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OCEAN WISE
Reducing EPS marine litter in the North East Atlantic
Cedre

OCEAN WISE

Assessment and comparison of potential impacts of expanded and extruded polystyrenes (EPS/XPS) and their alternatives on the marine environment

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INDEX

LIST OF ABBREVIATIONS

- ASTM: American Society for Testing and Materials
- CE: Circular economy
- CHARM : Chemical Hazard Assessment and Risk Management
- EPS: Expanded polystyrene
- FCM: Food Contact Material
- FSW: Filtered seawater
- HMCS: Harmonised Mandatory Control Scheme
- IS: Impact Score
- ISO: International Organization of Standardization
- MSFD: EU-Marine Strategy Framework Directive
- MP: Microplastics
- NIAS: Non-intentionally added substances
- NP: Nanoplastics
- OCNS: Offshore Chemical Notification Scheme
- OD: Optical density
- OECD: Organization for Economic Cooperation and Development
- PAH: Polycyclic aromatic hydrocarbons
- PBAT: Polybutylene Adipate Terephthalate
- PCB: PolyChloroBiphenyls
- PE: Polyethylene
- PHBH: Poly3-hydroxybutyrate-co-3-hydroxyhexanoate
- PLA: Polylactic Acid
- PP: Polypropylene
- PS: Polystyrene
- PVC: Polyvinyl chloride
- RAP-ML: OSPAR Convention’s Regional Action Plan on Marine Litter
- REACH: Registration, Evaluation, Authorization and restriction of Chemicals
- SBSE: Stir bar sorptive extraction
- XPS: Extruded polystyrene

EXTENDED SUMMARY

The OceanWise project

Foamed polystyrene, either expanded (EPS) or extruded (XPS), are materials regularly found as litter in the marine environment. OceanWise is a European project (Interreg Atlantic Area) that aims at reducing presence and impacts of foamed polystyrenes (PS) in the North-East Atlantic. It is an interdisciplinary project gathering 13 partners from five countries (France, Ireland, Portugal, Spain and United Kingdom) to develop concrete actions in the context of a circular economy (CE). Among the different project actions, one of work packages (WP7) aims at evaluating the promising solutions to replace the use of foamed polystyrene products, through analyses and real-life living-labs of eco-innovation on potential harm issues and usability pilots with target industries. Cedre is particularly involved in Action 2 that aims to assess and compare the potential impact of EPS/XPS and their alternatives. EPS and XPS have many qualities (*e.g.* high mechanical compressive strength, highly insulating, fireproof, easily shaped and processed, waterproof) explaining their global use in many sectors such as packaging (*e.g.* fish box, drink cups), leisure (*e.g.* surfboard, protective helmets), aquaculture/fishing (*e.g.* buoys) or construction (*e.g.* insulated panel). Due to its wide use, a part of EPS/XPS waste is mismanaged leading to releases in aquatic environments. Between 2018 and 2020, in OSPAR countries monitoring foamed polystyrenes (Denmark, the Netherlands, Germany, France, Ireland and Portugal), EPS/XPS pollution represented 15% of total plastics and 13% of total litter found on beaches, confirming these litter types are abundant in the North-East Atlantic .

Objectives of the study

In the context of work package 7.2, the present study aims to assess and compare the potential impact in the marine environment of foamed polystyrenes and newly developed biobased alternatives. This was done by compiling information existing in the literature and by conducting additional experimental work. The work was divided in two parts.

Firstly, a review of existing methods to assess potential impacts of plastic materials in the marine environment was conducted. Based on this review, a “toolkit” of assays covering the different risks associated with plastic materials was elaborated and a preliminary approach to assess the potential global impact of plastic materials in the marine environment was proposed.

Secondly, based on the toolkit and the approach proposed, a selection of assays was applied on three foamed polystyrene products found on the European market and three biobased and biodegradable alternatives currently developed to be put on the market, in order to assess:

- The potential impact of the three foamed polystyrene products on the marine environment;
- The relevance from an environmental point of view, to replace foamed polystyrenes by biobased alternatives.

Toolkit of assays and associated approach to assess the potential global impact of plastic materials on the marine environment

Information regarding the potential impacts of plastics and available assays (standardized or not) to assess them were compiled. The literature revealed that plastic litter in aquatic environments can induce a large panel of environmental impacts that can be classified into three main categories:

(i) Weathering (colonization leading to a species transport and degradation leading to a release of micro/nanoparticles);
(ii) Transfer of hazardous chemicals;
(iii) Toxicity on marine organisms.

In total, 48 assays that can be used to evaluate these three categories of impacts were identified. This selection constitutes a "toolkit" of assays to assess the potential impact on the marine environment of plastic materials.

Based on the literature review and the "toolkit", a preliminary approach to assess the global impact on the marine environment of plastic materials was proposed, in line with approaches already applied under existing regulations (*e.g.* for registration of chemicals intended to be used in offshore oil and gas applications). The proposed approach relies on the application of different assays and the combination of results obtained using an "Impact Score (IS)", traducing the potential impact on the marine environment of tested materials. It consists of different steps which are detailed below:

1) Selecting a minimum of 8 assays in the toolkit: 2 assays in the category "Weathering", 3 in the category "Transfer of chemicals" and 3 in the category "Toxicity on marine organism",
2) Selecting of a minimum of two materials to be tested,
3) Conducting the 8 assays on the two materials and statistical comparison of results obtained for each assays,
4) Attributing scores to each material for each assay,
5) For each impact category ("Weathering", "Transfer of chemicals" and "Toxicity on marine organisms"), calculating the average of scores obtained for the different assays,
6) Calculating the resulting Impact Score for each material by summing the 3 average scores obtained for the three impact categories,
7) Interpreting results and ranking the potential global impact of the two materials knowing that the lower the score, the lower the potential global impact on the marine environment.

The elaboration of this toolkit and associated impact score approach should be seen as a first step in the development of a tool to help decision-makers in the mitigation of plastic pollution impact on the marine environment. It has currently several limitations, which are related to existing methodological and knowledge gaps. For instance, the approach can only be applied to perform relative impact assessment by comparing at least two materials, due to the absence of standardized thresholds and mathematical models.

Overall, this type of approach is needed to assess the potential global impact exhibited by a material likely to reach the marine environment but also to allow pragmatic comparisons of the potential impact between conventional plastic items commonly found as aquatic litter and proposed

alternatives. This type of information is also interesting to feed life-cycle analysis and circularity assessment tools as developed in OceanWise work package 6.

Case study on six foamed polystyrenes and alternatives

Based on the toolkit and the associated approach proposed in Part 1, a case study was conducted with six materials: two EPS, one XPS, and three biobased alternatives (Table 1) to provide first data on (i) their potential global impacts on the marine environment and (ii) the relevance from an environmental point of view to replace conventional foamed polystyrenes by biobased alternatives.

Table 1. Materials tested in the study

Material	Biobased	Biodegradable	Code	End product	Food contact materials
Expanded Polystyrene	No	No	EPS-F	Fish box	Yes
Expanded Polystyrene	No	No	EPS-I	Insulating plate	No
Extruded Polystyrene	No	No	XPS-O	Food box	Yes
Polylactic Acid	Yes	Yes In industrial composting facilities[1]	PLA-F	Fish box	Yes
Polylactic Acid + Polybutylene Adipate Terephthalate	Yes	Yes In industrial composting facilities[1]	PLA+PBAT-F	Fish box	Yes
Poly3-hydroxybutyrate-co-3-hydroxyhexanoate	Yes	Yes In the marine environment[2]	PHBH-F	Fish box	Yes

[1] certified by the NF EN 13432, [2] certified "OK Biodegradable MARINE", certificate from Green Planet™

The six materials were tested using 13 assays, combining both field and laboratory experimentations, selected in the toolkit described in Part 1. These assays are:

(I) For the "Weathering" category (3 assays):

- Level of colonization in seawater,
- Degradation of the materials in an aging chamber,
- Degradation of the materials on a beach.

(II) For the "Transfer of hazardous chemicals" category (3 assays):

- Level of intrinsic contaminants,
- Capacity to leach intrinsic contaminants in seawater,
- Capacity to adsorb environmental contaminants during a deployment in the field.

(III) For the "Toxicity on marine organisms" category (7 assays):

- Cytotoxicity of solvent extracts on bacteria,
- Cytotoxicity of solvent extracts on fish cell lines,
- Estrogenic potential (*i.e.* endrocrine disruption) of solvent extracts,

Assessment and comparison of potential impacts of EPS/XPS and their alternatives on the marine environment

- Reprotoxicity of solvent extracts on zebrafish early life stages,

- Cytotoxicity of seawater leachates on bacteria,
- Cytotoxicity of seawater leachates on microalgae,
- Reprotoxicity of seawter leachates on oyster early life stages.

The different assays were conducted either by Cedre in its premices (Brest, France) and in Brest Marina du Château or in collaboration with French partners laboratories, namely the EPOC lab (UMR 5805, Talence), the LEMAR lab (UMR 6935, Plouzané) and the LUBEM lab (UR 3882, Plouzané).

Results obtained from the different assays were combined by calculating the "impact score" for each material which provides a ranking from the less to the more potentially impacting for the marine environment.

It must be noted that this approach and associated results must be considered with caution since it is a first attempt to assess a global impact of plastic materials on the marine environment. The proposed approach and associated assays have a number of limitations due to existing knowledge and methodological gaps and further developments are needed to increase their robustness and accuracy.

Key results of the case study:

1. Weathering assessment

Foamed polystyrenes and alternatives can be colonized in the marine environment. After 3 months of deployment in the harbor area of Brest (France), results revealed that conventional EPS/XPS and alternatives are rapidly colonized by macrofauna with no apparent differences among materials. Plastic ended up in the marine environment can be colonized by macro- and micro-organisms, which can impact marine ecosystems through the transport of non-indigenous/invasive species and diseases. Results obtained in the present study did not show differences in terms of colonization between the six materials suggesting they are all able to transport macrofauna. However, further analyses are needed to confirm this hypothesis (analysis of microorganisms, characterization of the diversity attached to materials).

Degradation could be faster for foamed polystyrenes compared to alternatives. The six materials were placed for three months in an accelerated aging chamber at constant and controlled temperature (45°C) and illumination (55 W m^{-2}). Observations at the end of the exposure showed a greater yellowing of the surface of conventional foamed PS compared to the three alternatives, as well as a greater release of powder. These results support the hypothesis that conventional foamed PS degrade more rapidly. This could pose a threat to the marine environment through a more rapid and significant release of micro/nanoplastics and toxic chemical compounds initially added during material production (*i.e.* additives). However, further analyses are needed to confirm this hypothesis (study in outdoor or immersed conditions, longer exposure, assessment of biodegradation capacity, analyses of particles release).

2. Transfer of hazardous chemicals

Conventional foamed polystyrenes could adsorb more organic contaminants than alternatives. The six materials were deployed for three weeks in seawater in the harbor area of Brest (France) to evaluate their ability to adsorb chemical contaminants (PAHs, PCBs). Results showed an increase in the amount of PAHs adsorbed on the surface of the three conventional PS compared to the alternatives that displayed no change in the PAH content during the deployment. No change in the PCB content was found for the six materials, suggesting low PCB contamination at the deployment site. Overall, results suggest a higher capacity of conventional foamed PS to adsorb hydrophobic contaminants than tested alternatives, representing a higher risk to the marine environment.

Laboratory analyses found higher concentrations of chemicals (PAHs and additives) in foamed polystyrene than alternatives. Targeted chemical analyses revealed higher amounts of PAHs in conventional PS (average value= 45,154.0 ± 18,573.6 ng g^{-1}) compared to alternatives (average value= 2,379.2 ± 483.1 ng g^{-1}), which could be explained by the petroleum origin of polystyrenes. The EPS insulating plate (66,144.9 ± 1,223.9 ng g^{-1}) had the highest amount of PAHs compared to EPS Fishbox and XPS foodbox (average value= 34,658.5 ± 5,387.8 ng g^{-1}) while no statistical difference was detected among the three alternatives. Regarding the amount of additives, statistical differences were detected among the six materials with higher amounts for EPS insulating plate (12,344.3 ± 1,395.3 ng g^{-1}) and XPS foodbox (16,350.6 ± 1,255.4 ng g^{-1}) compared to other materials (average value = 5,078.1 ± 789.5 ng g^{-1}). In addition, more chemicals were detected in conventional foamed PS (33 for the EPS fishbox, 43 for EPS insulating plate, 41 for XPS foodbox) compared to alternatives (26 for the PHBH fishbox, 27 for PLA fishbox, 23 for PLA+PBAT fishbox). Furthermore, analyses revealed the presence of specific compounds considered as hazardous in conventional foamed PS such as benz[a]anthracene, anthracene and benzothiophene. Higher concentrations of hazardous compounds were found in conventional foamed PS such as naphthalene, 1-methylnaphthalene, biphenyl, phenanthrene and tricresyl phosphate. Overall, chemical analyses of the six materials suggest that fewer or different chemicals are present in the three alternatives compared to conventional materials. However, only targeted analyses on a limited number of chemicals were performed in the present study, which do not represent the chemical diversity found in tested materials. It can't be excluded that different additives, not analyzed in the present study, are used in alternatives. Therefore, to confirm results obtained, new analyses should be conducted to deeply characterize the diversity of chemicals in conventional foamed PS or alternatives, including additives, degradation products and non-intentionally added substances (NIAS).

No differences were recorded in the amount of chemicals released in seawater over a short leaching time (24h). Compiling all the data, the quantity of leached PAHs is less than 8% and less than 1% for the additives whatever the material. To confirm the same capacity of leaching among the tested materials, it would be interesting to repeat our experiment using longer leaching durations (weeks, months). Nonetheless, when looking at chemicals individually, the leaching pattern is different among materials. The individual visualization highlights higher amounts of hazardous chemicals (naphthalene, 1-methylnaphthalene, 2-methylnaphthalene and phenanthrene) in leachates from conventional foamed PS.

3. Toxicity on marine organisms

Toxicity experiments performed on different organization and trophic levels suggested a higher intrinsic toxic potential for conventional foamed PS, especially for the EPS not certified for food contact. A set of assays was performed to characterize the cytotoxicity, genotoxicity and reprotoxicity of solvent extracts of foamed polystyrenes and alternatives on different biological models (bacteria, microalgae, bivalves, and fish). Extracts obtained from conventional foamed PS showed a cytotoxic and estrogenic potential higher than the alternatives. This estrogenic potential suggests the presence of endocrine disruptors in the extracts of conventional foamed PS that could induce reprotoxic and long-term effects (inter/transgenerational effects). In this regard, reprotoxic effects were detected in zebrafish larvae (decrease in the percentage of normal *Danio rerio* larvae) while the alternatives had lower effect. The EPS insulating plate displayed the highest toxicity potential in comparison to the other tested materials. This material is the only among those tested which is not certified as a food contact material (FCM). Overall, the weathering (on the beach or in aging chamber) leads to a slight increase in intrinsic toxicity of materials, however effects remain limited over the study period.

Experiments conducted with leachates suggested low effects without differences among materials even if remarkable effects can be observed on specific biological model (*e.g.* PLA leachate inhibited completely oyster embryogenesis). This suggests that the six materials leached small amount of toxic compounds after 24h of leaching period, which is in agreement with results of chemical analyses described above. It could be interesting to repeat the experiments with leachates obtained from a wide range of leaching duration (from 24h to several weeks/months) to monitor the kinetics of potential effects associated to the release of toxic compounds by the six materials.

Global risk assessment and conclusions

The diversity of results obtained in this case study confirmed the need to conduct different assays to obtain a robust assessment of impacts on the marine environment of plastic materials.

Through all assays performed in the three environmental impact categories identified, the impact score (IS) proposed by the preliminary approach for harmonized impact assessment of plastic materials allowed to obtain a ranking available in Table 2. Based on assay results, higher IS were obtained for the three conventional foamed materials (average IS= 1.5 ± 0.3) compared to the alternatives (average IS= 0.4 ± 0.1), suggesting replacement of foamed PS by selected alternatives is a relevant option from a marine environment point of view.

This work is, to our knowledge, the first attempt to provide an approach with guidelines to characterize potential environmental impacts of plastics likely to reach the marine environment. This case study confirms the added-value of this type of approach as it provides (i) a harmonized framework allowing comparisons among studies and materials and (ii) data to help decision-makers in the mitigation of the impact of plastic pollution on the marine environment.

However, the proposed approach has limitations due existing methodological and knowledge gaps indicating that further developments are needed to make the approach and associated results more robust and accurate. For instance, it appears necessary to (i) develop more standardized assays to assess the different impacts of plastic materials, (ii) elaborate thresholds allowing to assess the level of impacts for each assay, (iii) further develop the impact score assessment method by specifying the number and types of assays to conduct, the weighting of each test, the calculation method to use…).

Table 2. Impact Score (IS) of the six materials regarding the three categories of impacts.

Environmental Impacts	EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Weathering	0.7	0.7	0.7	0.3	0.3	0.3
Transfer of hazardous chemicals	0.3	0.5	0.7	0	0	0
Toxicity on marine organisms	0.3	0.6	0.4	0	0.1	0.1
Impact Score	**1.3**	**1.8**	**1.8**	**0.3**	**0.4**	**0.4**

INTRODUCTION

1. Context

The global plastic production showed an exponential pattern since 1950 to reach 367 million tons (Mt) in 2020 (Figure 1; PlasticsEurope, 2021). Plastics are polyvalent and used in all sectors such as packaging (40.5% of the demand), building, automotive, electrical/electronics, and agriculture owing to numerous qualities (*e.g.* resistant, durable, malleable, low cost). Associated with this wide use, unmanageable amounts of plastic waste are produced every year. Of the 8,300 Mt of plastic waste produced from 1950 to 2015, 9% were recycled, 12% were incinerated, and 79% were disposed in landfills or in natural environments (Geyer et al., 2017).

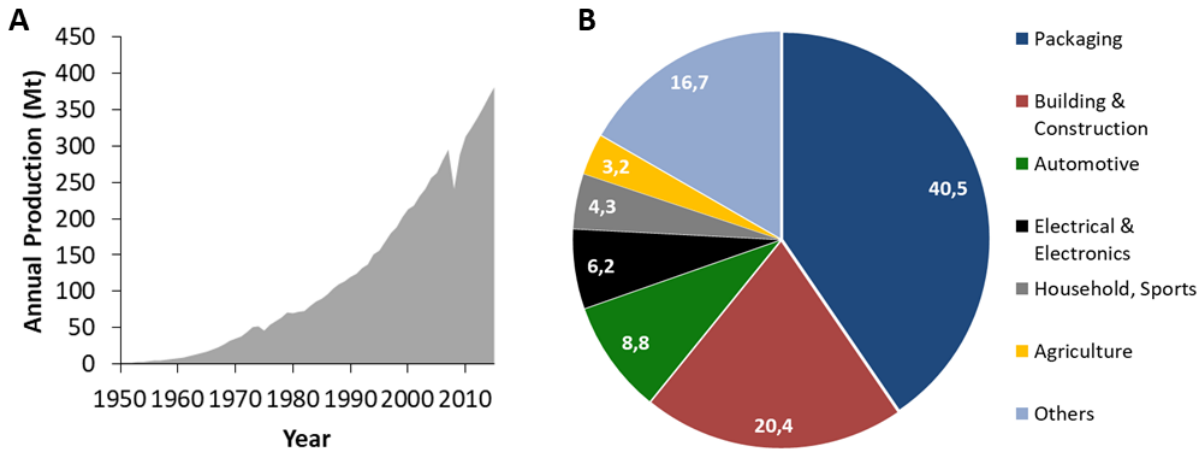

Figure 1. (A) Worldwide annual production of plastic resins from 1950 to 2015, (B) Plastics production by sectors in 2020 (data from Geyer et al. (2017) and PlasticsEurope (2021)).

Oceans constitute the final recipients of mismanaged plastic waste (which include discarded, disposed of, abandoned or lost waste). Yearly, it is estimated that 9 – 14 Mt of plastics are emitted to oceans by different ways (*e.g.* direct discharges, wastewater discharges, atmospheric transports, run-offs from land) (Lau et al., 2020; Figure 2). Through a business-as-usual scenario, this estimation will increase to 23 – 37 Mt in 2040 (Lau et al., 2020). Due to their intrinsic properties, plastic litter is relatively resistant to environmental stressors (*e.g.* biodegradation, physical damages), therefore, mismanaged plastic litter tends to persist and accumulate in environments. Today, plastic litter is ubiquitous across the Earth. In oceans, plastic litter is found everywhere (surface, sediments, biota), including in remote areas (polar regions, deep-seas, desert islands) (Paul-Pont et al., 2018). Furthermore, plastics are the most abundant anthropogenic litter in oceans (>70% of harvested litter are plastics; Hardesty et al., 2017; Lusher et al., 2017).

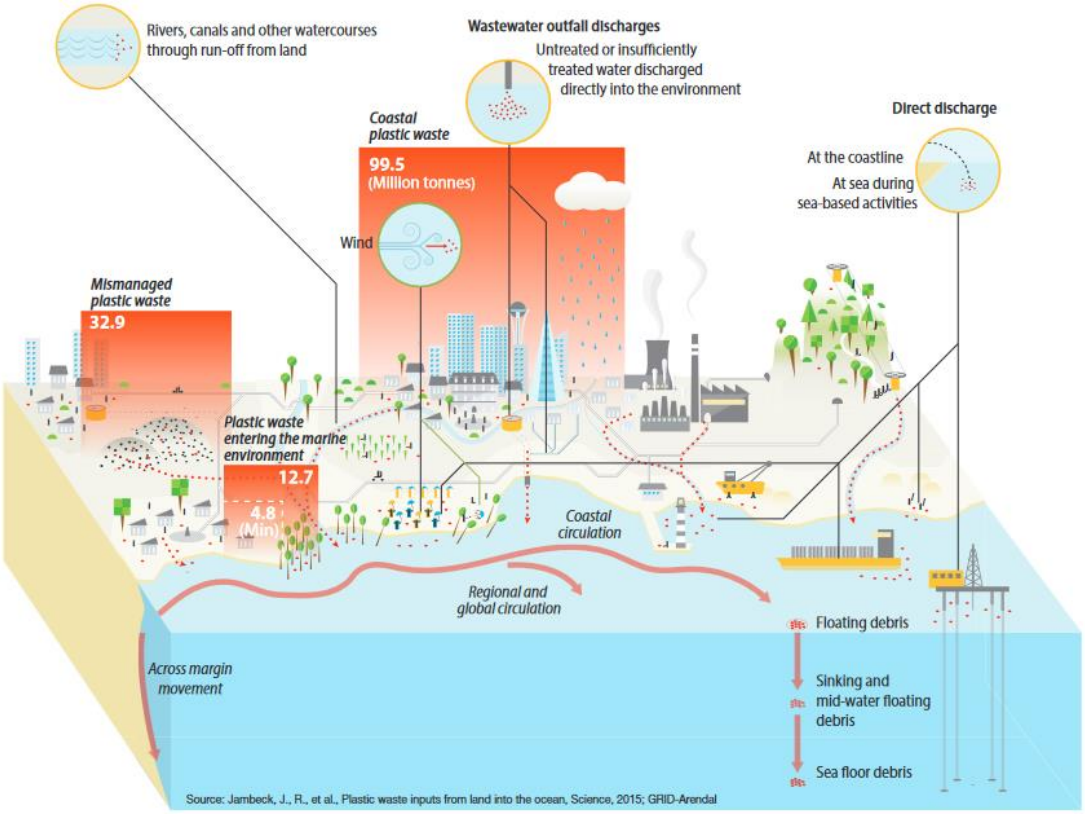

Figure 2. Pathways of plastic litter to the marine environment (Grid-Arendal & UNEP, 2016).

2. The case of expanded and extruded polystyrenes (EPS and XPS)

There are two main categories of polymers, differing by their chemical structures allowing specific features: the thermoplastics and the thermosets (UNEP, 2015). Under temperatures higher than their melting point, thermoplastics can be melted into liquids. Conversely, thermosets are cross-linked, preventing any remolding. Thermoplastics constitute 90% of the production and include the most frequently used polymers such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) or polystyrene (PS).

Commercialization of PS began in 1930. Today, PS constitutes 6.1% of the global plastic production (PlasticsEurope, 2021). This polymer is created by styrene polymerization with the addition of additives to have specific features (*e.g.* color, resistance). These additives are highly diversified, including flame retardants, blowing agents, nucleating agents, antioxidants, surfactants, plasticizers, and pigments, depending on the final use of the material. There are four types of PS: (1) Crystal PS, the most common; (2) high impact polystyrene (HIPS); (3) expanded polystyrene (EPS) and (4) Extruded polystyrene (XPS). EPS and XPS are both foamed PS with the same composition but produced by different industrial processes leading to distinct physical properties. For instance, XPS has smaller air pockets than EPS, leading to a higher resistance, explaining its use for construction. Overall, EPS/XPS exhibit several qualities (*e.g.* high mechanical compressive strength, highly insulating, fireproof, easily shaped and processed, waterproof) explaining their common use by different sectors such as packaging (*e.g.* fish box, drink cups), leisure (*e.g.* surfboard production), aquaculture/fishing (*e.g.* buoys) or construction (*e.g.* plate use for insulation) (OceanWise WP5.2, Godet et al., 2018; Figure 3). As there are foamed,

EPS/XPS are particularly lightweight. In mass, foamed PS represent only 3.2% of the plastic production in Europe (PlasticsEurope, 2021). However, in terms of volume, they constitute 60% of the production in Europe yearly while volumes of polyethylene and polypropylene represent 7% and 12%, respectively (OceanWise WP5.2, Godet et al., 2018).

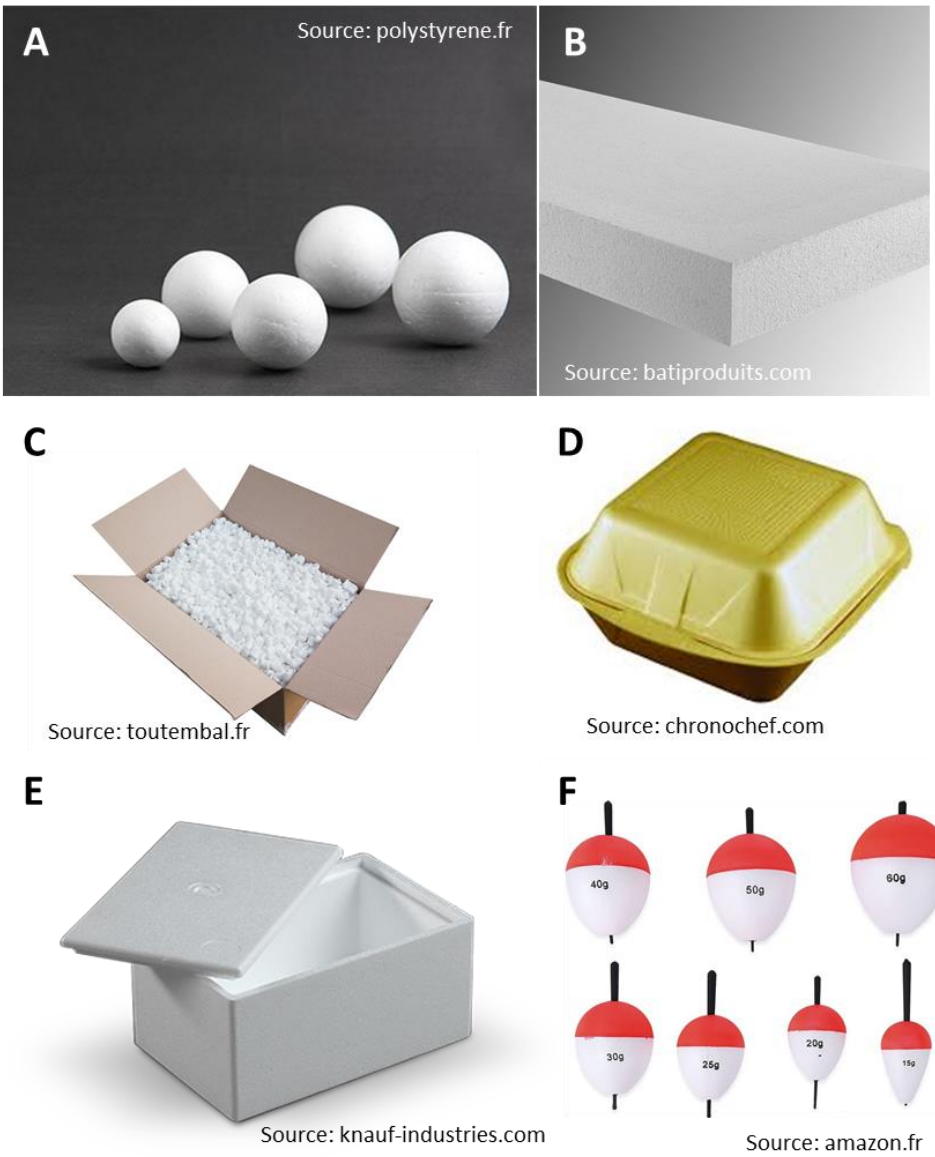

Figure 3. Examples of expanded (A, B, C, E, F) or extruded (D) polystyrene products.

Associated with this wide use, a part of EPS/XPS waste is mismanaged – notably because they are seldom recycled – leading to releases in aquatic environments. Hence, EPS/XPS litter is commonly found in the marine environment (Figure 4). Between 2018 and 2020, in OSPAR countries monitoring foamed polystyrenes (Denmark, the Netherlands, Germany, France, Ireland and Portugal), EPS/XPS pollution was representing 15% of total plastics and 13% of total litter found on beaches (OceanWise WP5.4, André et al., 2022). This fact calls for risk assessments and improvements of industrial/individual practices to reduce this major issue considering the potential hazards of PS for humans/wildlife (Chae et al., 2020; Kedzierski et al., 2020; Thaysen et al., 2018).

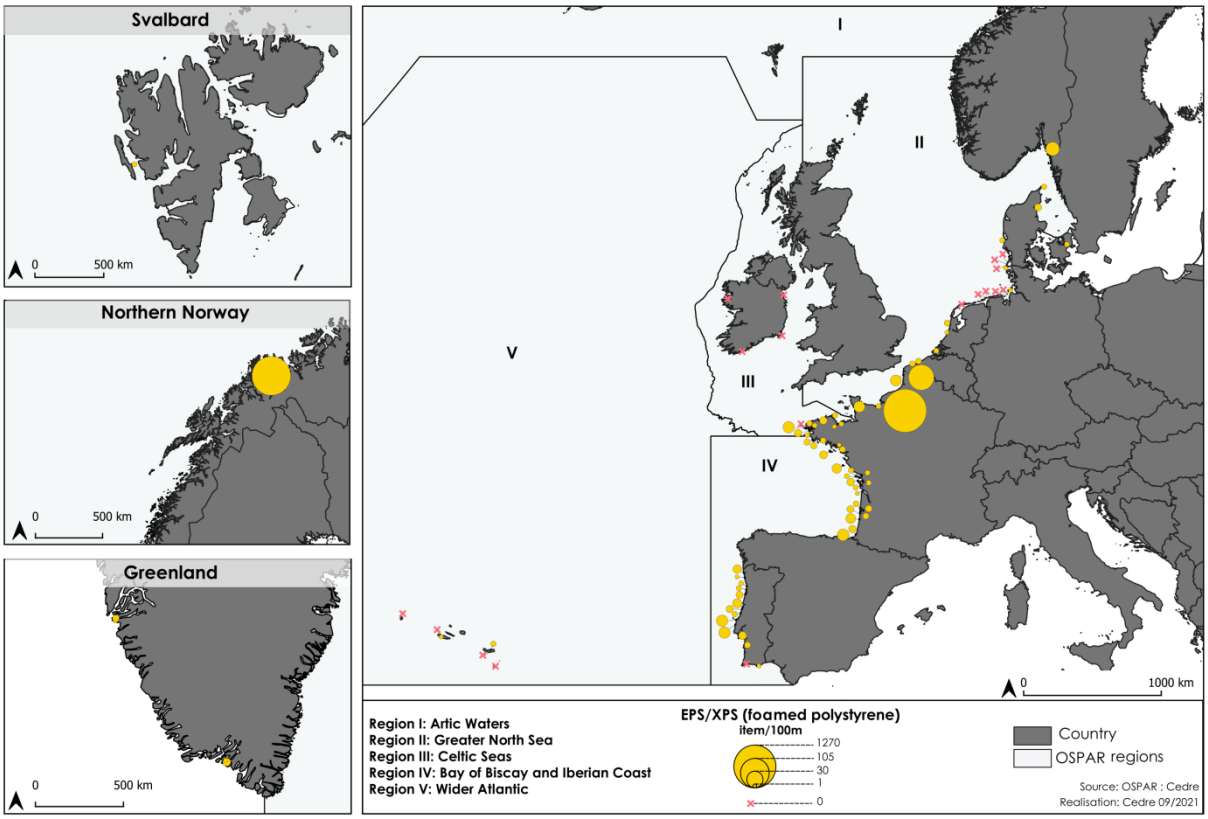

Figure 4. Foamed polystyrene median abundances (number of items/100m) on OSPAR beach litter monitoring sites in Norway, Denmark, the Netherlands, Germany, France, Ireland and Portugal, between 2018 and 2020 (OceanWise WP5.4, André et al., 2022).

3. The OceanWise project

I. Objectives

OceanWise is a European project (Interreg Atlantic Area) that aims to reduce presence and impacts of foamed PS in the North-East Atlantic. It is an interdisciplinary project gathering 13 partners from 5 countries (Ireland, United Kingdom, France, Spain and Portugal) to develop concrete actions in the context of a circular economy (CE). Thus, OceanWise has 4 main objectives:

(1) Identify EPS/XPS products and their sources that are more likely to reach the marine environment and impact on its ecosystems;

(2) Propose and test plausible options (reduce, reuse, recycle, recover) to achieve better environmental outcomes within different sectors;

(3) Engage producer and designer communities on the sustainability of specific applications and to explore more circular models;

(4) Develop circular economy-oriented methodologies to assess new opportunities, barriers and policy options.

OceanWise is driven by the EU-Marine Strategy Framework Directive (MSFD) and the OSPAR Convention's Regional Action Plan on Marine Litter (RAP-ML). There is a strong impetus to devolve

ideas and commitments to national, regional & local political levels. In particular, in alignment with the OSPAR RAP-ML, the project supports RAP action no.49, consisting in "assessing the prevalence and impact of EPS in the marine environment, and engaging with industry to make proposals for alternative materials and/or how to reduce its impacts".

II. Workpackage 7.2

Among the different project tasks, the purpose of the work package (WP) 7 is to evaluate the promising solutions to replace the use of EPS/XPS materials, through analyses and real-life living-labs of eco-innovation on potential harm issues and usability pilots with target industries. Cedre (France), with the support of Cefas (United Kingdom), is particularly involved in Action 2 that aims to assess and compare potential impacts on the marine environment of EPS/XPS and newly developed biobased alternatives.

Nowadays, most impact assessments on plastics rely only on a limited number of assays. However, as plastic materials can exhibit different types of impacts, it appears necessary to combine different assays to obtain a more realistic impact assessment and thus provide more accurate data to help stakeholders in decision-making regarding the mitigation of plastic pollution impact on the marine environment.

In this context, the present study proposed an innovative, but preliminary, approach to assess the potential global impact on the marine environment of EPS/XPS and alternative materials. To reach its objective, the study and the present report are divided in two parts.

1) **Part 1: Proposal of a toolkit of assays and an associated approach to assess EPS/XPS potential global impact on the marine environment**

Firstly, a literature review was conducted in order to identify potential environmental impacts that EPS/XPS could induce in aquatic environments. Secondly, existing assays allowing an assessment of these impacts were reviewed based on the literature and available normalized methods from OECD, ISO and ASTM International Guidelines. Based on this review, a "toolkit" of assays allowing an assessment of the different risks associated with plastic materials, was elaborated. Among this "toolkit", a minimum selection of assays was proposed along with a calculation method to combine obtained results under an "impact score" traducing the potential global impact of each tested material.

To our knowledge, this study is the first to propose an approach aiming at assessing the global impact on the marine environment of plastic materials.

However, it must be noted that this approach and associated results must be considered with caution since it is only a first attempt in conducting a global impact assessment for plastic in the marine environment. The proposed approach has, at this stage, a number of limitations due to existing knowledge and methodological gaps. It will need further developments to increase its robustness and accuracy.

2) **Part 2: Application of the approach to assess the potential global impact on the marine environment of three EPS/XPS materials and three biobased alternatives**

Assessment and comparison of potential impacts of EPS/XPS and their alternatives on the marine environment

In this part, three conventional EPS/XPS materials used respectively as a fish box (EPS), a food box (XPS) and an insulating plate (EPS) were selected along with three biodegradable and biobased alternatives newly developed to make fish boxes.

The potential global impact on the marine environment of these six materials was assessed by applying the approach proposed in Part 1. To do so, the six materials were submitted to 15 assays, conducted either in a laboratory or in the field, providing data on different impact indicators (weathering, transfer of hazardous chemicals, toxicity on marine organisms). Results were combined by calculating an "impact score" for each material, which provides a ranking from the less to the more potentially impacting for the marine environment. As said above, these results must be considered with caution as they are only a first attempt to provide a global impact assessment for plastic materials with known knowledge and methodological limitations. Further works will be needed to improve results accuracy and robustness.

The different steps of the study are synthesized in Figure 5.

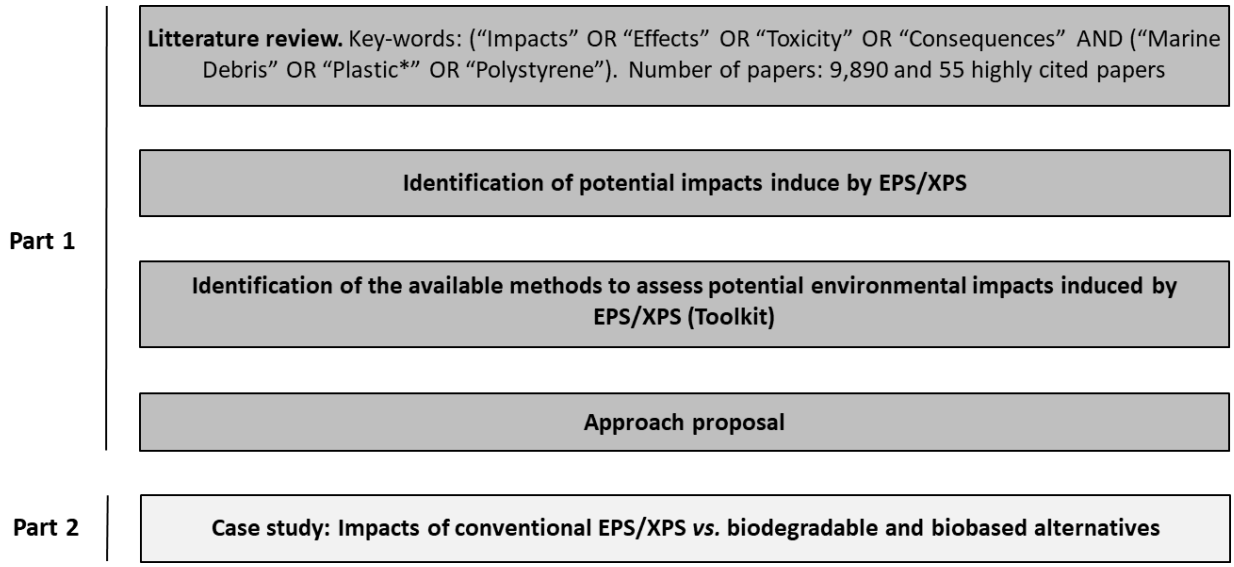

Figure 5. Presentation of the study.

PART 1: PROPOSAL OF A TOOLKIT OF ASSAYS AND AN ASSOCIATED APPROACH TO ASSESS EPS/XPS POTENTIAL GLOBAL IMPACT ON THE MARINE ENVIRONMENT

1) Review of impacts of plastic litter in the marine environment

The literature analysis was performed using the Web of Science database using several key-words: ("Impacts" OR "Effects" OR "Toxicity" OR "Consequences") AND ("Marine Debris" OR "Plastic*"). A total of 9,890 papers were proposed but we used the 55 highly cited papers to identify the main environmental impacts. These impacts can be classified in three main categories: (I) Weathering (colonization and degradation); (II) Transfer of hazardous chemicals; (III) Toxicity for marine organisms (see below for details).

I. Weathering of plastic litter

During its journey as litter, the potential impacts induce by a piece of plastic evolve with its weathering in the natural environment. This weathering implies two main processes.

On one hand, the weathering implies short and mid-term phenomena modifying the surface of the debris. Different substances can rapidly adhere on plastic debris – such as proteins, nutrients, organic matter, or hydrophobic contaminants – forming an eco-corona as well as bacteria, microalgae forming a biofilm (Figure 6). In addition, large debris can be colonized by a large diversity of organisms such as algae, bivalves, tunicates, barnacles, bryozoans. These phenomena affect the behavior of plastic debris and the potential interactions with aquatic life as the corona and biofilms defined the biological identity of plastic debris and can facilitate ingestion (Procter et al., 2019; Savoca et al., 2016). Therefore, plastic litter can be quickly incorporated into food webs.

On the other hand, the weathering implies processes of degradation, generally on a longer-term, leading to erosion and fragmentation of plastic litter in smaller pieces (microplastics (MP) and nanoplastics (NP); Figure 6) (MacLeod et al., 2021). Ultimately, the degradation processes could lead to a complete mineralization but this timeframe is currently unknown (Napper and Thompson, 2020). The rate of degradation depends on the location of the plastics litter. For instance, oxidation by UV-light is more important for plastic debris accumulating on beaches in comparison to the seafloor due to the low levels of oxygen and the reduced amount of solar radiation. Degradation is almost inexistent below the euphotic zone (Andrady, 2015, 2011). In addition to the production of MP and NP, the weathering can induce the release of chemicals added intentionally (additives) or non-intentionally added substances (NIAS; *e.g.* transformation products) in environment or organisms (during the gut transit) (*e.g.* Hermabessiere et al., 2017).

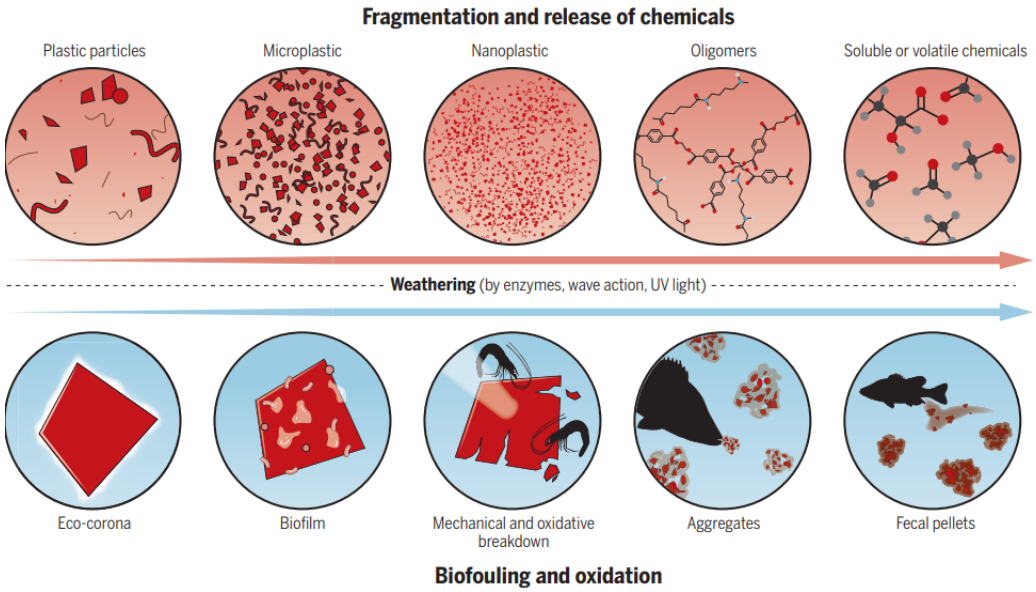

Figure 6. Weathering processes of plastic litter in aquatic environments (MacLeod et al., 2021).

Plastic debris can harbor communities of organisms on their surface (*e.g.* micro-organisms, algae, invertebrates, reptiles; Kiessling et al., 2015; Paul-Pont et al., 2018). They can carry non-indigenous species potentially invasive in ecosystems. Invasive species represent a high risk for ecosystems as they can disrupt their functioning and lead to the decrease/removal of native species at the origin of ecosystem services (especially cultural or economic). Overall, it is estimated that anthropogenic litter double the opportunities for invasion, especially in high latitudes (Barnes, 2002). In addition, special events can induce a massive transport of new species. For instance, 289 Japanese species were introduced on the west coast of United States following the Tsunami that affected the Japanese coast in 2011 (Carlton et al., 2017). The diversity of microorganisms on plastic litter constitutes the "Plastisphere" (Zettler et al., 2013; Figure 7).

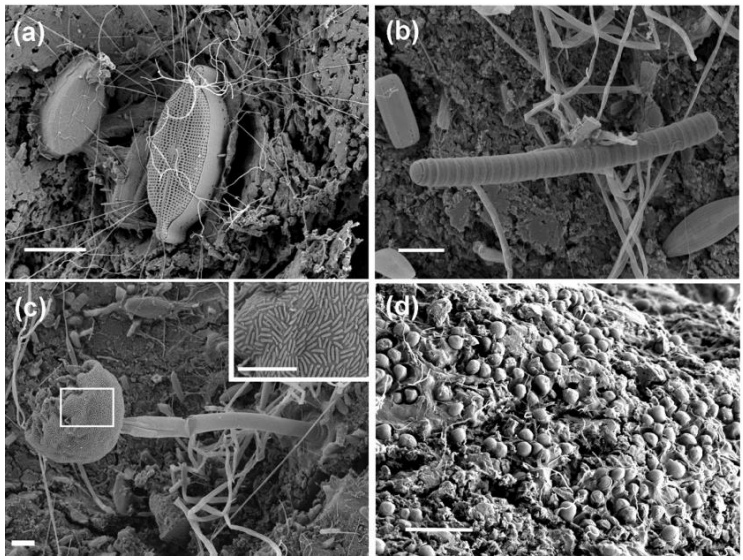

Figure 7. Scanning electron microscopy observations on plastic debris found in oceans (Zettler et al., 2013).

This Plastisphere can include toxic species such as harmful algal blooms (Masó et al., 2003) or toxic bacterial species for life of human health (Zettler et al., 2013). Therefore, plastic debris can act as vectors of pathogens affecting the functioning of marine ecosystems. For instance, the presence of plastic litter increases by 85% the probability of coral diseases in South-East Asia (Lamb et al., 2018). In addition, the worldwide dissemination of microorganisms by plastic litter could contribute to the increase in antibiotic resistance (Laganà et al., 2019). Interestingly, studies demonstrated that the diversity of microorganisms on plastic debris depends on the polymer type (*e.g.* Frère et al., 2018).

II. Transfer of hazardous chemicals

During the plastic production, chemicals (*i.e.* additives) are added to provide specific properties to the material (*e.g.* color, flame retardants, resistance, flexibility; Hermabessiere et al., 2017). On average, additives constitute 7% of the plastic mass although this value can be extremely high for some products (*e.g.* additives represent 50% of the mass of tires; Geyer et al., 2017; Wagner et al., 2018). Plastic additives can reach the aquatic environments and constitute a risk for living organisms as many additives are considered hazardous (*e.g.* bisphenol A). A recent study identified more than 10,000 substances used in plastics, some of which (> 2,400) considered as substances of potential concern (Wiesinger et al., 2021). On the one hand, additives can be released during the use of plastic items and reach oceans by industrial/municipal wastewaters, urban runoffs, atmospheric deposition, and inland waterway transports. On the other hand, plastic litter can release their additives during their weathering in the environment and contaminate organisms. For instance, it is estimated that approximately 190 tonnes (t) of 20 chemical additives entered the oceans in 2015 as constituents of 7 common plastic debris items (bottles, bottle caps, expanded polystyrene (EPS) containers, cutlery, grocery bags, food wrappers, and straws or stirrers; Frond et al., 2019).

In addition to the intentionally added additives, many non-intentionally added substances (NIAS) can also be present in plastics and released in aquatic environments (Tian et al., 2020; Wiesinger et al., 2021). For instance, annual mortality events of coho salmons in western North America are related to a highly toxic quinone transformation product of a tire rubber antioxidant (6PPD-quinone) released by road traffic (Tian et al., 2020).

Environmental contaminants (*i.e.* hydrophobic organic chemicals : polycyclic aromatic hydrocarbons – PAH; polychlorinated biphenyls - PCBs) can sorb on plastic litter which then acts as a vector of dissemination in the environment because the continuation of the weathering processes can allow a desorption of these contaminants to another place or in living organism (Koelmans et al., 2016; Rochman et al., 2013b). For instance, 190 tons of PCBs entered annually in oceans when considering only 7 common plastic debris (bottles, bottle caps, EPS containers, cutlery, grocery bags, food wrappers, and straws or stirrers; Frond et al., 2019). However, to date, the risk associated to this hazardous process is potentially negligible in comparison to other vectors available in aquatic environments (*e.g.* colloids, bacteria, detritus, phytoplankton; Koelmans et al., 2016). Nevertheless, in the context of the "Plastic Toxicity Debt Concept" proposed by Rillig and al. (2021), *i.e.* the risk associated to plastic debris will increase inevitably in the future decades due to the increase use of plastic items and the continuous fragmentation in oceans leading to higher and higher concentrations of small debris, it is therefore important to understand the adsorption/desorption mechanism of environmental contaminants and the differences among plastic materials.

III. Toxicity of plastic litter on marine life

Marine animals can ingest accidentally plastic litter by mistaking them for their natural preys. All trophic levels can ingest plastic litter, including zooplankton (Desforges et al., 2015; Sun et al., 2018), mollusks (Davidson & Dudas, 2016; Li et al., 2015; Van Cauwenberghe & Janssen, 2014), annelids (Van Cauwenberghe et al., 2015), fish (Foekema et al., 2013; Lusher et al., 2013), mammals (Fossi et al., 2012, 2014; Nelms et al., 2019), birds (Van Franeker et al., 2011), reptiles (Duncan et al., 2018). In 2021, literature reported 1,288 marine species ingesting plastic litter (Santos et al., 2021; Figure 8). Ingestion is reported all environments (temperate, tropical, polar, deep-sea; Santos et al., 2021). To date, at least one plastic is found in 66% of marine mammals, 50% of marine birds and 100% of marine turtles (Duncan et al., 2019; Rochman et al., 2016).

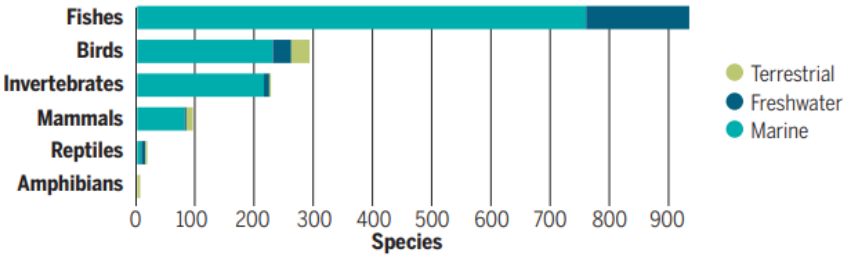

Figure 8. Number and type of species ingesting plastic litter (Santos et al., 2021).

The risk of ingestion depends on several features such as the size of plastic litter, the size of the mouth, the feeding mode (*e.g.* filter-feeding organisms, carnivorous organisms, deposit-feeding organisms), the shape of plastics litter (*e.g.* beads, fragments, fibers, film, foam) as well as the presence of biofilm on the debris (Cole and Galloway, 2015; Gray and Weinstein, 2017; Procter et al., 2019; Savoca et al., 2016; Scherer et al., 2017; Sussarellu et al., 2016). Transfer of non-excreted plastics can occur along the trophic chain as suggested by laboratory experiments (Farrell and Nelson, 2013; Setälä et al., 2014).

Ingested plastics or their associated chemicals can induce a wide range of detrimental effects, from subcellular disruptions to the death in the worst case implying potential consequences at higher biological organizations (populations/ecosystems; Galloway et al., 2017; Wright et al., 2013; Figure 9). On the one hand, ingested plastics can induce injuries in the digestive tract (*e.g.* dysbiosis, tissue damages) (Jin et al., 2018; Paul-Pont et al., 2018; Pedà et al., 2016; von Moos et al., 2012; Wright et al., 2013). In the case of small plastics debris, such as the nanoplastics, translocation can occur, *i.e.* the passage of particles through the epithelia and membranes, allowing colonization of various organs and favoring bioaccumulation (Paul-Pont et al., 2018). For instance, nanopolystyrene beads are found in various organs (*e.g.* gut, gonad, muscle) in the Great scallop (*Pecten maximus*) after a 6h exposure (Al-Sid-Cheikh et al., 2018). These authors estimate an annual bioaccumulation of 123 ng NP g^{-1}, 12 µg NP g^{-1} and 1.8 mg NP g^{-1} for constant concentrations of 1 pg L^{-1}, 100 ng L^{-1} and 15 µg L^{-1}, respectively. Translocation probabilities depend on the surface properties (*e.g.* corona composition; Canesi and Corsi, 2016; Galloway et al., 2017; Paul-Pont et al., 2018). One the other hand, ingestion of plastics can obstruct digestive organs affecting the feeding that can lead to deleterious consequences on the energy balance regulating the growth, the reproduction and the maintenance of animals (Cole et al., 2015; Gardon et al., 2018; Ogonowski et al., 2016; Sussarellu et al., 2016). For instance, ingestion of polystyrene beads disrupted the energy balance of zooplankton or oysters under lab exposures, leading to reprotoxic effects (*e.g.* decreases in hatching success, gametes and larvae qualities; Cole et al., 2015; Sussarellu et al., 2016) that may induce deleterious consequences at the population level

(*e.g.* recruitment). In addition to ingestion, physical interactions between plastic litter and external organs can induce damages to organisms such as entanglements/smothering (Gregory, 2009) or disruption of essential mechanisms (*e.g.* respiration, Watts et al., 2015).

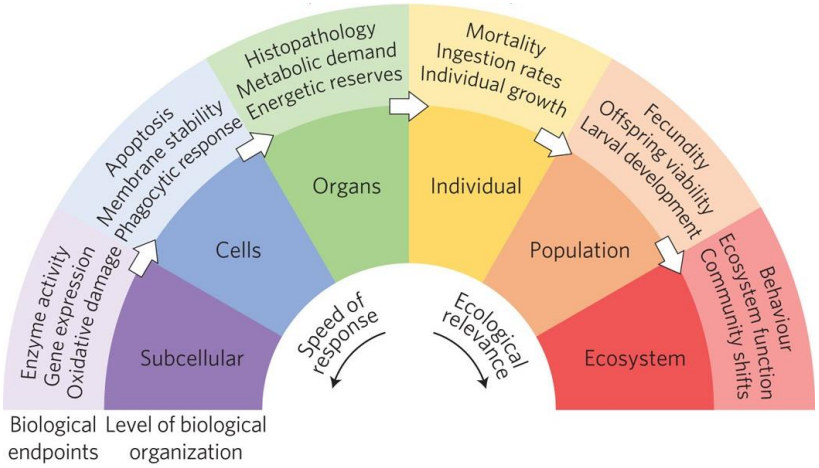

Figure 9. Potential impacts induced by plastic litter and associated chemicals (Galloway et al., 2017)

IV. Synthesis of impacts identified

The different impacts associated with the three main categories identified are synthesized in the table below.

Table 3. Summary of the potential environmental impacts induced by plastic litter

Environmental Impacts	Sub-categories of impacts
Weathering (colonization and degradation)	• Colonization by macro- and microorganisms, transport of pathogens and non-indigenous species • Fragmentation and release of micro/nanoparticles
Transfer of hazardous chemicals	• Adsorption of contaminants on the surface and release in new environment or marine biota; • Release of harmful additives or NIAS
Toxicity on marine organisms	• Ingestion and associated physical effects and toxicity (Modification of feeding behavior; Disruption of cell defenses; Reprotoxicity; Alteration of the population growth affecting the community structure) • Toxicity associated with leaching of contaminants (Modification of feeding behavior; Disruption of cell defenses; Reprotoxicity; Alteration of the population growth affecting the community structure)

2) Proposal of approach to assess the potential global impact of plastic litter

I. Context

Since a decade, the scientific community investigates the different impacts of plastic litter, notably the difference of risks among polymers. To date, it is known that weathering, transfer of hazardous chemicals or toxic effects on marine organisms are potential impacts of plastic materials that can vary among polymers or plastic items (Frère et al., 2018; Lozano et al., 2021; Tallec et al., 2022). Nonetheless, scientific papers are generally focused only on one impact category whereas it is not enough to assess the global impact footprint of a material and support decision-makers. Indeed, it appears essential to combine different analyses to have a global view of the potential impacts induce by a material or a substance considered as problematic for the environment or by a new material put on the market and susceptible to reach the environment.

To date, no policies or regulations target directly the production and marketing of plastic products (new, recycled, or alternatives) except for the chemicals added in the product (*e.g.* the European Union REACH regulation EC 1907/2006, REACH standing for "Registration, Evaluation, Authorization and restriction of Chemicals"). Thus, unlike chemicals, there is no harmonized risk assessment procedure for marketed polymers. In addition, there is no regulation or tool available for plastic litter likely to end up in the marine environment or for plastic items directly used at sea (*e.g.* aquaculture gears), as it exists for chemicals transported by ship (GESAMP, 2019) or for chemicals intended for use and discharge in the exploration, exploitation and associated offshore processing of petroleum in the UK and Netherlands (Offshore Chemical Notification Scheme (OCNS)). For instance, the goal of the OCNS is to register chemicals by performing a risk assessment notably based on the OSPAR Harmonised Mandatory Control Scheme (HMCS). The purpose of the HMCS is to obtain a ranking by calculating hazard scores using when possible a mathematical model (CHARM - Chemical Hazard Assessment and Risk Management) that uses data provided by a set of assays performed to assess the main potential impacts of the tested substances (toxicity, biodegradation and bioaccumulation (see details here:). A similar approach could be interesting for plastic litter likely to end up in the marine environment, for plastic items directly used at sea or to pragmatically validate alternatives to replace a product likely to end up at sea. Data obtained with such an approach would help stakeholders in decision-making regarding the mitigation of plastic pollution impact on the marine environment. These data would be also interesting to feed life-cycle analysis and circularity assessment tools as developed in OceanWise WP6.

In this context, the objective of this section is to propose a first approach to assess the potential global impact on the marine environment of plastic litter

II. Presentation of the approach

Based on approaches used for chemicals intended to be used by the offshore petroleum industry in the UK and Netherlands (OCNS) or transported by ship (GESAMP, 2019) (see Annex 1 for an illustration of the OCNS approach), a first approach for plastic materials is proposed and shown in Figure 10. The approach is divided in three main parts: (i) the risk assessment, (ii) the impact assessment, (iii) recommendations. The first part allows assessing if the material presents a risk for the marine environment *i.e.* plastic items used directly at sea environments or plastic items found as a major

category of marine litter. The second part relies on a harmonized impact assessment combining different assays covering the three main categories of impacts associated with plastic litter (weathering, transfer of chemicals, and toxicity on marine organisms). Results of the different assays are then combined under an "Impact Score" (IS) that can be used to rank different materials. In absence of standardized thresholds or standardized mathematical models (as the CHARM), it is currently not possible to assess the impact for only one material, only relative impact can be obtained by comparing different materials. This gap should be viewed as a priority for future years. Finally, results of comparative analyses allow the elaboration of recommendations, *i.e.* a recommendation of substitution if the alternative material has a better ranking than the original material, or the need of new developments (*e.g.* new alternatives, new recycling methods) or regulations by stakeholders.

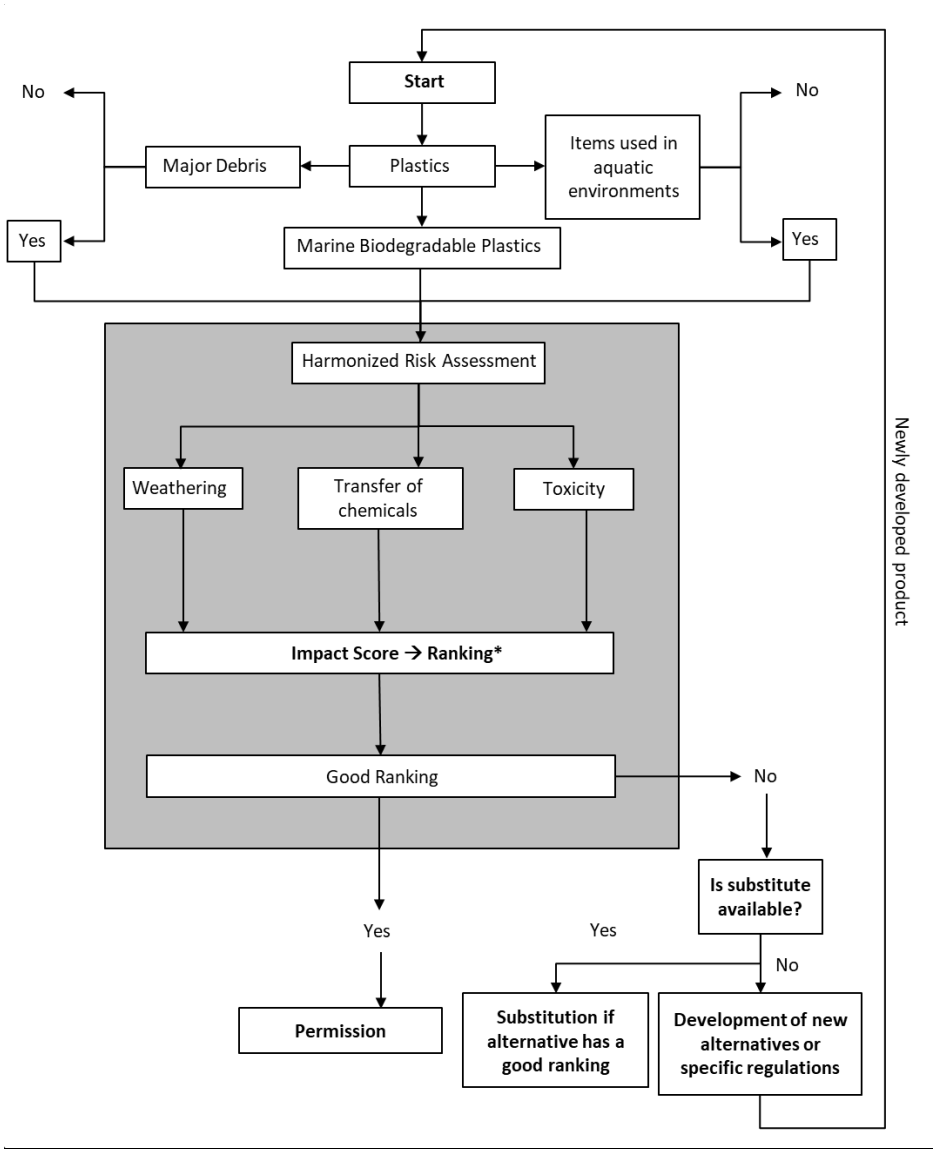

Figure 10. Approach proposal to study environmental impacts of plastic items used directly in aquatic environments or plastic debris and their alternatives. * = in absence of adopted threshold to assess the level of impact, only comparative analyses can be conducted.

III. Toolkit of assays for the impact assessment

For the harmonized impact assessment, a literature review allowed to make an inventory of tools and methods available to assess environmental impacts of plastic litter. In total, 48 assays were identified and selected to make a “toolkit” of assays to assess the impact on the marine environmental of plastic materials (Table 4). Only 11 normalized methods (from ISO, OECD or ATSM International Guidelines) were found. These latter were not all designed originally for plastic materials as several are for chemicals testing, though they could be adapted for plastic materials. This highlights the lack of normalized methods to conduct a harmonized impact assessment of plastic materials. It must be noted that the list of assays proposed in Table 4 is a first proposal; this “toolkit” of assays must be completed and improved in future years to obtain a better coverage of existing impacts.

Table 4. Toolkit of assays to assess potential impacts on the marine environment of plastic materials. When the methodology referred to ASTM, ISO or OCDE protocols, this means that standardized protocol are available.

Identified risks associated with plastic debris	Indicators	Measured Parameters	Methodology references	Scores		
				0	1	2
Weathering (Colonization & Degradation)	Material colonization by macrofauna	Material surface coverage (%)	ASTM-D3623; Fazey and Ryan, 2016	0 - 33%	33 - 66%	66 - 100%
		Mass of macroorganisms attached on materials	ASTM-D3623; Fazey and Ryan 2016	Low Mass	Intermediate Mass	High Mass
	Material colonization by microfauna	Toxic/Invasive organisms attached on materials	Zettler et al., 2013; Frère et al., 2018	No	/	Yes
		Abundance of bacteria on materials	Carson et al., 2013; Hossain et al., 2019	Low Abundance	Intermediate Abundance	High Abundance
		Bacterial communities attached on materials	Frère et al., 2018; Kirstein et al., 2019	Low Diversity	Intermediate Diversity	High Diversity
	Material persistence on a beach	Chemical properties, Mass Loss, Appearance	Corcoran et al., 2009; Fotopoulou and Karapanagioti, 2012	No change	Low changes	High changes
	Material persistence in seawater	Chemical properties, Mass Loss, Appearance	ISO 15314:2018	No change	Low changes	High changes
	Material persistence in aging chamber	Chemical properties, Mass Loss, Appearance	Julienne et al., 2019	No change	Low changes	High changes
	Material biodegradation on a beach	Carbon dioxide production	ASTM D7991-15	No degradation	Low degradation	High degradation
	Material biodegradation in seawater	Carbon dioxide production	OCDE Test 306 / ASTM D6691-17 / ISO/TR 15462:2006	No degradation	Low degradation	High degradation

Transfer of hazardous chemicals	Intrinsic contamination	Characterization of chemicals in plastic items	Gardon et al., 2020; Sussarellu et al., 2016; Völker et al., 2022; Zimmermann et al., 2021, 2020, 2019	Low Level	Intermediate Level	High Level
	Release of chemicals	Characterization of chemicals leached in seawater/sediment	Tallec et al., 2022; Gardon et al., 2020 ; Zimmerman et al., 2021	Low Level	Intermediate Level	High Level
		Characterization of chemicals leached in seawater/sediment from weathered materials	Tallec et al., 2022; Gardon et al., 2020	Low Level	Intermediate Level	High Level
	Adsorption of chemicals in seawater/sediments	Characterization of chemicals/metals adsorbed on new materials	Mato et al., 2001; Rochman et al., 2014, 2013a	Low Level	Intermediate Level	High Level
		Characterization of chemicals adsorbed on weathered materials	Mato et al., 2001; Rochman et al., 2014, 2013a	Low Level	Intermediate Level	High Level

Toxicity (particles/debris or extracts/leachates)	Cellular effects	Endocrine disruption	Kedzierski et al., 2018; Zimmermann et al., 2021	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Oxidative stress	Tallec et al., 2022; Zimmermann et al., 2021	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Genotoxicity	Jiang et al., 2020; Pannetier et al., 2020	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Viability	ISO 11348-3:2007 Fringer et al., 2020; Jiang et al., 2020; Kedzierski et al., 2018; Tallec et al., 2020	No or Low Toxicity	Intermediate Toxicity	High Toxicity
	Reprotoxic effects	Fertilization success	Martínez-Gómez et al., 2017; Tallec et al., 2022; Yaripour et al., 2021	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Embryogenesis and larval development	Cormier et al., 2021; Della Torre et al., 2014; Tallec et al., 2021; OECD TG 236 ; ISO 17244:2015	No or Low Toxicity	Intermediate Toxicity	High Toxicity
	Energy allocation	Feeding	Cole et al., 2013; Cole and Galloway, 2015; Sussarellu et al., 2016	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Respiration	Pousse et al., 2020; Tallec et al., 2021	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Absorption efficiency	Tallec et al., 2021; Sussarellu et al., 2016 ; Pousse et al., 2020	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Energy modeling	Sussarellu et al., 2016; Cole et al., 2015	No or Low Toxicity	Intermediate Toxicity	High Toxicity
	Behavior	Mobility	ATSM E 1440-91; ISO 14669:1999 ; Mattsson et al., 2017; Tallec et al., 2020	No or Low Toxicity	Intermediate Toxicity	High Toxicity
		Velocity	Cormier et al., 2021; Tallec et al., 2020 ; Mattsson et al., 2017 ; Pannetier et al., 2020	No or Low Toxicity	Intermediate Toxicity	High Toxicity
	Survival	Survival Yield	ATSM E 1440-91; ISO 14669:1999; OECD 221; OECD 203	No or Low Toxicity	Intermediate Toxicity	High Toxicity
	Population	Population growth	OECD 221; OECD 201; Martins and Guilhermino, 2018; Schür et al., 2021	No or Low Toxicity	Intermediate Toxicity	High Toxicity

IV. Proposal of an impact assessment approach for plastics

For the impact assessment, as said above, only relative impact can be assessed so it is necessary to have a least two materials to test (e.g. a conventional plastic versus a biobased alternative).

To compare the potential global impact of two plastic materials, it is proposed to select several methodologies to apply on materials to be tested. The minimum number of assays to conduct for a robust impact assessment is still to be determined. For instance, in the OCNS guidelines, 6 assays are recommended within three categories (1 assay of biodegradation, 2 assays of bioaccumulation and 3 assays of toxicity) but these guidelines can be adapted according to the context (*e.g.* when compounds are sinkers, an additional toxicity assay are require to test toxicity on a sediment reworker). Based on this international guideline, in the present approach, as a first attempt, it is proposed to select a minimum of 8 assays as follows: 2 assays for the weathering, 3 assays for the transfer of chemicals and 3 assays of toxicity, among assays in the toolkit (Table 4). However, conducting more assays is expected to increase the robustness of results obtained.

Below is presented an example of a combination of assays that could be selected to perform an assessment of the potential global impact for two plastic materials (see Table 4 for details and references):

- Impact category 1: Weathering
    - Assay 1: Mass of organisms and/or surface coverage after deployment in seawater to investigate the possibility of the item/debris to act as substrate of biodiversity with potential transport of species across oceans.
    - Assay 2: Assessment of the material persistence (*i.e.* degradation) to determine the resistance and the durability of the material in environment. The assay depends on the study site (beach, seawater or sediment).
- Impact category 2: Transfer of hazardous chemicals
    - Assay 1: Assessment of the intrinsic contamination to determine the amount of chemical compounds in the material, especially PAHs and additives. It is currently difficult and highly expensive to characterize all chemicals inside plastics with high-throughput non targeted chemical analyses (Tallec et al., 2022), a list of compounds could be proposed according to the available data in order to target major compounds added in plastics.
    - Assay 2: Assessment of the amounts of chemicals leached in seawater. The same compounds are targeted in the assay 1 and 2 to quantify the percent of leached compounds.
    - Assay 3: Assessment of the amounts of chemicals adsorbed on the plastic items/debris after a deployment in the marine environment.
    - *Additional assay: Assessment of the amounts of chemicals leached in sediment if plastic items/debris are sinkers.*
- Impact category 3: Toxic Effects. Regarding this impact, the use of several assays with different biological model is particularly appropriate to take into account possible biological variability as recommended by the OCNS. In addition, the use of several assays allows the study of various endpoints (*e.g.* survival, growth, reprotoxicity).
    - Assay 1: Effects on bacteria bioluminescence.
    - Assay 2: Effects on microalgae population growth.

- Assay 3: Effects on bivalve or fish development.
- *Additional assay: Effects on arenicola or amphipods survival/feeding if the plastic litter is a sinker (with a density higher than seawater).*

Once assays have been conducted, a score is attributed to each material for each assay. As a first attempt, the scoring is as follows: 0 for a low impact, 1 for a medium impact and 2 for a high impact. In absence of thresholds for the different assays, it is proposed to attribute scores based on statistical differences between the different materials tested and the control. For instance, if two materials A and B are tested, the score would be deduced as follows:

- If results obtained are not statistically different from the control, the score of the two materials A and B is 0,
- If results obtained are not statistically different from the control for material A but different for material B, scores are respectively, 0 for A and 1 for B,
- If for both materials A and B, results obtained are statistically different from the control but the difference is not significant between A and B, the score of the two materials is 1,
- If for both materials A and B, results obtained are statistically different from the control and that A has a significantly higher impact than B, scores are respectively, 2 for A and 1 for B.

Then, in order to weight the three main impact categories defined above (i.e. Weathering, Transfer of hazardous chemicals and Toxicity on marine organisms), it is proposed to calculate the average score for each of the three categories. This is done to ensure each impact category contributes equally to the Impact Score.

Then, it is proposed to calculate the Impact Score, which traduces the relative potential global impact of each material tested by summing the three averaged score obtained for categories “Weathering”, “Transfer of hazardous chemicals” and “Toxicity on marine organisms”.

The global impact assessment approach proposed in the present study could be synthesized as follows:

1) Selection of a miminum of 8 assays in the toolkit: 2 assays in the category “Weathering”, 3 in the category “Transfer of chemicals” and 3 in the category “Toxicity on marine organism”,
2) Selection of a minimum of two materials to be tested,
3) Conduction of the 8 assays on the two materials and statistical comparison of results obtained for each assays,
4) Attribution of scores to each material for each assay,
5) For each impact category (“Weathering”, “Transfer of chemicals” and “Toxicity on marine organisms”), calculation of the average of scores obtained for the different assays,
6) Calculation of the resulting Impact Score for each material by summing the 3 average scores obtained for the three impact categories,
7) Interpretation of the results and ranking of the potential global impact of the two materials knowing that the lower the score, the lower the potential global impact on the marine environment.

This work must be viewed as a first attempt to propose an approach to assess the potential global impact of plastic material on the marine environment. It should be considered with caution since it has numerous limitations due to knowledge and methodological gaps. It will need further developments to increase its robustness and accuracy.

PART 2: ASSESSMENT OF THE POTENTIAL GLOBAL IMPACT ON THE MARINE ENVIRONMENT OF 3 EPS/XPS MATERIALS AND 3 BIOBASED ALTERNATIVES

1) Selection of tested materials

A total of six materials were tested in the study: three types of foamed polystyrene (EPS or XPS) and three foamed alternatives, which are biobased and biodegradable (Figure 11, Table 5).

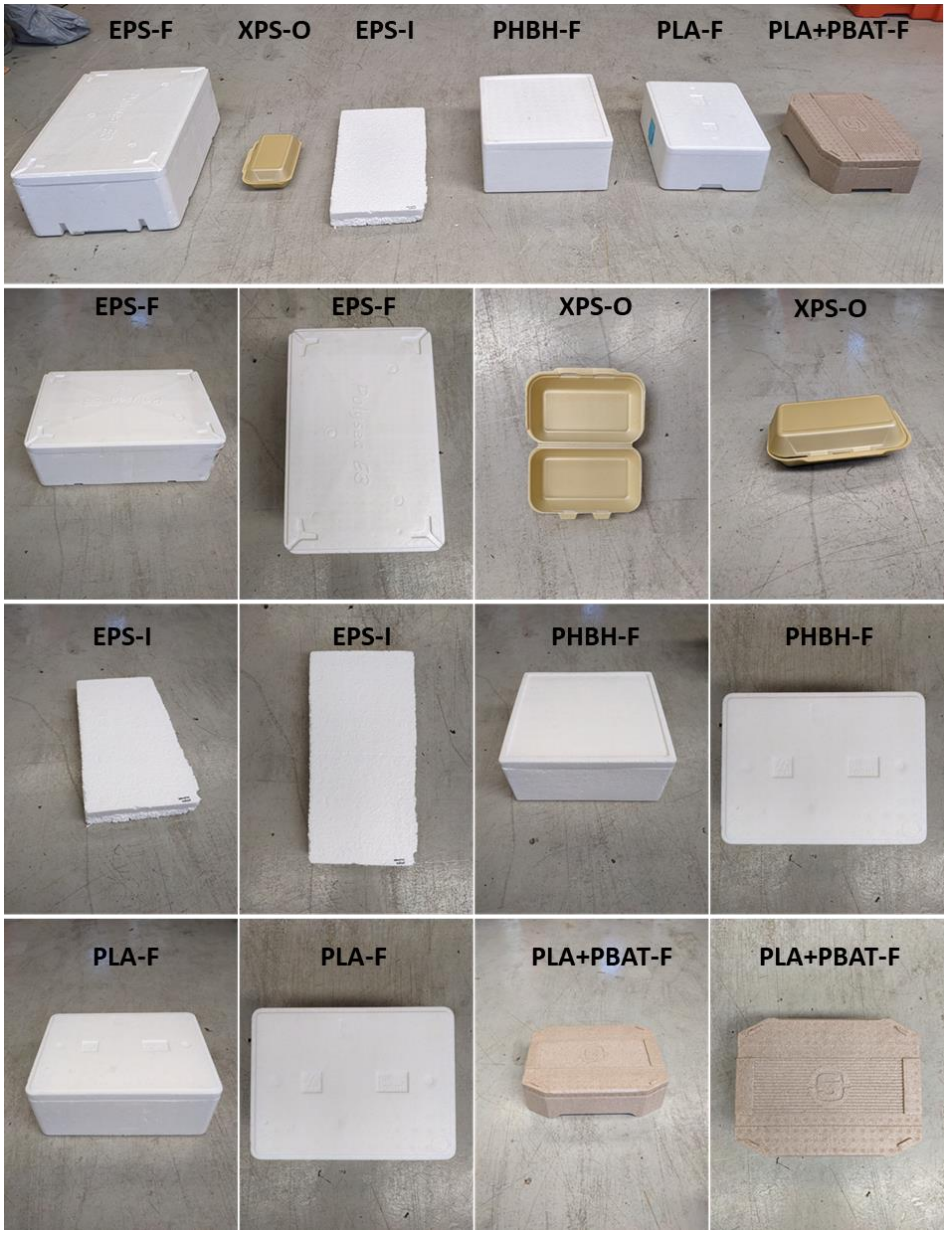

Figure 11. Photos of the six materials tested in the case study

Table 5. Materials tested in the study

Material	Biobased	Biodegradable	Code	End product	Food contact materials
Expanded Polystyrene	No	No	EPS-F	Fish box	Yes
Expanded Polystyrene	No	No	EPS-I	Insulating plate	No
Extruded Polystyrene	No	No	XPS-O	Food box	Yes
Polylactic Acid	Yes	Yes In industrial composting facilities[1]	PLA-F	Fish box	Yes
Polylactic Acid + Polybutylene Adipate Terephthalate	Yes	Yes In industrial composting facilities[1]	PLA+PBAT -F	Fish box	Yes
Poly3-hydroxybutyrate-co-3-hydroxyhexanoate	Yes	Yes In the marine environment[2]	PHBH-F	Fish box	Yes

[1] certified by the NF EN 13432, [2] certified "OK Biodegradable MARINE", certificate from Green Planet™

The three conventional foamed PS were purchased in stores while the three alternatives were kindly provided by three different producers.

The six materials were tested using 13 assays, combining both field and laboratory experimentations, selected in the toolkit described in Part 1. Theses assays are:

(I) For the "Weathering" category (3 assays):

- Level of colonization in seawater,
- Degradation of the materials in an aging chamber,
- Degradation of the materials on a beach.

(II) For the "Transfer of hazardous chemicals" category (3 assays):

- Level of intrinsic contaminants,
- Capacity to leach intrinsic contaminants in seawater,
- Capacity to adsorb environmental contaminants during a deployment in the field.

(III) For the "Toxicity on marine organisms" category (7 assays):

- Cytotoxicity of solvent extracts on bacteria,
- Cytotoxicity of solvent extracts on fish cell lines,
- Estrogenic potential (*i.e.* endrocrine disruption) of solvent extracts,
- Reprotoxicity of solvent extracts on zebrafish early life stages,

- Cytotoxicity of seawater leachates on bacteria,
- Cytotoxicity of seawater leachates on microalgae,
- Reprotoxicity of seawter leachates on oyster early life stages.

The different assays were conducted either by Cedre in its premices (Brest, France) and in Brest Marina or in collaboration with French partners laboratories, namely the EPOC lab (UMR 5805, Talence), the LEMAR lab (UMR 6935, Plouzané) and the LUBEM lab (UR 3882, Plouzané).

Materials and methods used and results obtained for the different assays are presented below. All results obtained are expressed as mean ± standard deviation (SD). Statistical analyses and graphical representations were conducted with the R Software (R Core Team, 2016). Differences are considered significant when *p*-values < 0.05.

2) Weathering of EPS/XPS and alternatives

I. Colonization

a. Materials and methods

i. Field experiment

A field experiment of 9 weeks was conducted in the Marina du Château in Brest (June – July 2021; 48°22'49.6"N 4°29'21.6"W) to compare the colonization potential between the six selected materials. This assay was conducted in collaboration with the LUBEM lab. All materials were cut to have similar dimensions in term of sizes (4-4.5 × 3 cm) and thickness (0.5-0.8 cm). Materials were immersed inside stainless steel cages (Figure 12).

Figure 12. (A) Marina of Brest, (B & C) Stainless steel cages immersed in seawater to assess colonization and environmental contaminants adsorption (see section 3.II for details).

ii. Macrocolonization

Every 3 weeks, 3 pieces per material were sampled to monitor the colonization trend. All samples were wrapped in aluminum foil and stored at -20°C. Once frozen, samples were scraped to collect the attached macroorganisms which were then placed at 50°C for 4 days to measure a dry mass (mg).

b. Results and discussion

Plastic litter or plastic products used directly and lost at sea (*e.g.* aquaculture gears) can be colonized by macro- and microorganisms with potential impacts for marine ecosystems due to the transport of non-indigenous/invasive species and diseases (*e.g.* Carlton et al., 2018; Frère et al., 2019; Lamb et al., 2018). In our experiment, a rapid colonization, notably by algae, was observed for all materials from the first sampling (Figure 13). The mass of colonizing organisms has a trend to increase over time but no statistical difference (*p*-value > 0.05) was detected between materials, which can be explained by a high variability among replicates (Figure 14). **Over the whole experiment, no statistical difference in macrocolonization (*p*-value > 0.05) was observed among materials. Overall, these results suggest that all tested materials can act as substrate of biodiversity and can transport species**.

Based on these results, the score attributed to each material for this assay is 1.

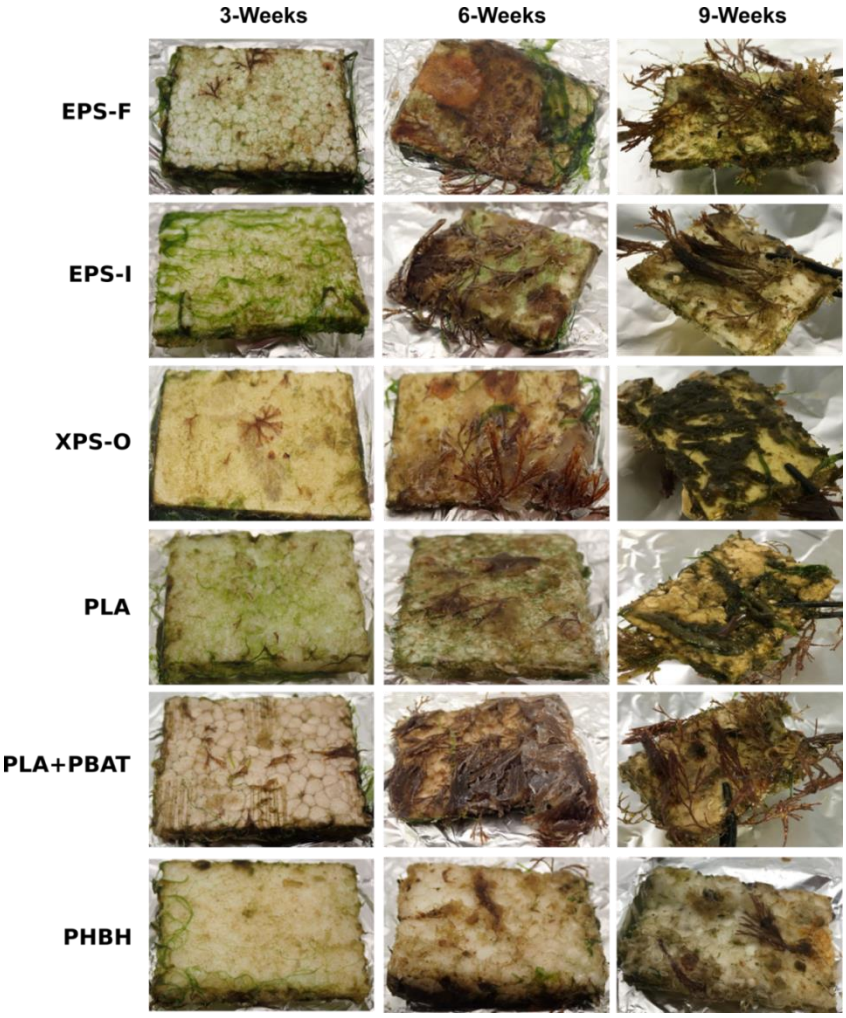

Figure 13. Photos of the six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PHBH-F, PLA-F, PLA+PBAT-F) after 3, 6 and 9 weeks of deployment in the Marina of Brest.

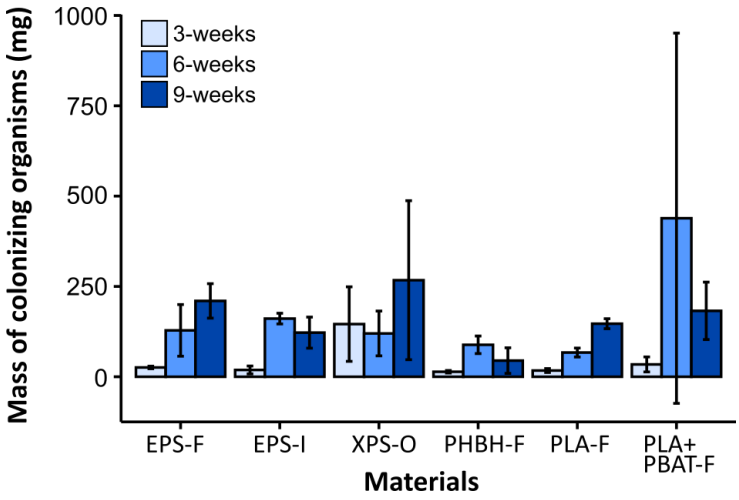

Figure 14. Mass (mg) of colonizing macro-organisms on the six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PHBH-F, PLA-F, PLA+PBAT-F) after 3, 6 and 9 weeks of deployment in the Marina of Brest. ANOVA was used to compare treatments at the 5% level (n=3).

Furthermore, the absence of difference in the mass of colonizing organisms suggests that all materials have the same capacity of colonization. Nevertheless, a high variability was observed between replicates. Thus, we recommend for future similar experiments the use of a larger number of replicates (>15 replicates) to consider the variability and conduct more efficient statistical analyses. In addition, our analysis did not consider the potential taxonomic variability among materials. Therefore we cannot exclude that the diversity varies significantly among conventional PS and their alternatives, with a potential increase of the risk if non-indigenous/invasive species colonize preferentially certain polymers. In the same way, we recommend a novel analysis for future studies with the characterization of the microcolonization (abundance and diversity) as it is well know that the diversity of microorganisms varies among polymers and can include pathogens (Frère et al., 2018; Pinto et al., 2019). This approach could allow identification of differences in the adhesion probability of harmful species.

II. Degradation in an aging chamber

a. Materials and methods

This assay was conducted in Cedre premices. The six materials were placed for three months in an aging chamber (Atlas Suntest XLS+) with constant and controlled temperature (45°C) and lighting (55 W m^{-2}). Dimensions of the materials placed in the chamber are described in Table 6. In addition, small pieces of each materials were sampled after 3 months in the aging chamber to perform a Microtox© assay in order to check an evolution in the toxicity potential after photodegradation (see section 4.I).

Table 6. Size of material pieces used in the aging chamber experiment.

Materials	Weight (g)	Size (cm)	Thickness (mm)
EPS-F	1.2	27.7 × 4.3	5
EPS-I	1.2	28 × 4.3	5
XPS-O	1.8	17.9 × 8.2	3
PLA-F	3.6	27.8 × 4	5
PLA+PBAT-F	1.7	25.9 × 3.6	5
PHBH-F	3.5	27.9 × 4.3	5

b. Results and discussion

Observations at the end of the material weathering revealed visual alterations with higher yellowing of the surface of conventional foamed PS compared to alternatives, as well as a higher release of powder (Figure 15 and 16). These qualitative results support the hypothesis that conventional foamed PS could degrade more rapidly. This could pose a threat to the marine environment through a more rapid and significant release of micro/nanoplastics and toxic chemical compounds initially added during material production (*i.e.* additives). Nonetheless, further studies are necessary to obtain quantitative data and validate this hypothesis.

Based on these results, scores attributed to foamed PS and alternatives, are respectively 1 and 0.

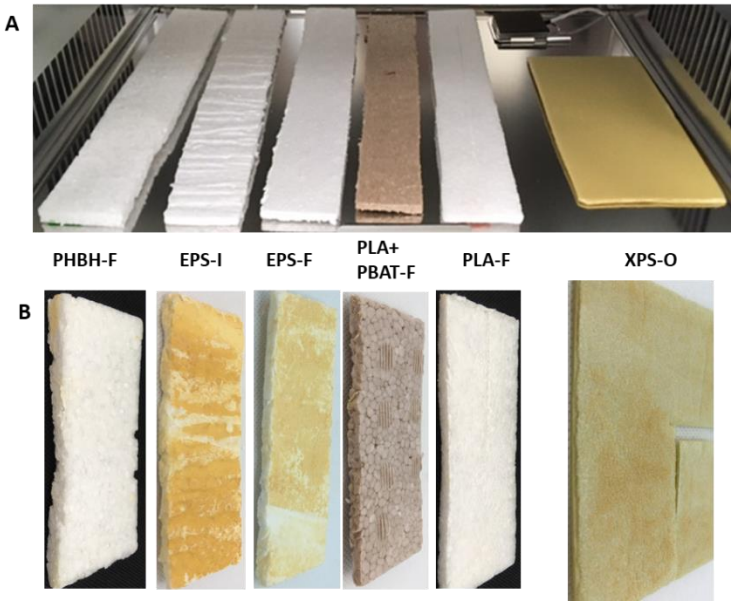

Figure 15. Visual observations of the materials at the beginning of the experiment (A) and after 3 months in the aging chamber (B).

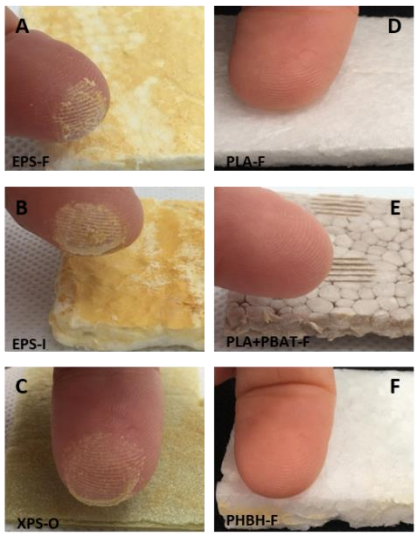

Figure 16. Release of the weathering powder from the six foamed materials (three conventional PS: (A) EPS-F, (B) EPS-I, (C) XPS-O; three alternatives: (D) PLA-F, (E) PLA+PBAT-F, (F) PHBH-F) after 3 months of weathering in the aging chamber.

III. Degradation on a beach

a. Materials and methods

This assay was conducted in Cedre premices. The six materials (Size: 1 × 1 cm; Thickness: 3 mm; n= 23-34 pieces per material) were deployed for eight months on Cedre's artificial beach (Figure 17). All pieces were numbered and weighted before deployment. Three pieces per materials were sampled after 1 week, 2 weeks, 1 month, 3 months, 6 months and 8 months to monitor the mass loss. During the exposure, the oxidation state was monitored by infrared spectroscopy (Nicolet Summit FTIR Spectrometer equipped with an ATR Everest Diamant). In total, six samplings were performed throughout the experiment, after 7, 14, 28, 92, 183 and 259 days. Microtox© assays were performed on samples collected after 3 and 8 months of deployment (see section 4.II).

Figure 17. Experimental area on Cedre artificial beach

b. Results and discussion

Mass analyses showed no significant differences (*p*-values > 0.05) over the monitoring on Cedre's beach for either conventional foamed PS or alternatives (Figure 18). For conventional foamed PS, EPS-F, EPS-I and XPS-O had a mass of 43.2 ± 4.6 mg, 49.8 ± 6.7 mg, 54.0 ± 6.4 mg before the deployment and 46.8 ± 3.1 mg, 58.2 ± 10.4 mg, 56.2 ± 3.2 mg after 8 months of deployment, respectively. Regarding alternatives, PLA-F, PLA+PBAT-F and PHBH-F had a mass of 84.2 ± 10.4 mg, 58.1 ± 8.9 mg, 83.1 ± 11.3 before the deployment and 84.3 ± 7.8, 65.9 ± 3.8, 104.3 ± 14.1 after 8 months of deployment, respectively.

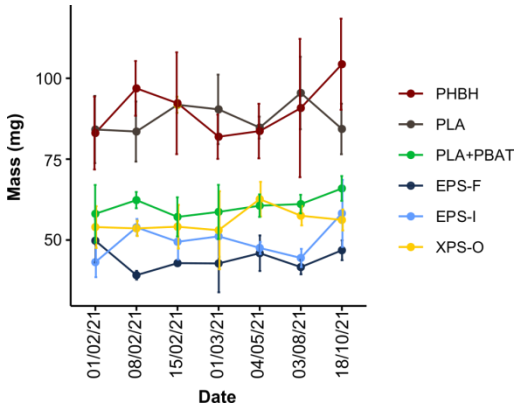

Figure 19A. Evolution of the mass of the six foamed materials (three conventional PS: EPS-F – dark blue, EPS-I – light blue, XPS-O – yellow; three alternatives: PLA-F – grey, PLA+PBAT-F – green, PHBH-F – red) over 8 months of deployment on Cedre's beach. ANOVAs were conducted to check significant differences over time (*p*-values > 0.05).

Analysis of spectral data showed three clusters: (1) the three conventional foamed PS, (2) PLA and PLA+PBAT, (3) PHBH (Figure 19A), in line with the different polymers composition of materials. **No significant evolutions (*p*-values < 0.05) were detected for all materials regarding their spectral signatures over the 8 month of deployment on Cedre beach (Figure 19B). These results suggest that the duration of deployment on the Cedre beach was too short to observe degradation patterns, future studies will require higher deployment duration.**

Based on these results, the score attributed to each material for this assay is 0.

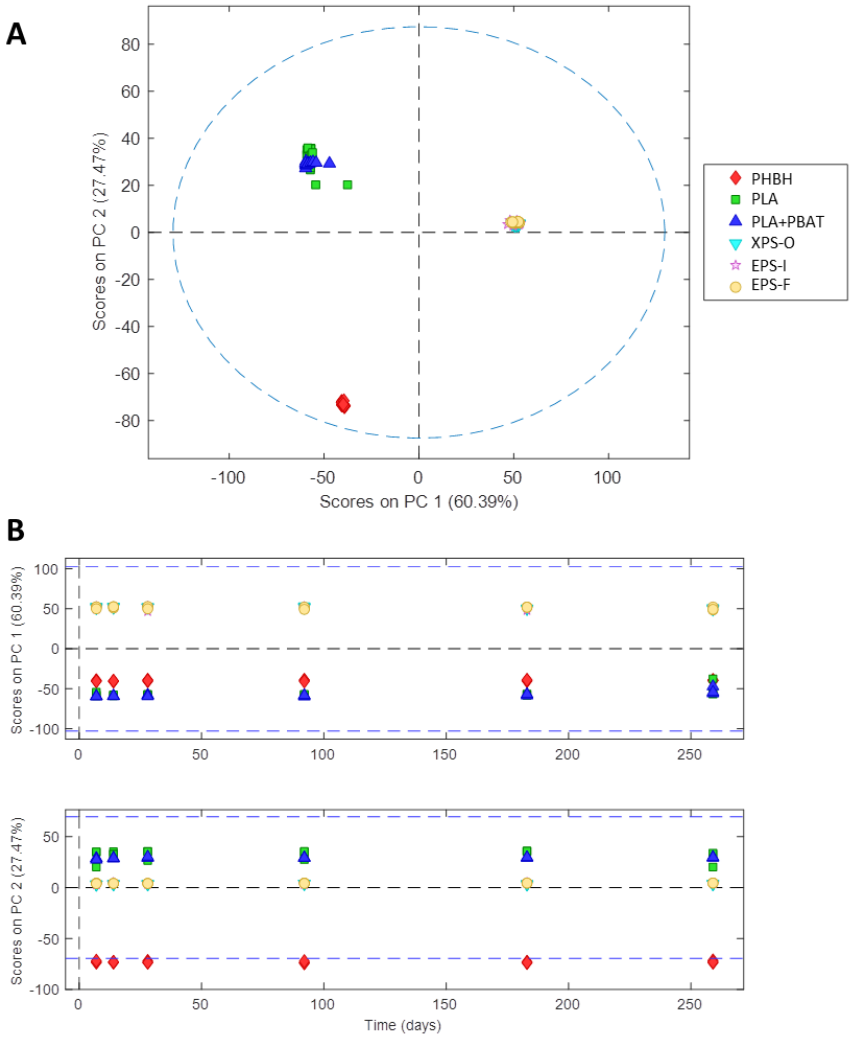

Figure 19B. Principal component analysis (PCA; A) and evolution (B) of the spectral data obtained from six foamed materials (three conventional PS: EPS-F – light blue, EPS-I – pink, XPS-O – yellow; three alternatives: PLA-F – green, PLA+PBAT-F – blue, PHBH-F – red) over 8 months of deployment on Cedre's beach.

IV. Impact Score obtained for the "Weathering" category

Based on results obtained in the different assays assessing the weathering of the six foamed materials, the calculated impact score (IS) for this impact category is presented in Table 7. The data compilation gave a higher IS for the three conventional materials owing to the results obtained in the aging chamber where they displayed a yellowing associated to a release of powder suggesting a higher degradation rate for these materials while alternatives did not exhibit these effects.

Table 7. Scores obtained after assays performed to assess the "Weathering".

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F	Observations
Colonization in the marina of Brest		1	1	1	1	1	1	All materials were rapidly colonized without differences among them
Degradation	Assay in aging chamber	1	1	1	0	0	0	Higher yellowing and release of powders for conventional EPS/XPS while no effect was visually detected for alternatives
	Assay on beach	0	0	0	0	0	0	No difference among materials
Average Score		**0,7**	**0,7**	**0,7**	**0,3**	**0,3**	**0,3**	

3) Transfer of hazardous chemicals for EPS/XPS and alternatives

I. Intrinsic contamination and leaching in seawater

Though they are presented in the same section, it must be noted that the assessment of intrinsic contamination and leaching in seawater are considered as two different assays.

These assays were conducted in Cedre premices. Intrinsic contamination was performed by analyzing materials powder extracted with a solvent whereas leaching in seawater was done by analyzing seawater leachates of the materials. For these assays, it was chosen to conduct targeted analyses on contaminants known to present in certain plastic materials. These contaminants include a selection of PAHs and additives, which are listed in Tables 8 and 9.

Table 8. List of PAHs targeted by the chemical analyses.

PAHs	Abbreviations
naphthalene	N
1-methylnaphthalene	1-MN
2-methylnaphthalene	2-MN
benzothiophene	BTBPE
biphenyl	B
acenaphtylene	ANY
acenaphtene	ANA
fluorene	F
Dibenzothiophene	DBT
Phenanthrene	P
anthracene	A
fluoranthene	FL
pyrene	PY
2-methylfluoranthene	2-MFL
Benz[a]anthracene	BaA
chrysene	C
benzobfluoranthene	B(b)FL
benzokfluoranthene	B(k)FL
benzoepyrene	B(e)PY
benzoapyrene	B(a)PY
perylene	PE
indeno123cdpyrene	IN
dibenzoahanthracene	DBA
benzo(ghi)perylene	BPE

Table 9. List of additives targeted by the chemical analyses.

Additives	Abbreviations	Families
Tripropyl phosphate	TPrP	Flame retardant + plasticizers
Tributyl phosphate	TBP	Flame retardant + plasticizers
Butylated hydroxy toluene	BHT	Antioxydant
4-tert-octylphenol	4-tOP	Plasticizer + antioxydant
Mix nonylphenols	NPs mix	Plasticizer + antioxidant
4-nonylphenol	4-NP	Plasticizer + antioxidant
Nonylphenol mono-ethoxylate	NP1OE	Plasticizer + antioxidant
Nonylphenol diethoxylate	NP2OE	Plasticizer + antioxidant
Tributyl Acetyl Citrate	ATBC	Flame retardant + plasticizer
Tris(1,3-Dichloro-2-propyl)phosphate	TDCPP	Flame retardant + plasticizer
Dibutyl phtalate	DBP	Plasticizer
Di-allyl phtalate	DAIP	Plasticizer
Dimethyl phtalate	DMP	Plasticizer
Di-n-hexylphtalate	DHP	Plasticizer
Benzyl butyl phtalate	BBP	Plasticizer
Dioctyl phtalate	DOA	Plasticizer
Diisoheptyl phtalate	DIHP	Plasticizer
Di-nonyl phtalate	DNP	Plasticizer
Dicyclohexyl phtalate	DCHP	Plasticizer
Di-n-octyl phtalate	DIOP	Plasticizer
Trinuvin 326	UV 326	UV Stabilizer
Trinuvin 328	UV 328	UV Stabilizer
Trinuvin 327	UV 327	UV Stabilizer
2,4,4'-tribromodiphenyl ether	BDE 28	Flame retardant
Tricresyl phosphate	TCP	Flame retardant
Tricresyl phosphate	TCrP	Flame retardant
Tri-o-tolyl phosphate	TToP	Flame retardant
2,2',4,4',6-pentabromodiphenyl ether	BDE 100	Flame retardant
2,2',4,4'-tetrabromodiphenyl ether	BDE 47	Flame retardant
2,2',4,4',5-pentabromodiphenyl ether	BDE 99	Flame retardant
2,2',4,4',5,6'-hexabromodiphenyl ether	BDE 154	Flame retardant
2,2',4,4',5,5'-hexabromodiphenyl ether	BDE 153	Flame retardant
2,2',3,4',5,6'-heptabromodiphenyl ether	BDE 183	Flame retardant
1,2-Bis(2,4,6 tribromophenoxy)ethane	BTBPE	Flame retardant

a. Materials and methods

i. Powder preparation and extraction

The six materials were grinded for 5 minutes with a frequency of 30 Hz (Restsch MM 400). Chemicals were extracted using 200 mg of powder per materials. Firstly, 5 mL of methanol was added and solutions were sonicated for 15 minutes. This step was repeated two times. Secondly, solutions were filtered on wool filters using Buchi flasks. Finally, 200 µL of dimethylsuifoxide (DMSO) was added before an evaporation step.

ii. Leachate preparation

Prior to the experiments, all glassware was rinsed with acetone and, whenever feasible, burnt in a muffle furnace for 6 h at 450°C. The six materials were grinded for 5 minutes (Restsch MM 400). The obtained powders were placed in amber glass bottles filled with 0.2 µm filtered seawater at a concentration of 10 $g.L^{-1}$. Mixtures were continuously stirred using an orbital shaker at 300 rpm and room temperature (20°C) for 24h. At the end of the leaching period, mixtures were filtered on glass-fiber filters (GF/A Whatman, porosity 1.6 µm). Leachates were kept at -20°C until use.

iii. Chemical analyses

A stir bar sorptive extraction (SBSE) of both powder extracts and leachates was performed before TD-GC-MS/MS analysis as described by Lacroix et al. (2014). At first, a polydimethylsiloxane stir bar (Twister 20 mm x 0.5 mm, Gerstel) was placed in the 100 mL leachate or diluted extract (= 1 mL extract + 99 mL seawater) then placed on a magnetic agitator (MIX15) for 16 h of extraction at 750 rpm in the dark at room temperature. After the extraction step, stir-bars were retrieved, rinsed with distilled water and placed on a gas chromatography system Agilent 7890 A coupled with an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies) and equipped with a Thermal Desorption Unit (TDU) combined with a Cooled Injection System (CIS) (Gerstel). Thermodesorption was performed at 280 °C for 6 min and samples were then cryofocused in the CIS at -10 °C. Injection in the GC-MS/MS system was carried out in splitless mode and the CIS was heated to 300°C at 12°C sec^{-1}. The GC temperature program was set as follows: 70°C for 0.5 min, then increase to 150°C at 20°C min^{-1} and finally increase to 300°C at 7°C min^{-1}, maintained for 5 min. An Rxi-5MS (Restek) capillary column was used. Helium was used as a carrier gas with a constant flow rate of 1 mL min^{-1}. The quantitative analysis of PAHs (Table 8) and additives (Table 9) was carried out by internal calibration in multiple reaction monitoring (MRM) mode with two transitions for each compound, one (Quantify) for quantification, the other (Qualify) to confirm the molecular nature by calculating the ratio Qualify/Quantify and by comparison with reference values of pure compounds. The acquisition frequency for each fragment was 2 cycles s^{-1}.

b. Results and discussion

In the materials (Figure 20A), chemicals analyses revealed higher amounts of PAHs in conventional foamed PS (average value = 45,154.0 ± 18,573.6 ng g^{-1}) compared to alternatives (average value = 2,379.2 ± 483.1 ng g^{-1}; *p*-value < 0.01). The EPS-I (66,144.9 ± 1,223.9 ng g^{-1}) had the highest amount of PAHs compared to EPS-F and XPS-O (34,658.5 ± 5,387.8 ng g^{-1}; *p*-value < 0.05) while no statistical difference was detected among the three alternatives. Regarding the amount of additives, statistical differences (*p*-values < 0.05) were detected among the six materials with higher amounts for EPS-I (12,344.3 ± 1,395.3 ng g^{-1}) and XPS-O (16,350.6 ± 1,255.4 ng g^{-1}) compared to other materials (average value = 5,078.1 ± 789.5 ng g^{-1}). Overall, chemical analyses of the six materials suggest that fewer or

different chemicals are present in the three alternatives compared to conventional materials. It can't be excluded that different additives, not analyzed in the present study, are used in alternatives. However, these results should be further confirmed as only targeted chemical analyses were performed in the present study. These types of analyses do not traduce the chemical diversity found in materials. Indeed, high-throughput non-targeted chemical analyses performed on bioplastics and plant-based materials found more than 1,000 chemical features in most samples (Zimmermann et al., 2020). Therefore, to confirm a lower number of chemicals in the alternative used in the present study, new analyses should be conducted to deeply characterize the diversity of chemicals in conventional foamed PS or alternatives. It must be noted that recent studies showed similar intrinsic chemical effects between conventional petroleum plastics, bioplastics and plant-based materials (Zimmermann et al., 2020, 2019). This fact could be related to less common compounds such as new additives, NIAs or transformation products that can display high toxic potential (Tian et al., 2020). The percentage of additive types in each material is described in the Table 10. Significant differences (p-values < 0.05) were found among all materials for each type of additives but no clear difference between conventional or alternative foamed materials was detected except for the additives used as flame retardants only with higher amounts (p-value < 0.05) in conventional materials (average value = 30.1 ± 17.4%) compared to alternatives (average value = 1.9 ± 2.4%).

Chemical analyses detected more chemicals in the powder of conventional foamed PS (33 for EPS-F, 43 for EPS-I, 41 for XPS-O) than alternatives (26 for PHBH-F, 27 for PLA-F, 23 for PLA+PBAT-F; Figures 20A&C). No major difference was recorded in the number of leached compounds (19 for EPS-F, 12 for EPS-I, 13 for XPS-O, 11 for PHBH-F, 12 for PLA-F, 15 for PLA+PBAT-F; Figures 20B&D). Five PAHs were only found in the three conventional foamed PS: benzo[a]anthracene (11.2 ± 8.2 ng g^{-1}), 2-methylfluoranthene (2.2 ± 2.4 ng g^{-1}), anthracene (441.9 ± 395.0 ng g^{-1}), acenaphtene (21.2 ± 6.2 ng g^{-1}), benzothiophene (31.7 ± 18.1 ng g^{-1}). Six PAHs displayed higher concentrations in conventional foamed PS compared to alternatives: naphatalene (1,368 ± 1,150 ng g^{-1} *vs.* 15.9 ± 12.7 ng g^{-1}), 1-methylnaphtalene (337.5 ± 255.5 ng g^{-1} *vs.* 39.3 ± 22.1 ng g^{-1}), 2-methylnaphtalene (453.6 ± 361.4 ng g^{-1} *vs.* 5.4 ± 1.9 ng g^{-1}), biphenyl (729.8 ± 272.5 ng g^{-1} *vs.* 3.8 ± 0.8 ng g^{-1}), fluorene (160.8 ± 50.4 ng g^{-1} *vs.* 8.8 ± 10.0 ng g^{-1}) and phenanthrene (868.8 ± 108.3 ng g^{-1} *vs.* 56.6 ± 42.7 ng g^{-1}). The presence of PAH in conventional foamed polysytyrene could be related to their probable petoleum origin. No PAHs were found only in alternative materials as well as no higher concentrations of PAHs compared to conventional materials. Two additives were only detected in conventional materials: Di-n-hexylphtalate (13.0 ± 0.6 ng g^{-1}; this compound was found in EPS-I and XPS-O but not in EPS-F) and Butylated hydroxy toluene (24.5 ± 8.7 ng g^{-1}). Four additives displayed higher concentrations in conventional foamed PS compared to alternatives: Tricresyl phosphate (TCrP; 1,785 ± 1,729 ng g^{-1} *vs.* 144 ng g^{-1}; not found in PHBH-F and PLA+PBAT-F), Tricresyl phosphate (TCP; 1,309 ± 1,510 ng g^{-1} *vs.* 21.2 ± 5.1 ng g^{-1}; not found in PLA+PBAT-F), Benzyl butyl phtalate (1,854 ± 1,410 ng g^{-1} *vs.* 470.4 ± 236.2 ng g^{-1}), Tributyl Acetyl Citrate (2,650 ± 774.2 ng g^{-1} *vs.* 1,364 ± 353 ng g^{-1}). One additive was found only in alternatives (PHBH-F and PLA+PBAT-F), the Tri-o-tolyl phosphate (28.2 ± 3.2 ng g^{-1}) while one additive (Mix nonylphenols) was detected in higher concentration (1,015 ± 536 ng g^{-1}) compared to conventional materials (80.5 ± 89.1 ng g^{-1}; not detected in EPS-F). **Therefore, the analyses suggest more hazardous chemicals in conventional EPS/XPS.**

Based on these results, one score was attributed per contaminant family (PAH and additives) for the intrinsic contamination. Attributed score are detailed below:

Table 12. Scores obtained for the intrinsic contamination

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Chemicals detected in the materials	Additives	0	1	2	0	0	0
	PAHs	1	2	1	0	0	0

In seawater leachates (Figure 20B), chemicals analyses revealed no statistical difference (*p*-values > 0.05) among the six materials, for either PAHs (average value = 356.7 ± 527.6 ng L^{-1}) or additives (average value = 172.4 ± 112.9 ng L^{-1}). By compiling the data set, the amount of leached PAHs is lower than 8% and lower than 1% for additives regardless of the material (Figure 19C). These results show therefore a low ability of all tested materials to leach their chemicals over 24h in seawater. To confirm the same capacity of leaching among the tested materials, it would be interesting to repeat the experiment using longer leaching durations (weeks, months). **Nonetheless, when looking at individual chemicals, the leaching pattern can be different among materials when comparing chemical quantifications in materials and in leachates.** For instance, acetyl tributyl citrate (ATBC) was found at higher concentrations in conventional materials than alternatives but alternatives leached more ATBC than conventional PS materials (123.9 ± 95.2 ng g^{-1} *vs.* 22.5 ± 2.8 ng g^{-1}; Figure 20D). ATBC is a biodegradable plasticizer approved for food contact materials and recommended for children's items explaining its global use. ATBC can induce toxic effects on aquatic organisms but EC_{50} (>1 mg L^{-1}; U.S EPA, 2015) largely exceed the amount of ATBC found in all leachates (<500 ng L^{-1}). Nevertheless, it cannot be excluded that accumulation of low and no toxic doses of chemicals lead to a "cocktail effect" causing detrimental effects on organisms (Escher et al., 2020). Furthermore, the individual visualization highlights that the leaching pattern is not consistent between the type of material (conventional or alternative), each material had an individual pattern (Figures 20B&D). Therefore, it is important to look at chemicals individually even if the global results suggest the same results among materials.

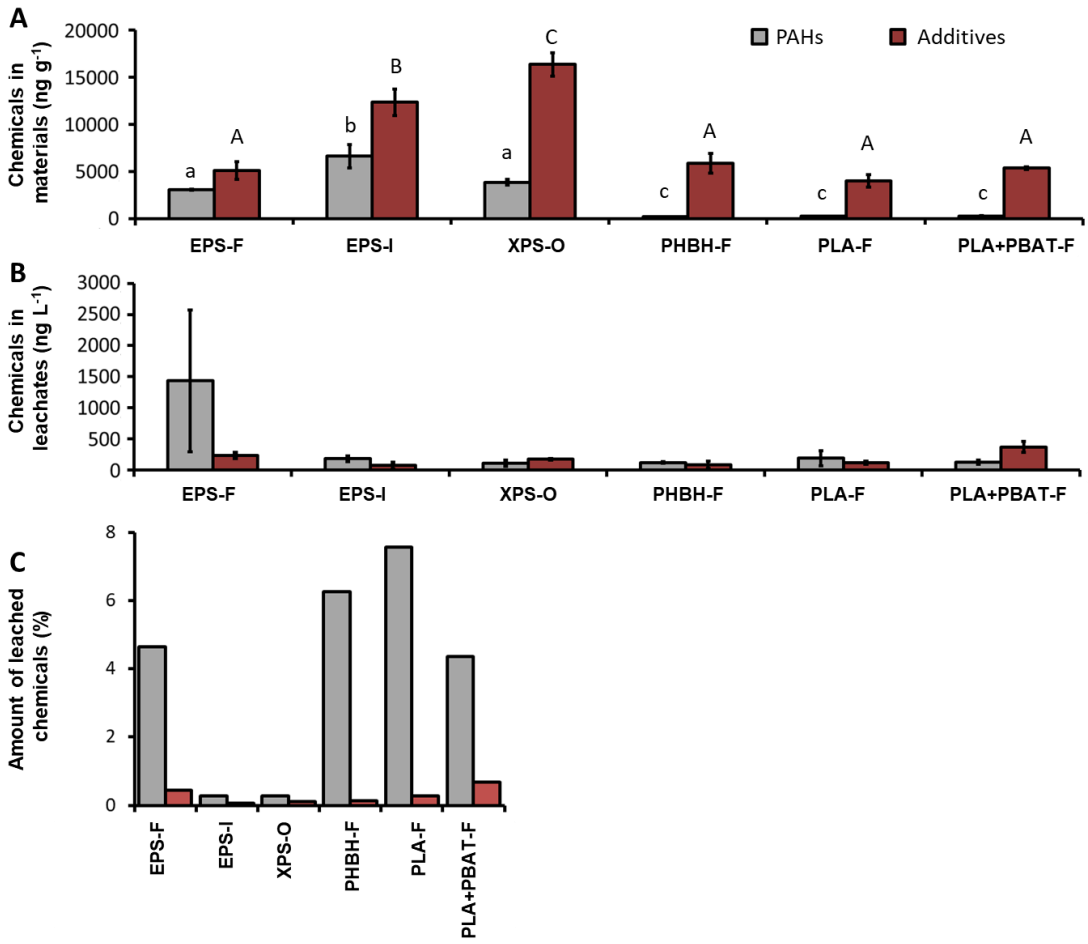

Figure 20. (A and B) Amounts of PAHs and additives quantified in the powder or leachates of six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) or in their leachates (10 g L^{-1}). (C) Proportion of leached chemicals compared to their amounts in the powder. ANOVAs were used to compare treatments with Tukey HSD for pairwise comparisons at the 5% level; homogeneous groups are indicated by the same letter.

Table 10. Mean percentage (%) of additive types in each material. ANOVAs were used to compare treatments with Tukey HSD for pairwise comparisons at the 5% level; homogeneous groups are indicated by the same letter. Analyses were performed for each additive type individually.

Type of Additive	EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Flame retardants + plasticizers	51.3 ± 4.2^{d}	29.2 ± 2.6^{ab}	15.6 ± 1.8^{c}	22.4 ± 1.0^{ac}	28.4 ± 6.4^{ab}	33.9 ± 0.8^{b}
Antioxydants	0.7 ± 0.0^{b}	0.1 ± 0.1^{a}	0.1 ± 0.4^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.1 ± 0.1^{a}
Plasticizers + antioxydants	3.4 ± 0.6^{d}	23.9 ± 2.8^{a}	2.3 ± 0.2^{d}	56.3 ± 5.9^{b}	42.4 ± 5.4^{c}	25.7 ± 4.7^{a}
Plasticizers	13.4 ± 0.6^{c}	33.3 ± 5.4^{ab}	33.9 ± 6.4^{ab}	19.8 ± 5.5^{ac}	24.0 ± 7.5^{b}	39.7 ± 5.6^{b}
UV Stabilisers	2.2 ± 0.4^{b}	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}	1.1 ± 0.3^{ab}	0.6 ± 0.7^{a}	0.0 ± 0.0^{a}
Flame retardants	29.0 ± 1.1^{d}	13.3 ± 3.8^{a}	48.0 ± 4.9^{c}	0.5 ± 0.2^{b}	4.7 ± 0.6^{b}	0.5 ± 0.0^{b}

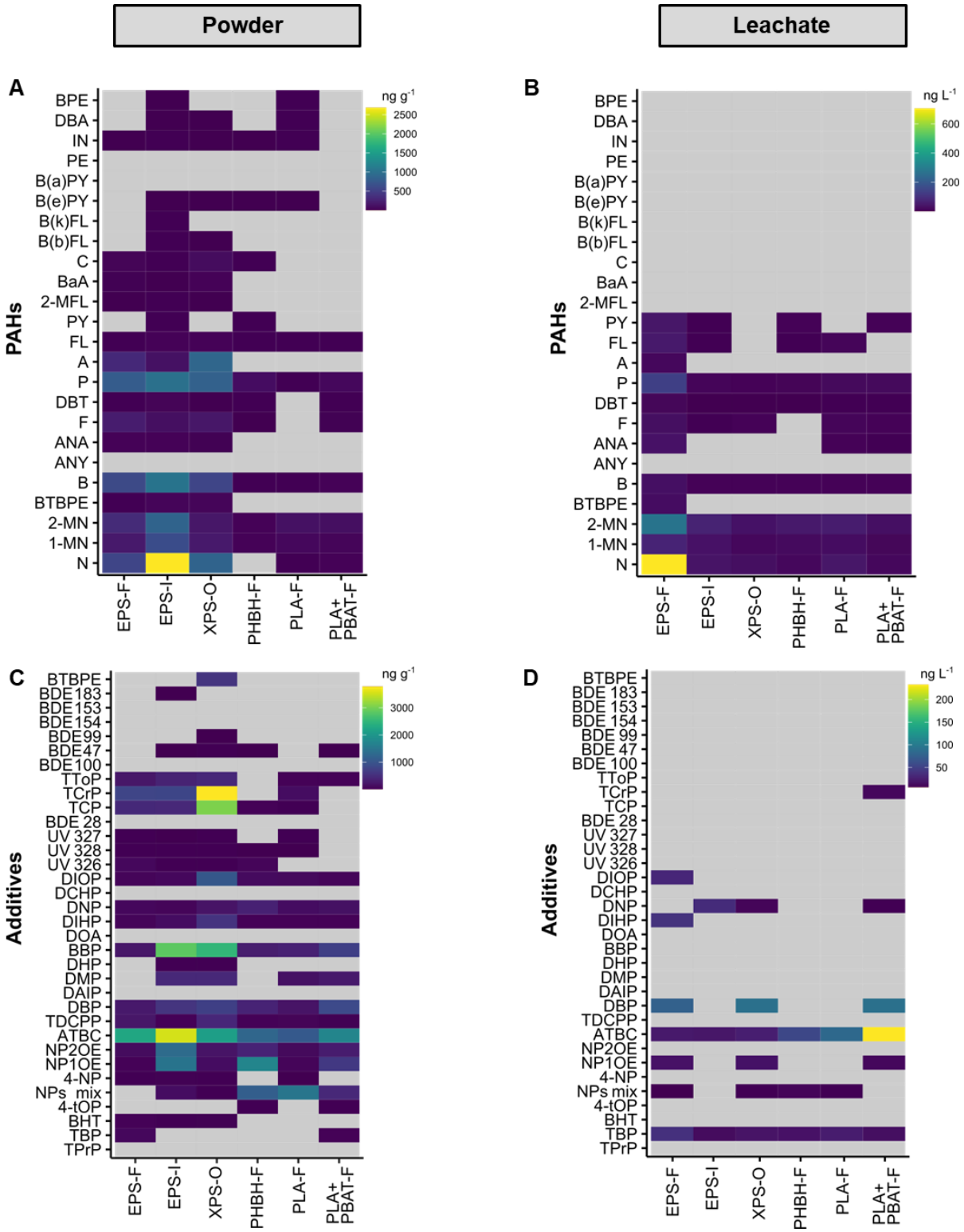

Figure 20. Heatmaps of PAHs (A&B) and additives (C&D) detected in powder (A&C; chemicals in ng g^{-1}) or leachates (B&D; chemicals in ng L^{-1}) of six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F). Gray boxes indicate compounds not detected.

Based on these results, one score was attributed per contaminant family (PAH and additives) for the leaching of contaminants in seawater. Attributed score are all 0, as shown below:

Table 12. Scores obtained for the leaching of intrinsic contaminants in seawater

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Release of chemicals after 24h of leaching in seawater	Additives	0	0	0	0	0	0
	PAHs	0	0	0	0	0	0

II. Adsorption of environmental contaminants using an *in situ* approach

For this assay, it was chosen to conduct targeted analyses on contaminants known to be present in the marine environment and adsorb on plastic materials. These contaminants include a selection of PAHs and additives, which are listed in Tables 8 and 11.

Table 11. List of PCBs targeted by the chemical analyses.

PCBs
PCB-7
PCB 28
PCB-52
PCB-35
PCB 101
PCB 77
PCB 135
PCB 118
PCB 153
PCB 105
PCB 138
PCB 156
PCB 180
PCB 169

a. Materials and methods

i. Field deployment

A field experiment of 3 weeks was conducted in the Marina of Brest in June 2021 to compare the adsorption of contaminants between conventional foamed PS and their alternatives. All materials were cut to display similar dimensions in term of sizes (4-4.5 × 3 cm) and thickness (0.5-0.8 cm). Materials were immerged inside stainless steel cages as detailed in section 2.I. Analyses were performed in triplicate on new and immersed (3-weeks in the field) materials.

ii. Chemical analyses

Material pieces (200 mg) were put in 10 mL of DMSO for 16h using an orbital shaker (230 rpm). Then, 1 mL were sampled from each samples and added in 125 mL glass vials filled with 99 mL of seawater. Finally, all samples were analyzed using the SBSE method described in the section 3.I.a.iii.

b. Results and discussion

Plastics can adsorb organic contaminants due to their hydrophobic surface (Rochman et al., 2013a). Overall, the two expanded PS (EPS-F and EPS-I) displayed similar amount of PAHs (average value= 1,338 ± 677 ng g^{-1}; *p*-value > 0.05) but significantly higher than other materials (average value= 135.4 ± 82.6 ng g^{-1}; *p*-value < 0.05; Figure 21A). The amount of PAHs adsorbed on EPS-F and XPS-O increased significantly (*p*-value < 0.05) between new and weathered pieces with values of 483 ± 146 ng g^{-1} *vs.* 1,641 ± 464 ng g^{-1} and 1,170 ± 357 ng g^{-1} *vs.* 2,060 ± 591 ng g^{-1}, respectively (Figure 21A). A similar trend was observed for the other conventional PS (EPS-I) with values of 127 ± 18 ng g^{-1} *vs.* 271 ± 88 ng g^{-1} without significant difference (*p*-value > 0.05). For all alternatives, the amount of PAHs did not evolve significantly (*p*-values > 0.05) between new and immersed pieces, with average values of 97 ± 28 ng g^{-1}, 48 ± 11 ng g^{-1}, and 198 ± 46 ng g^{-1} for PHBH-F, PLA-F and PLA+PBAT-F, respectively (Figure 21A). **This suggests that EPS/XPS materials display specific surface properties enhancing the adsorption of contaminants.** Regarding the amount of PCBs, no significant differences (*p*-values > 0.05) were detected among all materials and between new and immersed pieces (average value= 5.2 ± 4.2 ng g^{-1}). In sediments of the marina of Brest, the level of PCBs exceeds the sediment quality guidelines (Gauff et al., 2022). Nevertheless, sediments accumulate contaminants while in seawater the continuous renewal can allow low doses of PCBs. In addition, the level of contaminants varies inside the Marina of Brest (Gauff et al., 2022). Therefore, it could be interesting to deploy the materials in different locations of the Marina to confirm the low accumulation of PCBs in all conventional foamed PS and alternatives.

All materials are able to absorb new chemicals during their deployment, for instance the fluoranthene and pyrene are only found on EPS-F after the deployment in the marina of Brest (Figure 22). In total, 2, 5, 1, 9, 6, and 2 PAHs are detected only after the deployment on EPS-F, EPS-I, XPS-O, PHBH-F, PLA-F, and PLA+PBAT-F, respectively (see details in Figure 22). Conversely, 2, 1, 3, and 3 chemicals are not detected after the deployment on EPS-F, EPS-I, PLA-F, and PLA+PBAT-F, respectively (see details in Figure 22).

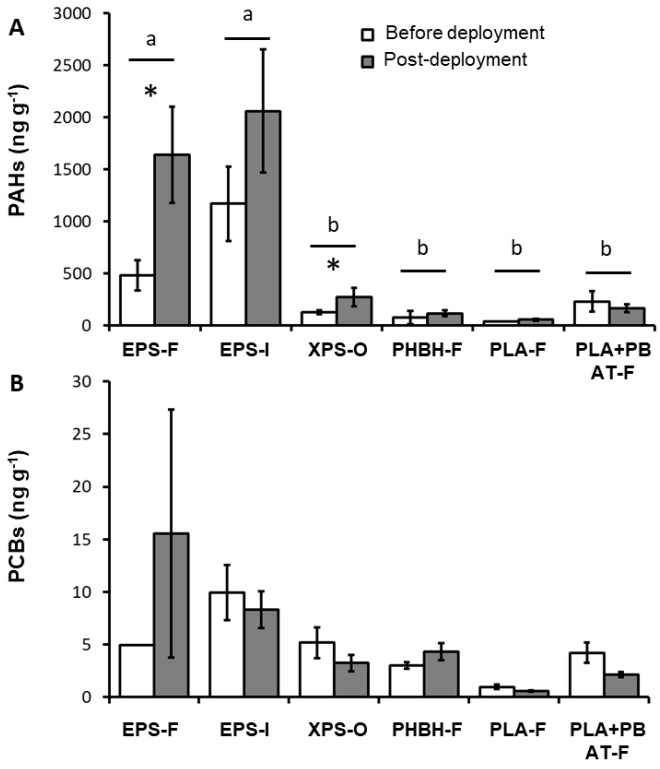

Figure 21. Amount of PAHs (A) and PCBs (B) quantified on six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) before a deployment in the field (white) and after 3 weeks of deployment in the Marina of Brest (grey). Student's tests were performed to compare the two samplings (* = *p*-value < 0.05). ANOVAs were used to compare treatments for each type of chemicals with Tukey HSD for pairwise comparisons at the 5% level; homogeneous groups are indicated by the same letter.

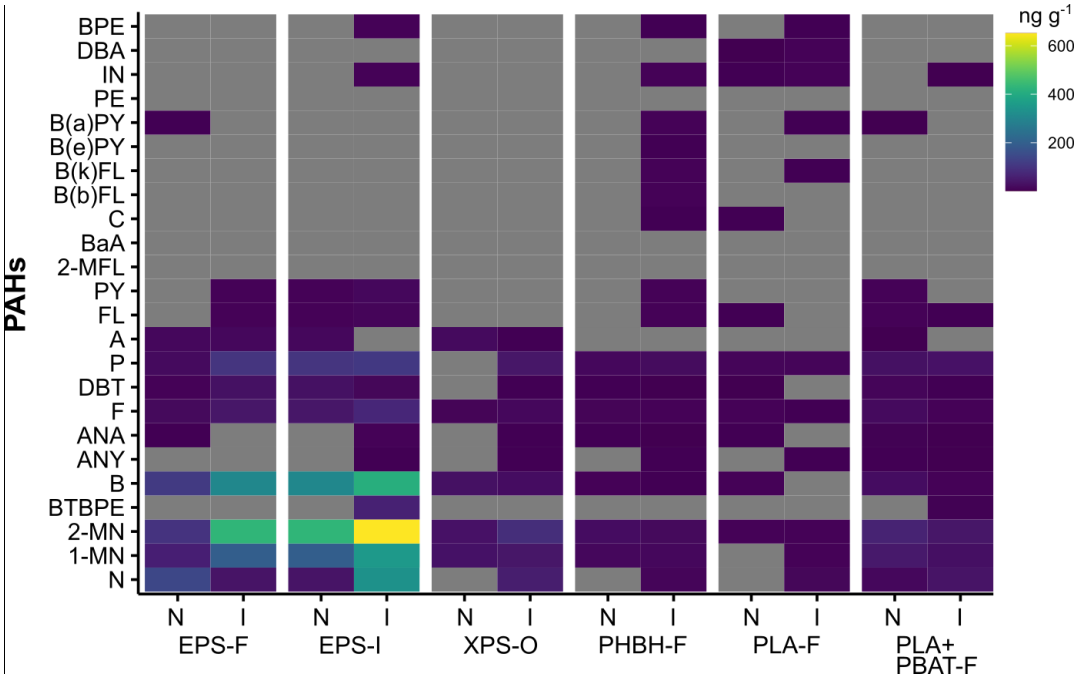

Figure 22. Heatmap of PAHs detected on new (N) or immersed (I; deployed 3 weeks in the marina of Brest) pieces of six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F). Gray boxes indicate compounds not detected.

Based on these results, one score was attributed per contaminant family (PAH and additives). Attributed score are described below:

Table 12. Scores obtained for the adsorption of environmental contaminants

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Adsorption of environmental contaminants	PAHs	1	0	1	0	0	0
	PCBs	0	0	0	0	0	0

III. Impact Score for the "Transfer of hazardous chemicals" category

Based on results obtained in the different assays assessing the transfer of hazardous chemicals of the six foamed materials, the calculated impact score (IS) for this part is available in Table 12. The data compilation gave a higher IS for the three conventional materials (XPS-O > EPS-I > EPS-F) notably because chemical analyses detected more chemicals in extracts in terms of numbers and concentrations. However, it must be noted that it can't be excluded that producers use different chemicals (e.g. additives) for alternative production which were not targeted in the present study.

Table 12. Scores obtained after the assays performed to assess the "Transfer of hazardous chemicals".

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F	Observations
Chemicals detected in the materials	Additives	0	1	2	0	0	0	The concentrations of chemicals were significantly higher in the three conventional foamed PS compared to alternatives except for the level of additives in EPS-F which was not different of the alternatives
	PAHs	1	2	1	0	0	0	
Release of chemicals after 24h of leaching in seawater	Additives	0	0	0	0	0	0	No difference detected in the amount of chemicals released in seawater among materials
	PAHs	0	0	0	0	0	0	
Adsorption of chemicals in the field	PAHs	1	0	1	0	0	0	Only EPS-F and XPS-O displayed a significant increase in the level of PAHs after the deployment
	PCBs	0	0	0	0	0	0	
Average Score		**0,3**	**0,5**	**0,7**	**0**	**0**	**0**	

4) Toxicity on marine organisms of EPS/XPS and alternatives

I. Assessment of intrinsic toxicity

The intrinsic toxicity was assessed by testing the toxicity of solvent extracts of material powder. It relies on 4 assays which are all detailed in the present section.

a. Materials and methods

i. Extracts

The method described in the section 3.I was used to prepare extracts using a mix of methanol and DMSO for the effects on bacteria bioluminescence. For the estrogenic potential, genotoxicity, cellular effects and embryo-larval toxicity, plastics were extracted only in DMSO. Extracts were obtained from new materials or materials weathered for 3 months in an aging chamber (see section 2.II). A extract of unsmoked cigarette filter was also prepared to be used as a positive control as unsmoked cigarette filters are plastic materials known to exhibit some toxic effects on aquatic organisms (Micevska et al., 2006).

ii. Effects on bacteria bioluminescence (Assay 1)

This assay was conducted in Cedre premices. A standardized assay (ISO 11348-3: 2009) was used to assess effects of foamed materials extracts on bacteria (*Aliivibrio fischeri)* using the B-Tox method of the Microtox© FX kit (ModernWater, UK). Briefly, the *Aliivibrio fischeri* reagent was mixed in 300 µL of Microtox diluent, forming the reconstituted reagent. In each vials, 100 µL of the reconstituted reagent was added. After 15 minutes, the bacteria bioluminescence was measured using a portable toxicity analyser Microtox© FX (ModernWater, UK). Then, 900 µL of all extracts were added in each vials. After a 30 minute-exposure, the bacteria bioluminescence was measured to obtain the percent of inhibition (%). This assay was performed in triplicate with extracts from new and aged materials.

iii. Estrogenic potential (Assay 2)

This assay was conducted by the EPOC lab in their premices. The T47D-KbLUC assay determines the estrogenic potential of plastic extracts by the detection of estrogen-like compounds. A modified human breast cancer cell line (T-47d hERα+β) was exposed for 24h to extracts of the six materials (concentration: 0.3% v/v). Estrogenic-like compounds stimulate the luciferase production allowing the estimation of the estrogenic potential. Results are expressed as estradiol equivalent per gram of plastic extracted (pg equivalent 17β-estradiol/g of plastic extracted) and compared to a calibration curve after exposures to 17β-estradiol determining a limit of quantification (LOQ). When the estradiol equivalent per gram of plastic extracted exceeds the LOQ, the plastic item is considered with a significant estrogenic potential.

iv. Cellular effects on raimbow trout cell lines (Assay 3)

This assay was conducted by the EPOC lab in their premices. The assay was performed using a rainbow trout (*Oncorhynchus mykiss*) liver cell line (RTL-W1; Lee et al., 1993).

The cell viability was measured by fluorimetry using the Alamar Blue assay (Le Bihanic et al., 2016). Cells were exposed to plastic extracts (from 0.01% to 1% extracts v/v) for 24h. Then cells were

incubated for 2h with Alamar Blue 10%. The fluorescence rate of resorufin is proportional to the metabolic activity of the cells. This rate is measured with the Fluostar Optima spectrofluorometer (BMG Labtech), at excitation/emission wavelengths of 540/590 nm. The results are expressed as percentage of cell viability compared to the control (cell culture medium).

The reactive oxygen species (ROS) production was measured using the chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA, Molecular Probes TM, Life Technologies) (Pannetier et al., 2018). RTL-W1 were seeded 48 h prior to chemical exposure then rinsed with HBSS and incubated at 20°C for 20 min with 125 µL of CM-H_2DCFDA (20 µM). Cells were exposed for 4h to 0.3% (v/v) extracts of the six materials or 5 mM of H_2O_2 used as positive control. The fluorescence was measured at 485 nm/520 nm (excitation/emission). Results are expressed as percent of ROS induction over 240 min relatively to the control treatment.

The level of DNA damage (*i.e.* genotoxicity) was assessed using the formamidopyrimidine glycosylase Fpg-modified comet assay (Pannetier et al., 2019). Cells were exposed to plastic extracts (0.3% extracts v/v) for 24h. Then, slides were prepared according to Kienzler et al. (2012). Slides were incubed for 35 min at 37 °C with Fpg enzyme. Electrophoresis was performed at 25 V and 300 mA for 20 min before a staining with 20 µL of ethidium bromide (BET; 20 µg/mL). Slides were analyzed using an epifluorescence microscope (×200; Olympus BX51) coupled with the Comet assay IV software (Instrument Perspective LtD; analysis of 100 randomly selected nucleoids were analyzed). DNA damages were expressed as percentage tail DNA, *i.e.* the percentage of DNA which has migrated from the head (Pannetier et al., 2019).

v. Embryo-toxicity assay: Case of zebrafish (Danio rerio) (Assay 4)

This assay was conducted by the EPOC lab in their premices. Embryos of zebrafish (*Danio rerio*) were exposed to the six extracts (0.1% v/v), DMSO (0.1%) and 3,4-dichloroaniline (4 mM; positive control CTL+) according to OECD (2013). Exposures were conducted on 20 embryos in glass petri dishes (n= 3 replicates per treatment) filled with 20 mL of E3 medium supplemented by the treatment under semi-static conditions with a daily renewal. The experiment lasted for 4 days using a thermoregulated enclosure (Snijders Scientific, the Netherlands) with a temperature of 26 ± 1°C and a photoperiod of 14 h light: 10 h dark. Embryos survival and hatched larvae were checked every day at a fixed time and dead embryos/larvae were removed to avoid a contamination. The hatching rate was calculated as: (number of hatched larvae ÷ number of embryos) × 100. At the end of the exposure, larvae were euthanized with 200 mg L^{-1} of benzocaine to evaluate the percentage of malformed larvae as: (number of malformed larvae ÷ number of larvae) × 100. The malformations and lesions analyzed are the following: axial skeletal anomalies (lordosis, scoliosis or kyphosis), head anomalies (atrophy or hypertrophy of the jaws, skull, eyes), malformation of the heart or blood circulation defect, and lesions such as edemas (heart, yolk sac) or hemorrhages (Cormier et al., 2019).

b. Results and discussion

i. Effects on bacteria bioluminescence

Extracts from conventional foamed PS induced an inhibition of *Aliivibrio fischeri* bioluminescence 3-fold higher than alternatives (*p*-value < 0.05; Figure 23). Unsmoked cigarette filters (used as a positive control) induced similar effects than conventional foamed PS (*p*-values > 0.05) except for the extract from aged EPS-I that caused a significant higher toxicity (+27.5%; *p*-value < 0.05). **Regarding the**

expanded PS (EPS-F and EPS-I), extracts from aged materials displayed higher effects than the new ones (+19% for EPS-F and +27% for EPS-I; *p*-values < 0.05). For other materials, the weathering in the aging chamber had no effects on the intrinsic toxicity.

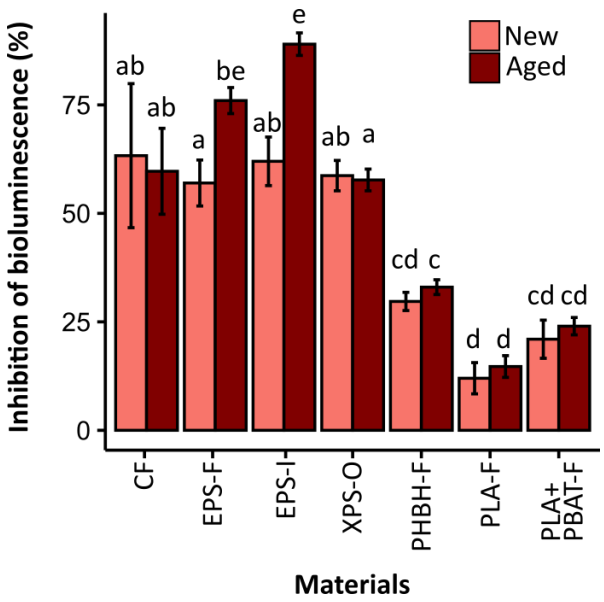

Figure 23. Effects of extracts from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber on the bioluminescence of the bacteria *A. fischeri*. CF = Extract from unsmoked cigarette filters used as a positive control. ANOVAs were used to compare treatments, with Tukey HSD for pairwise comparisons at the 5% level (n=3).

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed for the cytotoxicity assay on bacteria.

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Cytotoxicity on bacteria	1	1	1	0	0	0

ii. Estrogenic potential

All extracts from conventional foamed PS had a significant estrogenic potential while the extracts from the unsmoked cigarette filters or **foamed material alternatives displayed no estrogenic potential** (Figure 24). No difference (*p*-value > 0.05) between new and aged materials was detected except for the EPS-I with a higher (*p*-value < 0.05) estrogenic potential for new material (×1.8).

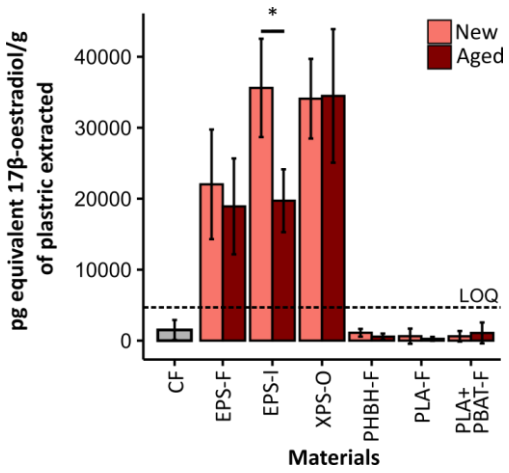

Figure 24. Estrogenic potential of six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber. CF = Extract from unsmoked cigarette filters. *T*-test were used to compare new and aged materials (n= 3).

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed for the estrogenic potential.

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Estrogenic potential	1	1	1	0	0	0

iii. Cellular effects on rainbow trout cell lines

Overall, exposures to all materials had no effect on the viability of rainbow trout cell lines, whatever the tested concentrations (*p*-values > 0.05; average value= 97 ± 3%; Figure 25). Similar results **(no effect) were obtained regarding the ROS production** (*p*-values > 0.05; average value = 1.01 ± 0.03 A.U.; Figure 26) **except for the extract from aged EPS-I that caused a significant increase** compared to the control (+9%; *p*-value < 0.05).

Regarding the Comet assay, two extracts induced slight but significant increases (*p*-values < 0.05) in **the percentage of tail DNA, traducing DNA damage**, compared to the control treatment: aged EPS-I (+6.0%) and aged PLA (+4.5%). Other treatments induced no effects on the percentage of tail DNA (*p*-values > 0.05; Figure 27).

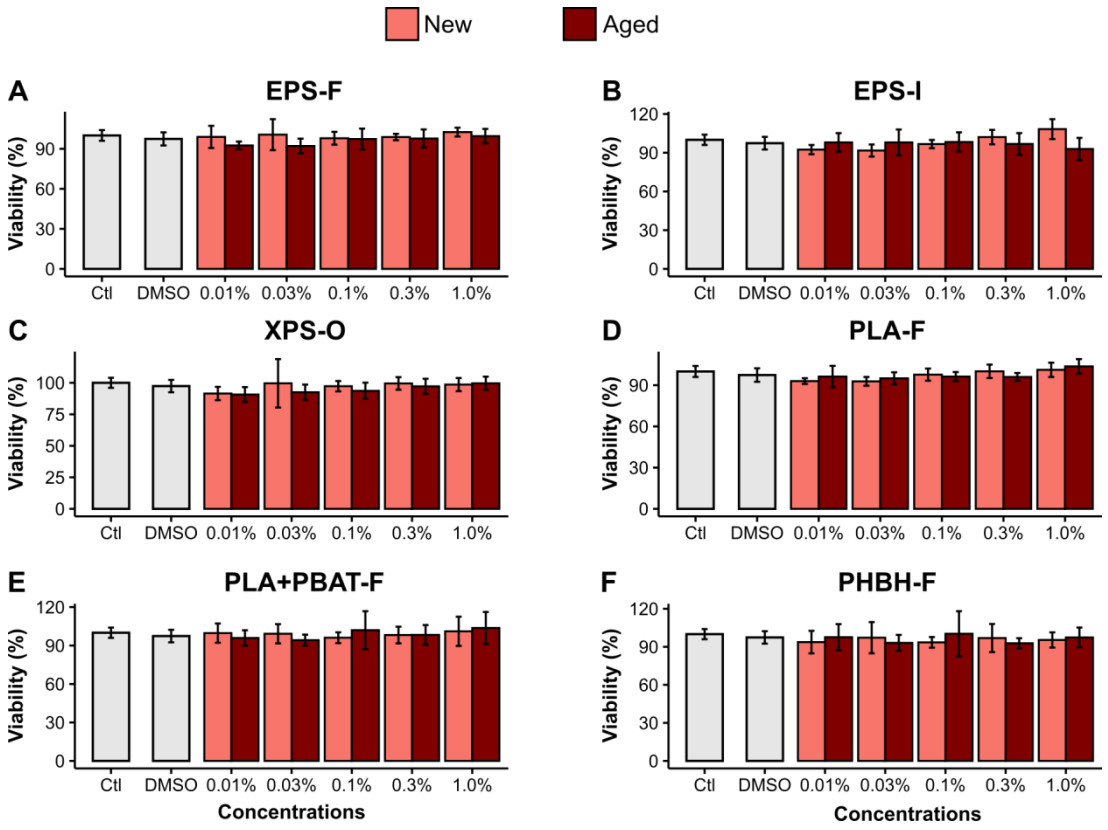

Figure 25. Effects of extracts from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber on the viability of rainbow trout cell lines. ANOVAs were used to compare treatments at the 5% level (n=3).

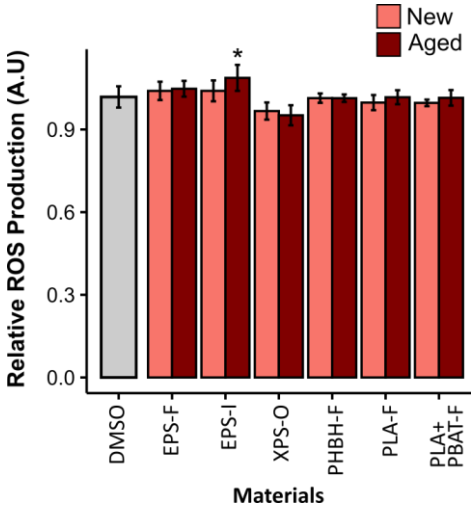

Figure 26. Effects of extracts from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber on the ROS (reactive oxygen species) production of rainbow trout cell lines. ANOVAs were used to compare treatments, with Tukey HSD for pairwise comparisons at the 5% level (n=3); * = *p*-value < 0.05.

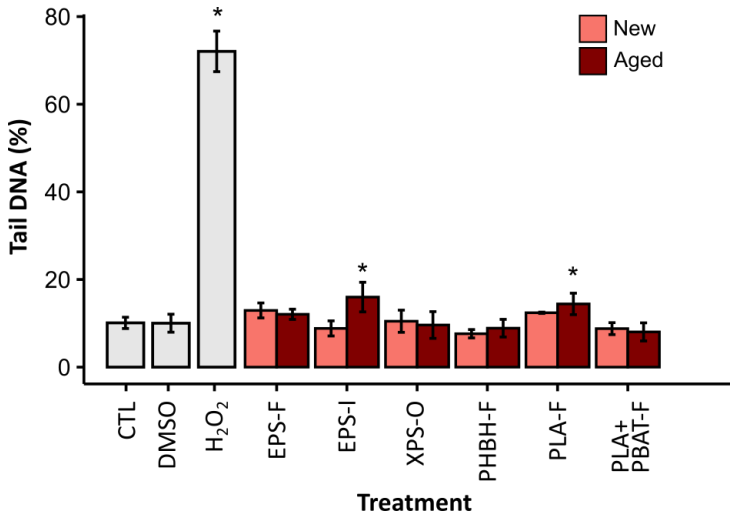

Figure 27. DNA damage (percentage of Tail DNA) on RTL-W1 cells after exposure to organic extract exposures to organic extracts (0.3%) from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber. *T*-tests were used to compare treatments with the control (n=3); * = *p*-value < 0.05.

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed for the cytotoxicity assay on fish cell lines.

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Cytotoxicity on fish cell lines	0	1	0	0	0	0

iv. Embryo-toxicity assay: Case of zebrafish embryos

Extract from new PLA+PBAT reduced significantly the hatching rate of *D. rerio* compared to the control treatment (-18%; *p*-value < 0.05; Figure 28A). By contrast, the extract from aged PLA+PBAT had no effect (*p*-value > 0.05). The other materials (new or aged) had no effects (*p*-values > 0.05; average value = 92.1 ± 5.8%; Figure 28A).

The EPS-I and XPS-O (new or aged) reduced significantly (*p*-values < 0.05) the percentage of normal larvae compared to the control treatment (-25%, -9%, -16% and -30% for new EPS-I, aged EPS-I, new XPS-O and aged XPS-O, respectively; Figure 28B). The other materials (new or aged) had no effects (*p*-value > 0.05; average value = 93.1 ± 3.3%; Figure 28B).

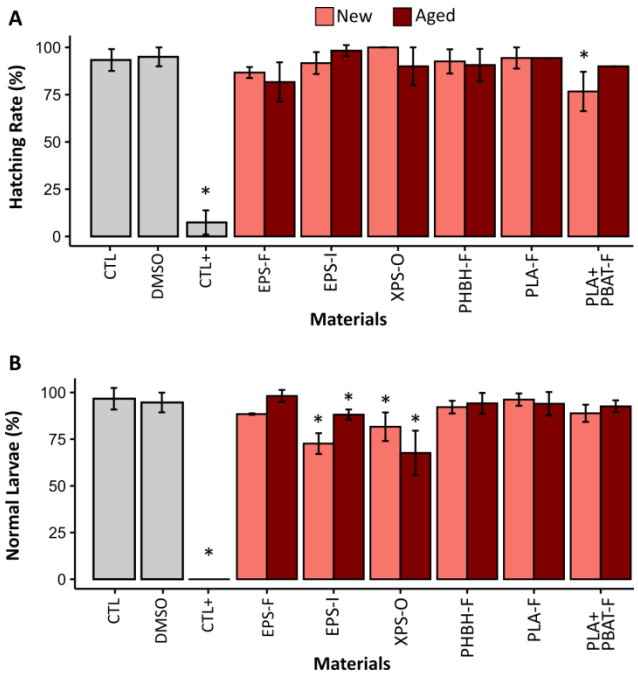

Figure 28. Hatching rate (%; A) and malformation rate (%; B) of *D. rerio* embryos/larvae after exposures to organic extracts (0.1%) from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained from new materials or after 3 months of artificial weathering in an aging chamber. Dunnett tests were used to compare treatments with the control (n=3); * = *p*-value < 0.05.

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed for the reprotoxicity assay on zebrafish.

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Reprotoxicity on zebrafish early life stages	0	1	1	0	0	1

v. Discussion

Cytotoxicity covers all actions impairing the cell functioning including oxidative stress, lipid peroxidation, membrane breakages, DNA damages (*i.e.* genotoxicity) that could lead to cell death (*i.e.* apoptosis). **In the present study, extracts from conventional foamed PS have high cytotoxicity on the bacteria *A. fischeri* but no effects on the viability and ROS production (proxy of impairment of the antioxidant defenses) on rainbow trout line cells (except a slight but significant increase of the ROS production with the aged EPS-I). This result signifies that the cytotoxic potential of conventional foamed PS depends on the biological model.** In addition, it is not surprising to have stronger effects on the bioluminescence of *A. fischeri* as this assay is considered as particularly sensitive to contaminants in comparisons to other cytotoxic assays (*e.g.* Zimmerman et al., 2019).

General conclusions cannot be drawn solely based on cytotoxicity tests, it is important to perform additional assays to obtain information about the toxicity potential on other biological organization. For instance, it appears important to determine estrogenic potential of materials as chemicals impairing normal estrogen signaling can act as endocrine disrupting chemicals and lead to the appearance of cancers, developmental defects, growth, metabolisms, inter/transgenerational effects (*e.g.* Adeel et al., 2017). Here, **only the three conventional foamed PS (new and aged) displayed high estrogenic potential. This suggests that they have the potential to induce stronger and long-term effects on living organisms in comparison to alternatives.** In this way, extracts of EPS-I and XPS-O are reprotoxic for *D. rerio* by reducing the percentage of normal larvae; this effect could be linked to the chemicals responsible of observed estrogenic potential as it is well known that these chemicals induce deformities in *D. rerio* (*e.g.* Moreman et al., 2017).

The EPS-I displayed the highest toxicity potential in comparison to the other tested materials. EPS-I is the only tested material that is not certified for use as food contact material (FCM) and it is therefore submitted to less regulations regarding chemicals content and release (Zimmerman et al., 2019). This could explain the higher toxicity observed for this material.

Overall, the weathering (on the beach or in aging chamber) leads to a slight increase in intrinsic toxicity of materials. However effects remain limited over the study period, especially for alternatives. Regarding the conventional foamed PS, the weathering increased the effects of the EPS-I and EPS-F extracts on the bioluminescence of *A. fischeri* while the same extracts of EPS-I increase significantly the ROS production of rainbow trout cell lines. This evolution in the toxicity potential could be linked to apparition of transformation products during the weathering increasing the intrinsic toxicity of the two materials as recently highlighted between new and used tires (Tian et al., 2020).

II. Toxicity of seawater leachates

This assessment relies on 3 assays which are all detailed in the present section.

a. Materials and methods

i. Leachates preparation

The leachates were prepared and stored as described in the section 3.I.

ii. Effects on bacteria bioluminescence (Assay 1)

This assay was conducted in Cedre premices. The standardized assay (ISO 11348-3: 2009) using the Microtox© FX kit described in the section 4.I was used to assess the toxicity of leachates obtained from the six materials. Microtox© assays were performed with leachates from new materials and leachates obtained from materials artificially aged during 1 month in the aging chamber or aged for 3 and 8 months on the Cedre artificial beach.

iii. Effects on microalgae (Assay 2)

This assay was conducted in Cedre premices. A standardized assay (ISO 10253: 2016) was used to assess effects of foamed material leachates on microalgae using the AlgalToxKit© (R-Biopharm®; Germany) based on the use of microalgae inoculum of the marine diatom *Phaeodactylum tricornutum*. Briefly, a culturing medium was produced using synthetic seawater (NaCl 26.4 g L^{-1}, KCl 840 mg L^{-1}, $CaCl_2$ 1670 mg L^{-1}, $MgCl_2$ 4600 mg L^{-1}, $MgSO_4$ 5580 mg L^{-1}, $NaHCO_3$ 170 mg L^{-1} and H_3BO_3 30 mg L^{-1}) enriched with nutrient solutions provided by the kit. Then, *P. tricornutum* were cultured in culturing

medium containing the different treatments (control or leachates) at 20°C. During a 3-days exposure, the optical density (OD) was measured using a spectrophotometer for measurements at 670 nm in order to assess the algal growth inhibition (%) at the beginning of the exposure (T_0), after 24h, 48h and 72h. In addition to the leachates, a solution of potassium dichromate (1 g L^{-1}) was used as a toxicant reference.

iv. Effects on oyster early-life stages (Assay 3)

Effects on oyster fertilization

This assay was conducted in collaboration with the LEMAR lab in their premices. Mature oysters (*Crassostrea gigas*; 18 months-old) were collected in July 2021 (Localization: 48°34'30''N, 4°36'18''W). Gametes were collected by stripping as described in Tallec et al. (2018). Spermatozoa and oocytes were diluted in 100 mL and 1 L of SW (20°C), respectively, and left for 1 h prior to use to ensure gamete quality (spermatozoon mobility and round shape of oocyte). Spermatozoa and oocyte concentrations were assessed by flow cytometry (EasyCyte Plus cytometer; Guava Merck Millipore). Spermatozoa and oocytes from three males and three females were placed in glass tube at a spermatozoa-to-oocyte ratio of 100:1 and a concentration of 1,000 oocytes mL^{-1} (final volume: 5 mL). This step was repeated in four replicate. After 1.5h, all samples were fixed with a formaldehyde-seawater solution (0.1% final) to estimate the fertilization yield (FY) under a microscope (Zeiss Axio Observer Z1; ×4-10 magnification; 100 oocytes observed per treatment). The FY (%) is calculated as: number of fertilized oocytes ÷ (number of fertilized + unfertilized oocytes) × 100. An oocyte is considered as fertilized when polar bodies and cell divisions are observed (Tallec et al., 2018).

Effects on oyster embryo-larval development

This assay was conducted in collaboration with the LEMAR lab in their premices. A standardized assay (ISO 17244:2015) was used to assess embryotoxicity of foamed materials leachates. We collected spermatozoa and oocytes from 12 males and 12 females using the same batch of oysters and methods described in section 4.II.iv. Gametes from 3 males and 3 females were placed in glass beaker filled with 0.2 filtered-seawater (FSW) at a spermatozoa-to-oocyte ratio of 100:1 and a final concentration of 1,000 oocytes mL^{-1}. This step was repeated four times. Once fertilization was achieved (FY > 90%), 2-cells embryos were transferred in glass vials filled with the different treatments at a concentration of 60 embryos mL^{-1} (final volume: 25 mL). After 36 h in dark conditions, all samples were fixed with a formaldehyde-seawater solution (0.1% final) to estimate the normal D-larval yield under a microscope (Zeiss Axio Observer Z1; ×4-10 magnification; 100 larvae observed per treatment). The normal D-larval yield (%) is estimated as: normal D-larvae ÷ (normal + abnormal D-larvae) × 100. Abnormal D-larvae referred to larvae exhibiting morphological deformations (*e.g.* mantle, shell, hinge) as well as development arrest during embryogenesis (Tallec et al., 2018).

b. Results and discussion

i. Effects on bacteria bioluminescence

After 1 month of weathering in the aging chamber, **no statistical differences were detected regarding the leachate toxicity on *A. fischeri* among all materials** (*p*-value > 0.05; average value = 29 ± 5%; Figure 29). Nonetheless, for the PLA, a significant increase (*p*-value < 0.05) in the toxicity was observed over time (×13.5).

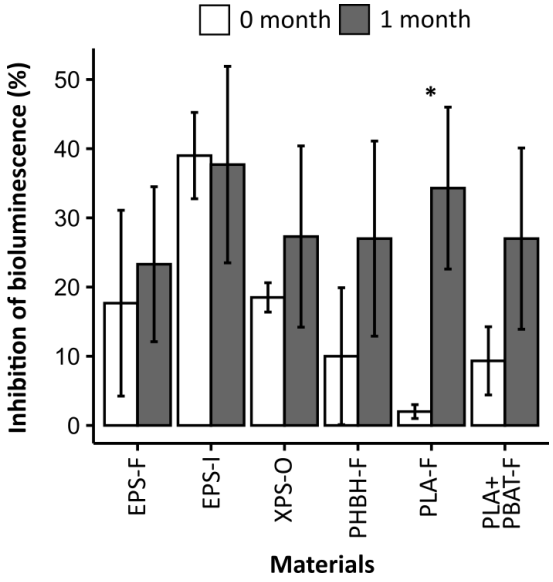

Figure 29. Inhibition of bioluminescence (%) of the bacteria *A. fischeri* after 30 min exposures to leachates from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained after 0 or 1 month in an aging chamber. ANOVAs were used to compare treatments, with Tukey HSD for pairwise comparisons at the 5% level (n=3); t-tests were performed to compare the time of weathering (* = *p*-value < 0.05).

After 3 and 8 months of weathering on the Cedre artificial beach, **no statistical differences were detected regarding the leachate toxicity on *A. fischeri* among all materials** (*p*-values > 0.05; average value = 34 ± 21%; Figure 30). Nonetheless, for two materials, a significant increase (*p*-values < 0.05) in the toxicity was observed over time (+410% for PHBH-F and +395% for PLA+PBAT-F).

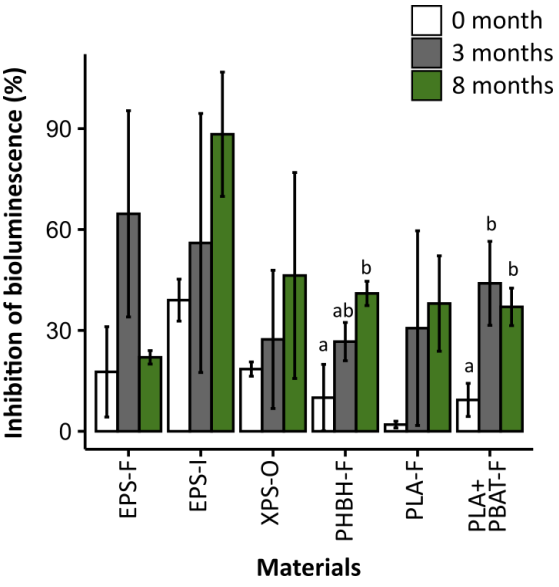

Figure 30. Inhibition of bioluminescence (%) of the bacteria *A. fischeri* after 30 min exposures to leachates from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F) obtained after 0, 3 and 8 months of deployment on the Cedre artificial beach. ANOVAs were used to compare treatments, with Tukey HSD for pairwise comparisons at the 5% level (n=3); homogeneous groups are indicated by the same letter.

Based on obtained results, scores detailed below were attributed.

Table 13. Score attributed for bacteria cytotoxicity assay

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Cytotoxicity on bacteria	0	0	0	0	0	0

ii. Effects on microalgae

Compared with the control treatment**, all tested materials did not alter the growth of *P. tricornutum*** (average value= 100.8 ± 5.3%; Figure 31). No difference was suggested between leachates from unsmoked cigarette filters and PS materials or alternatives (Figure 31). As expected, the potassium dichromate ($KrCrO_4$; 1 g L^{-1}) used as the reference toxicant reduced the microalgal growth (-68%).

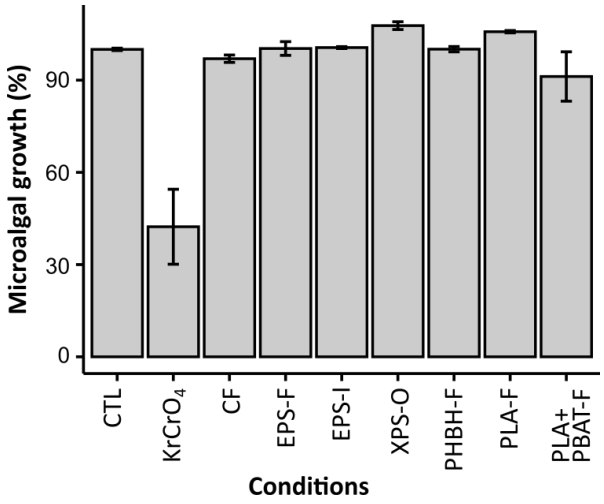

Figure 31. Growth (%) of *P. tricornutum* after 72h exposure to leachates from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F). $KrCrO_4$= Potassium dichromate. CF = Leachate from unsmoked cigarette filters.

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed for the microalgae cytotoxicity assay

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Cytotoxicity on microalgae	0	0	0	0	0	0

iii. Effects on oyster early life stages

Compared with the control treatment, all tested materials did not alter the fertilization success of *C. gigas* (average value= 72.6 ± 10.7%; *p*-values > 0.05; Figure 32A). Regarding the embryo-larval development success, the PLA-F reduced significantly the normal D-larval yield (-99%; *p*-value < 0.001)

compared to the control treatment (average value= 87.6 ± 6.7%) while other materials had no effects (average value= 78.4 ± 6.3%; *p*-value > 0.05; Figure 32B).

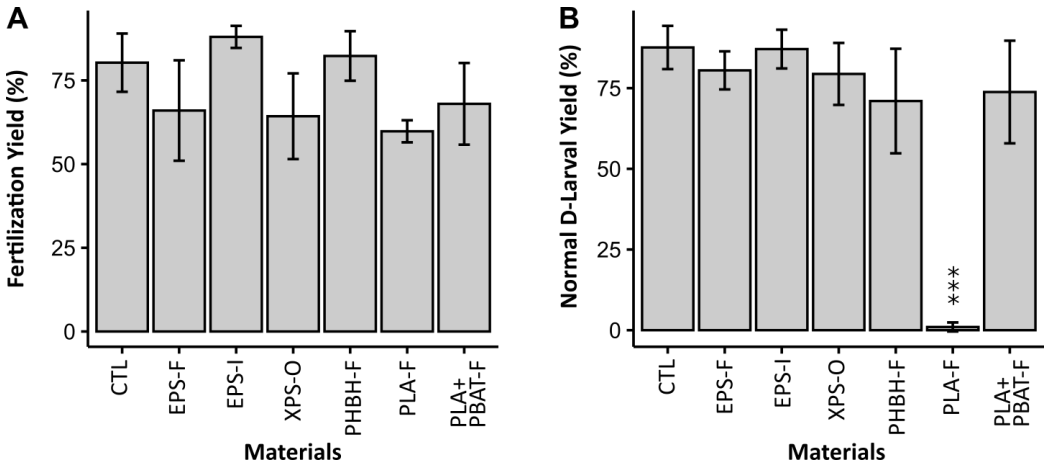

Figure 32. (A) Fertilization yield (%) and (B) Normal D-Larval Yield (%) of oyster *C. gigas* after 1.5h exposure to leachates from six foamed materials (three conventional PS: EPS-F, EPS-I, XPS-O; three alternatives: PLA-F, PLA+PBAT-F, PHBH-F). ANOVAs were used to compare treatments, with Tukey HSD for pairwise comparisons at the 5% level (n=4); ***= *p*-value < 0.001.

The experiments conducted with leachates suggested low effects without differences among materials even if remarkable effects can be observed on specific biological model (*e.g.* PLA leachate with oyster embryos). This suggests that the six materials leached small amount of toxic compounds, which is in agreement with the results of the chemical analyses described above. It could be interesting to repeat the experiments witch leachates obtained from a wide range of leaching duration (from 24h to several weeks/months) to monitor the kinetics of potential effects associated with the release of toxic compounds in the leachate of the six materials.

Based on obtained results, scores detailed below were attributed.

Table 13. Scores attributed to the oyster reprotoxicity assay

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Toxicity of extracts	Reprotoxicity on oyster early life stages	0	0	0	0	1	0

III. Impact Score

Based on the results obtained in the different assays assessing the toxicity of the six foamed materials, the calculated impact score (IS) for this part is available in the Table 13. The data compilation gave a higher IS for the three conventional materials (EPS-I > XPS-O > EPS-F) with a IS at least three times higher than the alternative's IS.

Table 13. Impact score obtained after the assays performed to assess the "Toxicity".

Assays		EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F	Observations
Toxicity of extracts	Cytotoxicity on bacteria	1	1	1	0	0	0	The 3 conventional foamed materials displayed higher cytotoxic potential than alternatives.
	Cytotoxicity on fish cell lines	0	1	0	0	0	0	
	Estrogenic potential	1	1	1	0	0	0	The 3 conventional foamed materials, but not alternatives, exhibited an estrogenic potential
	Reprotoxicity on zebrafish early life stages	0	1	1	0	0	1	Significant decreases observed only with EPS-I, XPS-O and PLA+PBAT-F
Toxicity of leachates	Cytotoxicity on bacteria	0	0	0	0	0	0	No difference among materials
	Cytotoxicity on microalgae	0	0	0	0	0	0	No difference among materials
	Reprotoxicity on oyster early life stages	0	0	0	0	1	0	Only the PLA leachates reduced significantly the oyster embryogenesis
Average Score		**0,3**	**0,6**	**0,4**	**0,0**	**0,1**	**0,1**	

5) Study overview and conclusion

Through the 13 assays performed for the three environmental impacts categories identified in the Part 1 of these report, the IS proposed in the preliminary approach for harmonized impact assessment of plastic materials allowed to obtain a ranking available in the Table 14. Based on the IS calculations, we obtained higher IS for the three conventional foamed materials (average IS= 1.5 ± 0.3) compared to the alternatives (average IS= 0.4 ± 0.1). These results suggest replacement of foamed PS by selected alternatives is a relevant option from a marine environment point of view.

Furthermore, all results demonstrate the importance to perform a large screening of toxicity assays to compare the risk of different polymers/materials. The use of a limited number of assays can lead to a misinterpretation and incorrect recommendations. For instance, if conclusions are based only on the oyster embryo-larval development assay, the conclusion is a higher risk for the PLA compared to other materials whereas the PLA extract and leachate had no effect in the other toxicity experiments.

Table 14. Impact Score (IS) of the six materials regarding the four categories of impacts.

Environmental Impacts	EPS-F	EPS-I	XPS-O	PHBH-F	PLA-F	PLA+PBAT-F
Weathering	0.7	0.7	0.7	0.3	0.3	0.3
Transfer of hazardous chemicals	0.3	0.5	0.7	0	0	0
Toxicity on marine organisms	0.3	0.6	0.4	0	0.1	0.1
Impact Score	**1.3**	**1.8**	**1.8**	**0.3**	**0.4**	**0.4**

This work is, to our knowledge, the first attempt to provide an approach with guidelines to characterize potential environmental impacts of plastics likely to reach the marine environment. This case study confirms the added-value of this type of approach as it provides (i) a harmonized framework allowing comparisons among studies and materials and (ii) data to help decision-makers in the mitigation of the impact of plastic pollution on the marine environment.

However, the proposed approach has limitations due existing methodological and knowledge gaps indicating that further developments are needed to make the approach and associated results more robust and accurate. For instance, it appears necessary to (i) develop more standardized assays to assess the different impacts of plastic materials, (ii) elaborate thresholds allowing to assess the level of impacts for each assay, (iii) further develop the impact score assessment method by specifying the number and types of assays to conduct, the weighting of each test, the calculation method to use…).

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APPENDIX

Appendix 1. Exemple of harmonised scheme used for offshore substances (source: https://www.cefas.co.uk/data-and-publications/ocns/)