Q, resistivity > 18.2 MΩ) to prevent contamination. The water was then transferred in 5 and 10 L glass bottles and brought back in the laboratory within one hour. Then, the seawater was directly filtrated in an ISO class 6 cleanroom (temperature: 22 °C; SAS pressure: +15 Pa; SAS brewing rate: 30 vol h^{-1}; lab pressure: +30 Pa; brewing rate: 50 vol h^{-1}) through precombusted (450 °C for 6 h) GF/C filters (1.2 µm retention size and 47-mm diameter, which was rinsed with 2 L of Milli-Q and 150 mL of sample prior to filtration) in a precombusted glass apparatus, transferred into 1-L glass bottles and stored for 2-3 h at 4 °C for further experiments. Physiochemical properties, bacterial abundance and ΣPAEs concentration of the sample are reported in Table S1.

PAE release from plastic material experiments

For the PAE release experiments, two commercially available plastic types were selected: one black plastic garbage bag (2 fragments of 2 cm × 2 cm × 10 µm, total mass of 0.4 g, 8.1 cm^2 surface area) and one insulation layer from an electrical cable (2 tube fragments of 1 cm length, 9 mm O.D., 5 mm I.D., total mass of 1.5 g, 4.8 cm^2 surface area). Both materials were analyzed by Fourier Transform Infrared Spectroscopy (FTIR attenuated total reflectance, Thermo Scientific Nicolet iS50 FT-IR, 4000-600 cm^{-1}, 16 scans per sample, 0.5 cm^{-1} resolution, Figure S1), which allowed for identifying the plastic bag as polyethylene (PE) and the electric cable as polyvinylchloride (PVC). The plastic bag and electric cable will hence be named "PE-bag" and "PVC-cable" in the rest of the document, respectively. PE is largely used for garbage bags, and is predominant among all plastic debris found in the Ocean, mainly at the Ocean surface.[12,15] Although less abundant than PE,[12] PVC is expected to sink rapidly through the water column to the seafloor due to its density > 1, therefore affecting its exposure to light and then colonization by biofilm. Each type of fragment was transferred into separate 1-L glass bottles that were previously filled with 600 mL of filtrated seawater (1.2 µm GF/C filters, see "Seawater sampling and pretreatment" section) and each bottle corresponds to one

incubation time. The bottles were filled to 60% of the bottles' volume to ensure well-oxygenated conditions. Before the experiment, plastic surfaces were cleaned with Milli-Q and cut into pieces with metal scissors that were previously cleaned with hexane, DCM and Milli-Q water. The plastic fragments were incubated for three months under various conditions of light and bacteria content. Experimental details are given in Table 1.

Table 1. Experimental design of PE-bag and PVC-cable exposure

Experiment name	Irradiation	Biology	Temperature (°C)
LA22	Light	Abiotic	22
DA22	Dark	Abiotic	22
DB22	Dark	Biotic	22

The artificial light inside the thermostatic room was left on for the light samples, whereas the dark samples were wrapped up with aluminum paper and kept in cardboard boxes. Then, all 'light' samples were not subjected to radiation in the UV range. The abiotic condition was obtained by poisoning the samples with 1 mL of 10 g L^{-1} $HgCl_2$ (17 mg L^{-1} in seawater), which has been successfully used to account for abiotic conditions in a series of degradation study of a wide variety of organic contaminants (e.g., pharmaceuticals, polycyclic hydrocarbon) in various matrices (e.g., soil, sewage effluent, estuarine waters)[47-49]. Temperature was controlled in a thermostatic room. The bottle samples were gently swirled for a few seconds three times a day and twice during the weekend. Duplicate samples were extracted for PAE after 0, 1, 2, 4, 7.5, 10, and 12 weeks of exposure. Briefly, 400 mL of the total 600 mL were transferred to another clean glass bottles, poisoned with sulfuric acid to a pH ~ 2 to avoid any biological activity, closed with polytetrafluoroethylene-lined (PTFE) screw caps and stored in the dark at 4 °C until analysis. The remaining 200 mL were used for dissolved organic carbon (DOC) measurements (10 mL in duplicate in glass vials, stored at 4 °C before analysis), and prokaryote abundance determination (1.8 mL transferred into cryovials and fixed with 2% (w/v final dilution) formaldehyde solution and -80 °C frozen until analysis).

PAE bacterial degradation experiment

For the PAE biodegradation study, 700 mL of filtered seawater (1.2 µm GF/C filters, see "Seawater sampling and pretreatment" section) was transferred into precombusted 1-L glass bottles, spiked with a

mixture of 7 PAEs' solution (grade > 98%, Supelco, Bellefonte) to reach a final concentration of 1 µg L^{-1} in seawater, and incubated in duplicate at 22 °C for two months in the dark in a thermostated laboratory. Only 2-thirds of the bottles were filled to ensure well-oxygenated conditions. The abiotic control samples were prepared in duplicate, poisoned with sulfuric acid to a pH ~ 2 to avoid any biological activity and measured at the end of the experiments to be able to attribute all the PAE loss to biotic processes. Aliquots of all samples were collected by using precombusted Pasteur pipettes at 0, 1, 2, 4, 7, 13, 21, 28, 35, 42, 49 and 60 days for the flow cytometry analysis, as detailed in the previous section.

Phthalate analyses

For PAE analyses, seawater samples were performed following a method described elsewhere.[33] Briefly, PAEs were extracted from seawater by solid phase extraction (SPE) with a precombusted 6 mL-glass reaction tube and 200 mg of Oasis HLB sorbent (Waters Corporation, 30 µm). After sample percolation, PAEs were eluted by 6 mL of ethyl acetate and then evaporated up to a final volume of 200 µL under a gentle stream of nitrogen (purity > 99.995%). The extractions were carried out in controlled air conditions in an ISO class 6 chemistry cleanroom. The seven phthalates that were studied included dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPP), di-isobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), benzylbutyl phthalate (BzBP) and di-(2-ethylhexyl) phthalate (DEHP). Before use, all the glassware was kept in an acid bath overnight (10% hydrochloric acid), combusted at 450 °C for 6 h and rinsed with methanol and dichloromethane. The analysis was performed using an Agilent Technologies 6850 gas chromatograph system coupled to an Agilent Technologies 5975C mass spectrometer (GC/MS) operated with electron impact ionization (70 eV). Chromatographic separation was achieved using an Agilent HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness). PAEs average recovery ranged from 90 % (DEHP) to 108 % (DiBP). Method detection limits ranged from 0.1 to 0.9 ng L^{-1} for DMP and DEHP, respectively. Although caution was paid to prevent contamination, DEP, DiBP and occasionally DnBP were detected in the procedural blanks at levels that remained below 0.4-2%, 2-3% and 0-4%, respectively, of the masses that were measured in different seawater samples.

Heterotrophic prokaryotes, DOC analyses and scanning electron microscopy (SEM)

For the heterotrophic prokaryote determination, seawater aliquots were analyzed by using the flow cytometry core facility PRECYM of the Mediterranean Institute of Oceanology (http://precym.mio.osupytheas.fr). Immediately after sampling, the samples were thawed at room temperature and stained using SYBR Green II (Molecular Probes®). The analyses were performed on a FACSCalibur flow cytometer (BD Biosciences®) equipped with an air-cooled argon laser (488 nm, 15 mW).[50] The DOC concentrations were measured using a Shimadzu TOC-5000 carbon analyzer.[51] The plastic pieces were analyzed with SEM at t_0 and t_f to obtain insights into the potential surface modification of the materials. To this end, the samples were carbon-coated before being examined on two different zones with a Zeiss Supra 40VP microscope with an accelerating voltage set at 10 kV and a working distance of 9 mm.

RESULTS AND DISCUSSION

Release from plastic fragments: light effect

Our results indicated that, regardless of the indoor light/dark conditions, both PVC-cable and PE-bag leached specific PAEs toward the surrounding seawater, with higher release rates for the latter. Only the DMP and DEP migrations (expressed as ng g^{-1} of plastic incubated) were detected from the PVC-cable, whereas only DiBP and DnBP were detected from the PE-bag (Figures 1). The absence of other targeted PAEs may be explained by i) their absence from the selected polymers or ii) the low release rate to the surrounding water phase due to high affinity with the polymer. In all experiments, the larger migration was measured within the first two weeks of incubation with a specific magnitude and trend for each individual treatment. LA22 were compared to DA22 treatment to isolate the effect of the light (Table 1).

Note that for the PVC-cable (Figure 1a-b), a higher migration was observed during the first 1-2 weeks (up to 6.6 ng g^{-1} and 23.2 ng g^{-1} for DMP and DEP, respectively), whereas the measured concentrations reached a plateau and remained stable in both the light- and dark-abiotic conditions throughout the following 6 weeks. After 8-10 weeks, the measured concentrations started to slightly decrease, most likely due to the glass bottle adsorption or hydrolysis[52], although late prokaryotic development and subsequent biodegradation cannot be precluded. Overall, our results showed that i) DEP was predominantly released from the PVC-cable over DMP (3.5 times more) and ii) the indoor light condition induced up to two times more DEP and DMP releases compared to the dark condition. In contrast, for the PE-bag experiments, a higher amount of PAEs, including DiBP followed by DnBP, were released (up

171 to 139 ng g^{-1}) mainly during the first week. Differently from the PVC-cable experiments, the PE-bag results
172 indicated no significant release differences between light- and dark-abiotic conditions (Dark-Abiotic: DiBP:
173 83.4 ± 12.5 ng g^{-1} and DnBP: 120.1 ± 18.0 ng g^{-1} and Light-Abiotic: DiBP: 103.6 ± 15.5 ng g^{-1} and DnBP:
174 138.8 ± 20.8 ng g^{-1}) during the time course experiment (Figure 1c-d), thus suggesting that only seawater
175 leaching promotes PAE release whatever the light conditions. Similar decreases for both dark and light
176 conditions during the last weeks of the experiment suggest that photodegradation in the visible radiation
177 range was not a significant process on freely dissolved DiBP and DnBP destruction. Therefore, the different
178 patterns observed for both PVC-cable and PE-bag could be rather linked to the 3-dimension configuration of
179 each plastic piece (i.e., 2 mm vs. 10 µm thicknesses, respectively). Indeed, the very thin PE-bag material
180 could release a large part of its PAE burden either with light or not. In contrast, photochemical oxidation
181 reactions may alter the PVC-surface, thereby making more PAE quantities water-accessible.

182 DOC leaching confirms the PAEs trend, with the PE-bag's highest release in the first week and small
183 differences between dark the and light conditions (24.4 x 10^3 and 24.6 x 10^3 ng C g^{-1} of plastic bag) and with
184 the PVC-cable's highest release after 1-2 weeks and higher release during the light experiment (13.4 x 10^3
185 and 21.9 x 10^3 ng C g^{-1} in the dark and light conditions, respectively) (Figure S2). The PAE carbon content
186 released from the PE-bag and PVC cable thus represented a small portion of the DOC that leached, i.e., only
187 0.05-0.09% of the DOC released from the PVC-cable and 0.15-0.17% of the DOC from the PE-bag. In
188 addition to PAEs, other groups of organic additive or oligomers could be leached from this plastic during the
189 experiment, thus increasing the concentration of DOC in the surrounding water. The amount of DOC leached
190 per surface area unit of the PE-bag in this study (5.5 and 5.6 µg C cm^{-2} of the plastic surface in the dark and
191 light conditions, respectively) are higher than the migration observed by Romera-Castillo et al. (2018) in PE
192 food packaging (0.26-0.31 µg C cm^{-2}), which is probably due to the lower amount of additives mixed in food
193 plastic resins, but is in the same range of LDPE and HDPE pellets' leaching (2.4-8.9 µg C cm^{-2}). In the same
194 study, similar leaching kinetics were reported, with the peak of leaching observed in the first week of the
195 experiment, which was followed by a sharp decrease of DOC migration during the first month. Interestingly,
196 we observed a second strong DOC leaching after 10-12 weeks (83-96 x 10^3 ng C g^{-1} for the PE bag and 28-38
197 x 10^3 ng C g^{-1} for the PVC-cable), which was probably due to the initial degradation of the plastic surface.
198 The lack of a strong weathering such as UV-exposure or a strong mechanical abrasion induced a slow

degeneration of the polymers and thus, part of the organic matter pool more strongly bounded to the polymer could be leached only when the fragments were affected by major surface modifications.

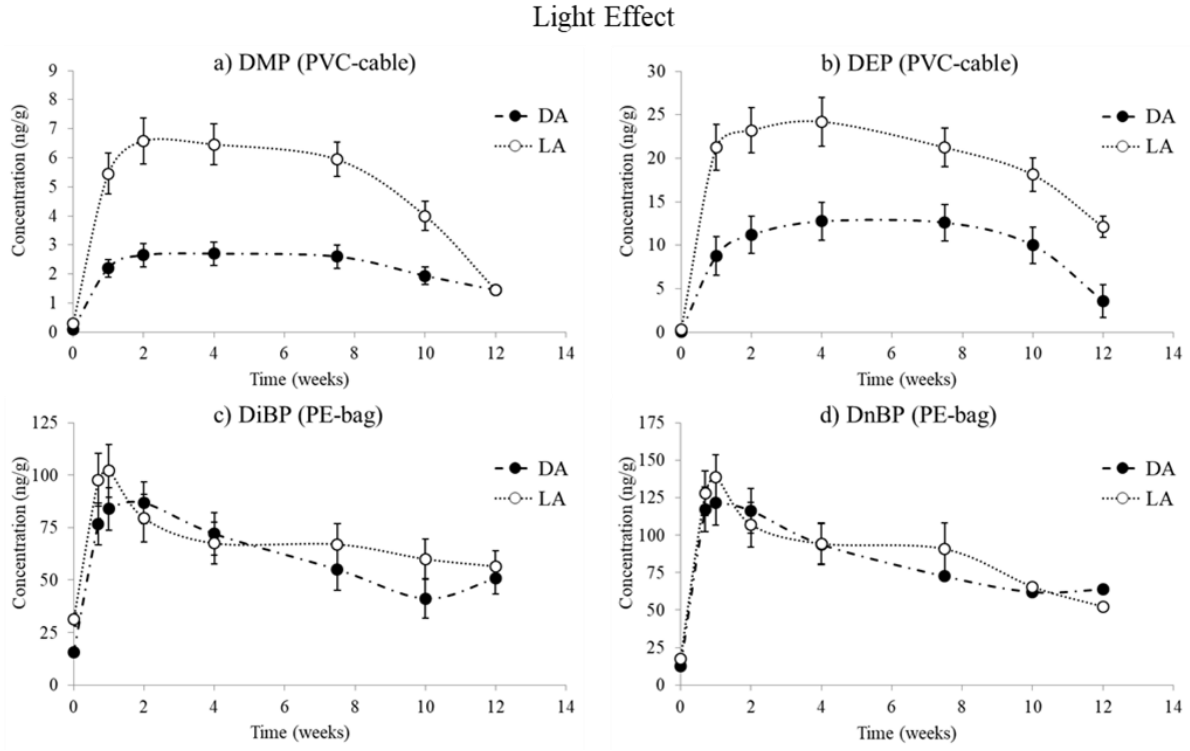

Figure 1. Graphical representation of the release kinetics of DMP (a) and DEP (b) from the PVC-cable experiments and of DiBP (c) and DnBP (d) from the PE-bag experiments. The two experimental conditions were Dark Abiotic (DA) and Light Abiotic (LA) incubated at 22 °C (*in situ* temperature). Curves are given to assist in the reading and do not represent data modeling.

Release from plastic fragments: Biotic effect

Biotic effects were studied by comparing the results of the previous abiotic conditions with the PAE release kinetics from the same plastic materials diluted in seawater comprising its natural prokaryote assemblage (biotic conditions, seawater filtered through 1.2µm GF/C and not poisoned with $HgCl_2$; Figure 2). The results indicated that DiBP and DnBP are more rapidly released and in higher proportions (up to 122 ng g^{-1}) from the PE-bag than the DMP and DEP from the PVC-cable (63.5 ng g^{-1}). Globally, the same PAEs were detected for both light and biotic experiments. However, 5-fold higher quantities of DMP/DEP were produced from the PVC-cable in the biotic conditions during the first month rather opposed to the abiotic conditions, thus indicating that PAE leachates were promoted by prokaryotic activity. In contrast, no influence of prokaryotes was observed on the initial release of DiBP and DnBP from the PE-bag. For the light effect, a PAE release catalyzed by bacterial communities seemed to be more efficient for the PVC-cable

than for the PE-bag. The large difference in PAE release between biotic and abiotic conditions observed in the case of PVC-cable was not observable in the case of PE-bag experiments. This could be attributed to i) the low thickness of the material, thus allowing for a complete release of PAE burden regardless of the conditions or ii) the low PE aging under the action of bacteria.

Interestingly, for both materials incubated with seawater prokaryote assemblages, an increase in the PAE concentration was followed by a net decrease of this PAE concentration, as low as almost zero after 4 and 12 weeks for the PE-bag and PVC-cable experiments, respectively, thus suggesting the subsequent assimilation of dissolved PAEs by prokaryotes. Re-adsorption of PAE by the plastic could also explain the decrease of PAE content in the dissolved fraction. Indeed, plastic surface modification during aging includes an increase of surface polarity,[53] and therefore changes the partition coefficient of individual PAEs between water and plastic fragments. It is of importance to note that the DiBP and DnBP released from the PE-bag are more rapidly consumed by prokaryotes than the DEP and DMP produced from the PVC-cable. After the beginning of the fragments incubation and PAE leaching, bacterial abundance increased probably as a result of the leached available for prokaryote consumption and growth (Figure 2). In PE-bag experiments, the lack of available PAEs after 4 weeks corresponds a decreasing of the prokaryotic abundance. This was not observed in PVC-cable experiments, where the growth ended after one week. The reason could be the smaller amount of leachate from PVC that may support a smaller community than the larger amount of leachates from PE-bag. The plastic fragments at t_0 and t_{final} exposed under dark biotic conditions were observed through SEM and showed a diffuse degradation of the PVC-cable surface, with characteristic cavities along the fragments after 3 months of incubation (Figure 3a-b) and no evident differences on the PE-bag surface at the end of the incubation (Figure 3c-d). This observation seems to confirm that PVC-cable fragments are a better substrate for prokaryote colonization and subsequent degradation. This outcome may probably explain the large differences observed between the biotic and abiotic samples for the PVC-cable experiments and the lack of differences for the PE-bag experiments, whether these differences are linked to the total or only the surface PAE release, regardless of the exposure conditions. Then, this experiment indicated that DiBP and DnBP are more rapidly released from the PE-bag and quickly exhausted by prokaryotes, whereas both processes are found to be slower in the case of the PVC-cable/ DMP/DEP experiment.

The observed DOC leached results are smaller or negligible compared with the 2 abiotic experiments in the incubation with bacteria. The DOC release of 7 x 10^3 ng C g^{-1} was measured from the PVC-cable in the first week, and no DOC leaching was observed from the PE bag in the first weeks of the experiments. This result is probably because the plastic derived DOC is immediately available for bacterial degradation and supports the bacterial growth. The prokaryotic consumption of the plastic-derived DOC agrees with a previous study.[15] Interestingly, the DOC from the PE and PVC plastics was characterized by large leaching after 10-12 weeks of incubation (23 x 10^3 and 36 x 10^3 ng C g^{-1} for the PVC-cable and the PE-bag, respectively), as already shown by the abiotic experiments. This kinetic is not supported by the PAE results and could be due to the release of organic substances derived from polymer degradation and weathering.

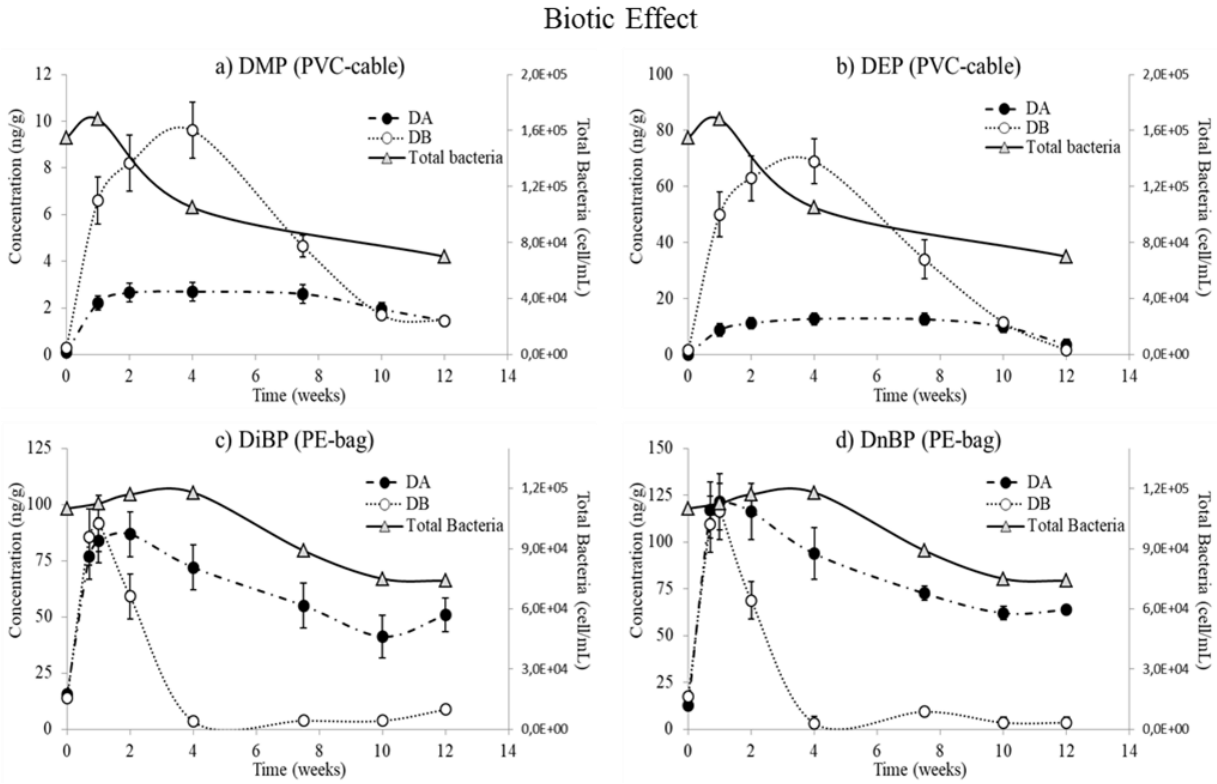

Figure 2. Graphical representation of the release kinetics of DMP (a) and DEP (b) from the PVC-cable experiments and of DiBP (c) and DnBP (d) from the PE-bag experiments. The two experimental conditions were Dark Abiotic (DA) and Dark Biotic (DB) incubated at 22 °C (*in situ* temperature). Total bacteria include LNA and HNA cell abundance. The curves are given to assist in the reading and do not represent the data modeling.

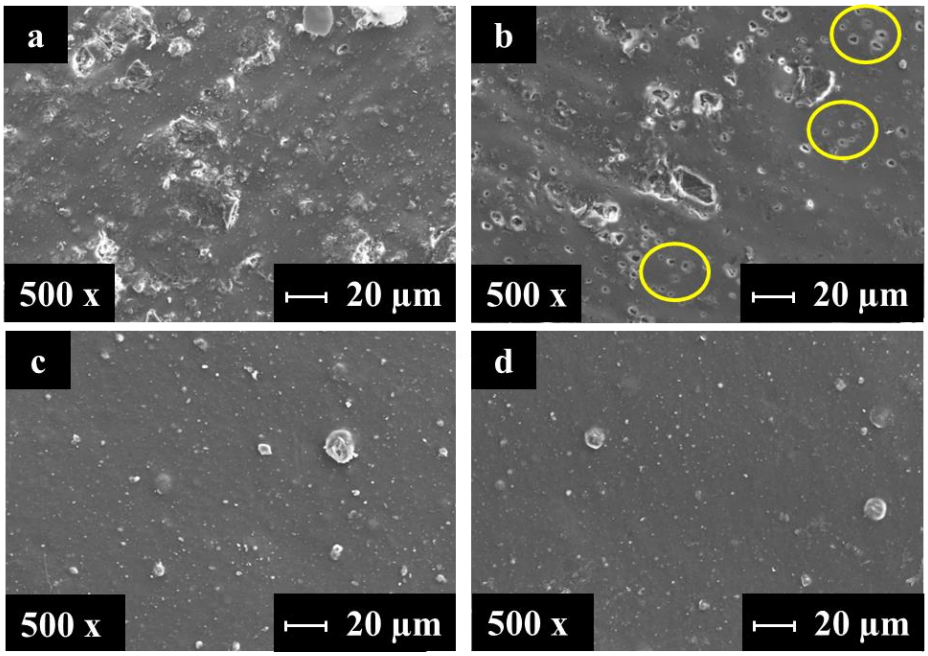

Figure 3. Surface of plastic fragments observed through SEM in the DB (Dark Biotic) condition at t_0 and t_{final} (3 months). a) PVC-cable fragments at t_0, b) PVC-cable fragments at t_{final}, c) PE-bag fragment at t_0, and d) PE-bag fragments at t_{final}. The yellow circles highlight the cavities on the PVC-cable fragments after three months of incubation.

PAE biodegradation in seawater

A dissolved phthalate biodegradation experiment was undertaken to study the biodegradability of PAEs that could have been released from any plastic fragments in the natural environment. Our results showed that the PAE concentrations in the dark under abiotic conditions (controls) remained relatively stable over the 60 days of exposure for all compounds (Figure 4). Indeed, minor concentration changes, ranging from -3.5% (DEP) to -6.1% (DEHP), were observed, thus suggesting no significant abiotic degradation and slight sorption on the glass bottle[52] during the time course experiment. However, under biotic conditions, 4 of the 7 target PAEs in seawater, including DnBP, DiBP, BzBP and DEHP, were almost completely degraded (> 85%) within 49 days of incubation (Figure 4), whereas 28-46% of DMP, DEP and DPP were degraded. No significant correlations were found between bacterial abundance and PAE consumption, either as individual PAEs or as total PAEs. A first order regression (Eq. 1) was applied to the data to estimate the degradation rate (i.e., k) and half-life ($t_{1/2}$, Eq. 2) (Table 1).

$$C_{(t)} = C_{(t=0)} \times e^{-kt} \quad (1)$$

$$t_{1/2} = \frac{ln2}{k} \qquad (2)$$

where $C_{(t)}$ and $C_{(t=0)}$ are the PAE concentrations at each time t or $t = 0$, respectively.

The calculated values of k ranged from 0.046 ± 0.005 d^{-1} (DnBP) to 0.009 ± 0.001 d^{-1} (DEP), thus resulting in $t_{1/2}$s ranging from 21 to (DnBP) to 79 days (DEP). It is of interest to note that the lowest values of k (0.009-0.013 d^{-1}) were observed for the shortest chain PAEs (DMP and DEP), whereas longer and branched chain PAEs exhibited higher values (0.024-0.046 d^{-1}) (Table 1), which is consistent with our PAE plastic release experiment (Figure 2). PAEs biodegradation rate has been reported to decrease with increasing of alkyl chain length as a result of the stereospecific blockade[54]. However, our results confirm this trend only between the longer chain PAEs and showed an extremely lower rate for the short chain PAEs. This behavior has been previously reported in another study, where DnBP was degraded faster than DEP, showing an inhibitory effect of DnBP on DEP, probably cause by the competition for the same enzyme active site[37]. Another reason might be the production of intermediate short chain-PAE products during the long chain-PAE degradation. Indeed, monobutyl phthalate and DEP have been reported as the two major intermediate compounds of the degradation of the DiBP, DnBP and DEHP by the primary degradation pathway and by the secondary pathway,[54-56] in which PAEs with longer side chains are converted to those with shorter chains by β-oxidation, which removes one ethyl group each time until getting DEP[56] and, eventually, by further transesterification, ethyl-methyl phthalate and then DMP.[57] Accordingly, DMP and DEP can be considered intermediate or end products of long chain PAE degradation oxidation reactions.

Additionally, the difference in the prokaryotic degradation is very likely the result of the specific abundance of the organisms with the specific ability to degrade individual PAEs.[54,58] Note that the DEHP and DnBP biodegradation by pure cultures of bacteria isolated from activated sludge, mangrove sediments and wastewater have been already reported,[59-63] whereas several microorganisms were identified for phthalate degradation, such as *Pseudomonas fluorescens*, *Rhodococcus rhodochrous* and *Comamonas acidovoran.*[64-67] The already published DnBP degradation rate and half-life of the isolated bacteria ranged from 0.018 to 0.035 h^{-1} and from 20 to 72 h, respectively.[37,43,55] However, most of the microorganisms have been isolated from terrestrial subsurface environments, and far less is known about their counterparts in marine environments. In addition, complete phthalate degradation is always carried out syntrophically by several members of microorganisms in natural environments.[68] The k of DnBP and DEHP reported in several studies

with mixed cultures in environmental conditions ranged from 0.015 to 0.024 d^{-1},[54-57,69] which is consistent with our findings (Table 1). Additionally, in an aquatic environment, PAE can also be degraded by the intra and extracellular enzymes of phytoplankton.[37,70]

Table 1. Degradation rates (k) and half-lives ($t_{1/2}$s) of 7 PAEs under dark biotic conditions. A first order regression was fitted to the experimental data using XLSTAT software. The RSD (relative standard deviation) is applicable for both k and $t_{1/2}$

Compound	k (d^{-1})	$t_{1/2}$ (d)	RSD (%)	R^2
DMP	0.013	53	11.4	0.905
DEP	0.009	79	9.2	0.932
DPP	0.024	29	20.1	0.727
DiBP	0.024	29	20.1	0.822
DnBP	0.046	15	10.2	0.964
BzBP	0.034	21	20.8	0.824
DEHP	0.027	26	8.3	0.963

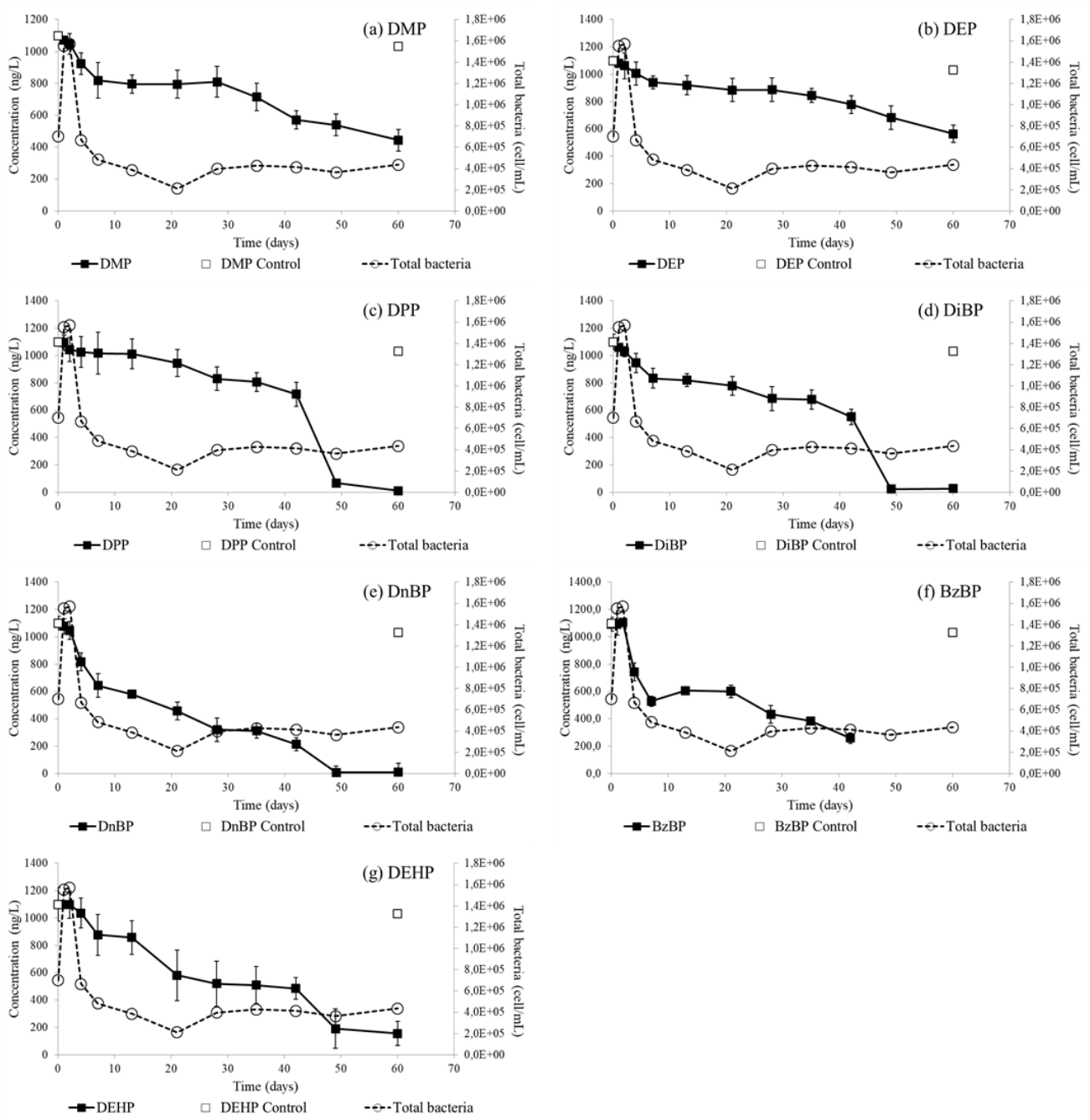

Figure 4. Bacterial degradation of the 7 PAEs in seawater at 22 °C and in the dark condition. (a) DMP, (b) DEP, (c) DPP, (d) DiBP, (e) DnBP, (f) BzBP and (g) DEHP kinetics of degradation. Abiotic samples poisoned with sulphuric acid were used as controls in this study at t_0 and t_{final}. Total bacteria include LNA and HNA cell abundance.

Release from plastic fragments, material effects

The two common plastic products that were studied here, including the LDPE plastic trash bags and PVC electrical cables, were found to release distinct PAEs in different ways during the time course

experiments. Note that an extension of these results must be taken cautiously because there is some variability in the chemical composition of these commercially available products. Indeed, trash bags, which are commonly manufactured, can be either made from plastic beads of low-density polyethylene including (LDPE) or HDPE (or both), whereas the insulation sheath of electric cables can also be made of a polymer composition comprising a polymer base resin of polyethylene, ethylene-propylene rubber (EPR) or polyvinyl chloride (PVC, this study). In addition, these materials layers usually contain large range additives to improve the physical proprieties and resistance to different surrounding conditions, which range from 0.5 to 5% of the weight of total polymer composition.[71]

PAE migration from plastic materials was already reported in cases concerning the potential release in food and water from bottles, packaging materials and disposable tableware.[72-81] The polymer has a three-dimensional porous structure in which the additives are dispersed, and the pore diameter and additive size are important parameters[82] that could determine a selective release of the lower molecular weight additives, which in this case are the DMP and DEP for the PVC-cable. In addition, the depletion of these PAEs from the resin surface and a negative concentration gradient from the inside to the surface may cause the migration.[82] In contrast, DEHP, which has the highest molecular weight phthalate target in this study, and the other high molecular weight PAEs are more resistant to migration due to their hydrophobicity and higher partitioning coefficient. The nature of the polymer of the insulation layer of electrical cables, which is compact and dense, and the tube-shape of the fragments used for the incubation experiments could be two factors involved in PAE selective migration in the surrounding medium. DMP and DEP could be better candidates for the migration process from this fragment of plastic if compared with DiBP, DnBP and DEHP. However, a significant DiBP and DnBP release was observed from the plastic bags. This material was constructed by a different polymer structure that was less compact and more flexible, and the fragments used for the incubation were characterized by a larger surface to mass ratio. In addition, the two plastic materials could be made of different amount of plasticizers since the purpose for which they have been produced and their necessary features are different. The release may take place during the service life of the plastics or their production as well as after their disposal. Moreover, due to the lower steric hindrance of DMP and DEP, it could be possible that this material has already lost most of its low molecular weight PAEs content before the incubation experiments.

Environmental implications

Overall, these results confirm that, according to the origin and aging of the material, plastic aquatic dilution may provide variable amounts of PAEs in their surrounding environments, including seawater and the guts of marine organisms, birds and mammals. During the study period (three months), the PE-bag provided approximately 1 order of magnitude more PAEs than the PVC-cable. PAE leaching from plastics and its subsequent effects might be important in areas with high plastic concentrations[3,11,12,83] and certainly contribute to the high PAE concentrations reported in coastal areas in the vicinity of large rivers and urbanized areas.[33,34,84] It has been estimated that between 4.8 x 10^6 and 12.7 x 10^6 MT of plastic entered in the oceans in the year 2010[15,85], with 28% and 5% being made of polyethylene and PVC, respectively.[2] By extrapolating our results to the oceans, our results would suggest that between 0.32 MT and 0.86 MT and between 0.02 MT and 0.05 MT of PAE leach in the first two months of their introduction into the oceans every year from plastic bags and PVC-cables, respectively, and it is important to understand that the myriad of plastic items in the oceans may release different types of PAEs. Our study suggests that most of the PAEs produced are exhausted by marine prokaryotes within one month (PE-bag) and 2.5 months (PVC-cable). Similarly, intense solar radiation in the surface water[15] may certainly modify the release and bioavailability of PAEs produced from plastics in the oceans, whereas high hydrostatic pressure in deep waters is able to modify the prokaryotic degradation of particulate organic matter[86] and certainly have a significant effect on the plastic aging deposited on the deep sediment. Considering that we found that PAEs that were released ranged from 71 ng g^{-1} to 241 ng g^{-1} and that plastics usually contain 0.5-5% of PAEs, our results suggest that, after three months, more than 90% of the PAEs in the plastic remain and will ultimately leach out over a longer period of time.

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