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TOPICAL REVIEW

Biodegradable plastics in aquatic ecosystems: latest findings, research gaps, and recommendations

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Abstract

The negative impact of plastic accumulation in aquatic ecosystems is a known and undeniable problem. However, while many of the scientific community's countermeasures against such accumulation target the effects of the most common commodity plastics, the consequences of so-called 'biodegradable' plastics in those ecosystems are seldom discussed. After all, though their alleged biodegradability sustains the widespread belief that they are harmless to the environment, because a material's fate determines its classification as biodegradable or not, many plastics classified as biodegradable do not in fact meet the required norms and standards of biodegradability in aquatic ecosystems. Furthermore, during the past five years, the scientific community has shown that the degradation of such plastics can generate bio-microplastics that have effects similar to or worse than those of conventional microplastics (MPs). Against that background, this review details the latest findings regarding how biodegradable plastics can influence aquatic ecosystems and thus cause adverse health effects in living organisms and/or act as vectors of chemical pollutants. Beyond that, it identifies the key aspects of such trends to be investigated in greater depth, including the need to consider a wider variety of biodegradable plastics and to develop systematic methods that allow quantifying and identifying the remains of those pollutants in living species. Other aspects worth considering include the arrival and mobilisation dynamics of MPs in oceans. The ways in which small animals fed by filtering (e.g. red crabs and other zooplankton organisms) move MPs through the water column and into food webs also merit attention, for those MPs are ingested by numerous species at different trophic levels, at which point bioaccumulation in tissues has to be considered as a factor of toxicity. This review closes with a series of recommendations and perspectives for future studies on 'biodegradable plastics' in aquatic ecosystems.

1. Introduction

Without a doubt, no one today can imagine life without plastics, those extraordinary materials that have surrounded us with lightweight, high-performance products for decades. Every year, plastic products are produced in massive quantities, many of them for packaging. In 2020 for example, global plastics production reached nearly 370 million tons [1]. However, much of that plastic ends its life in aquatic ecosystems that are consequently affected by the material's presence in various ways. In fact,

approximately 12 million tons of plastic enter Earth's oceans each year, most of which is waste from single-use packaging. Whereas fishing gear and other resources lost at sea, industrial losses, and illegal dumping represent only a fifth of marine litter, the other 80% comes from land [2]. Worse still, the situation is aggravated by the increasingly excessive use of single-use plastics, including personal protective equipment (e.g. gloves and masks) consumed during the COVID-19 pandemic, many of which have ended up in bodies of water [3].

Once plastic objects reach aquatic ecosystems, they remain in those environments for periods ranging from weeks to thousands of years depending on the plastic's source (i.e. conventional or biodegradable in that aquatic ecosystem) and the specific conditions that it is exposed to (e.g. sunlight, oxygen, and mechanical agents) [4–6]. During the time that plastics remain in those aquatic habitats, they can cause severe damage to living organisms, including the more than 1 million seabirds and more than 100 000 marine mammals that die each year due to plastics that have reached the sea [7]. One of the most visible consequences of the accumulation of those residues in Earth's oceans is the formation of so-called 'garbage patches' [8], a term used to describe areas of the ocean's surface that resemble rubbish islands, where floating waste accumulates in the middle of giant circulating current systems. Although replacing conventional plastics with other materials able to biodegrade in aquatic ecosystems may mitigate the problem, replacing such plastics with ones that are compostable or biodegradable in other environments is not a viable solution.

Interest in biodegradable plastics has enjoyed healthy growth in the past two decades (figure 1), and by now, the market for biodegradable plastics has become a reality [1]. The global production capacity for biodegradable plastics in 2020 was 1.23 million tons according to European Bioplastics, the most prominent being polylactic acid (PLA), polybutylene adipate terephthalate (PBAT), and starch mixtures [9–17]. Although only 0.33% of the approximately 368 million tons of plastic produced annually is biodegradable, demand for such materials is rising, and thanks to the wide range of emerging materials with superior properties, the market has already experienced dynamic development [1]. In particular, European Bioplastics estimates that the market for biodegradable plastics will expand by 50% by 2025 (figure 1), most likely propelled by the increased use of polyhydroxyalkanoates (PHAs) [1].

Although many biodegradable polymers have the characteristics needed to replace conventional plastics in different applications, not all of them biodegrade in aquatic ecosystems quickly enough to
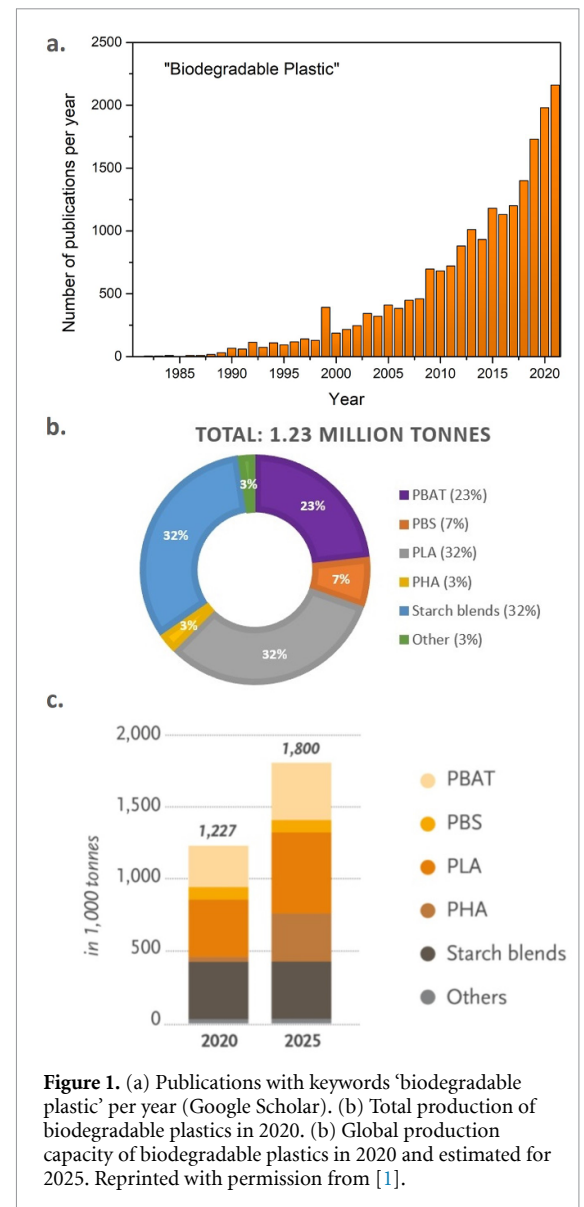

Figure 1. (a) Publications with keywords 'biodegradable plastic' per year (Google Scholar). (b) Total production of biodegradable plastics in 2020. (b) Global production capacity of biodegradable plastics in 2020 and estimated for 2025. Reprinted with permission from [1].

avoid accumulation [6]. In response, this review puts biodegradable plastics under the microscope to study how they might affect aquatic ecosystems if their implementation was widespread. To that purpose, we performed an extensive search in 'Google Scholar' and 'ScienceDirect' in English for the terms 'biodegradable plastic' and 'biodegradable microplastic' combined with 'sea,' 'ocean,' 'river,' or 'aquatic.' Results obtained regarding the biodegradation of biodegradable plastics in aquatic ecosystems, as well as their potentially dangerous effects for the living organisms in those habitats, were summarised and organised. Overall, our objective was threefold: to understand whether biodegradable plastics available today can significantly mitigate plastic pollution in aquatic ecosystems, to identify which aspects of the topic should be studied in greater depth or with a different approach, and to provide a series of final recommendations in this regard.

2. Biodegradable plastics: definition and classification

Green plastics, also known as *bioplastics*, are defined as biodegradable and/or bio-based polymers [18]. On the one hand, *bio-based polymers* are defined by the International Union of Pure and Applied Chemistry as macromolecular substances either obtained directly from plants, animals, or microorganisms or synthesised from them. On the other, *biodegradable polymers* are defined as macromolecular substances susceptible to degradation due to biological activity and, in the process, decreased molar mass. Polymers can be mineralised (i.e. converted into inorganic compounds) through the action of microorganisms, the final products of which are carbon and water. If that process occurs under anaerobic conditions (i.e. in the absence of oxygen), as in the case of the sediment of seas, rivers, lakes, ponds, marshes, and brackish waters [19], methane (CH_4) is emitted as well. Along with the presence of oxygen, other environmental parameters such as the availability of nutrients, temperature, humidity, microorganisms, and pH influence the biodegradability of plastics [20].

In a given environment, the biodegradability of a polymer depends on its molecular structure and resulting properties. For example, its crystallinity, molecular weight, stereochemistry, and hydrophilic or -phobic character, as well as the susceptibility of its bonds to enzymatic breaking, the flexibility of its chains, and the size of its amorphous regions, all strongly influence its potential to be degraded by microorganisms [21–24].

Commercial biodegradable plastics can be classified into three categories according to their raw materials and methods of synthesis. For one, biodegradable plastics can be obtained directly from renewable materials, as in the case of PHAs such as poly-3-hydroxybutyrate, poly-3-hydroxyvalerate, and their copolymers poly-3-hydroxybutyrate-co-3-hydroxyvalerate and poly-3-hydroxybutyrate-co-4-hydroxybutyrate, which are produced by a microbial fermentation process. Another possibility for obtaining biodegradable plastics is by polymerising renewable monomer precursors in industrial processes, as in the case of PLA, bio-based polybutylene succinate (bio-PBS), and polybutylene succinate-co-butylene adipate (PBSA). Last, biodegradable plastics can alternatively be synthesised from petrochemical resources; examples include PBAT, PBS, PBSA, polycaprolactone (PCL), and polyvinyl alcohol (PVA).

An important term in the context of biodegradability is *oxo-degradability* [18]. Materials that claim to be oxo-degradable, oxo-biodegradable, or oxo-fragmentable are composed of conventional plastics supplemented with specific additives to mimic biodegradation. However, those additives enable only fragmentation, not complete degradation, which generates tiny fragments of plastic that remain in the environment. In 2017, more than 150 organisations worldwide endorsed a proposal banning oxo-degradable plastic packaging worldwide [25].
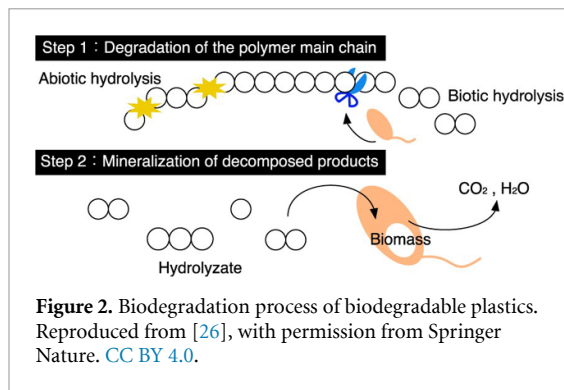
3. Potential problems in aquatic ecosystems caused by biodegradable plastics

3.1. Biodegradation profile

The degradation of biodegradable plastics occurs in two stages: chain cleavage and mineralisation (figure 2). In the first stage, chain cleavage occurs via hydrolysis, by way of either microbial hydrolytic enzymes (i.e. biotic hydrolysis) or non-biological hydrolysis (i.e. abiotic hydrolysis). Most biodegradable polymers contain hydrolysable bonds (e.g. ester, anhydride, amide, and/or carbonate bonds) in their leading chains that can be cleaved. The labile bond, the accessibility of bonds to water or enzymes, and other factors (e.g. glass transition temperature, hydrophilicity, morphology, crosslinking, pH, and presence of proteins) influence the rate of hydrolysis, a process in which molecules form in a size that microorganisms can assimilate. The second stage entails the mineralisation of those molecules during catabolism—that is, their conversion into carbon dioxide, nitrogen oxide, methane, and water [26].

The ways in which those processes unfold for many biodegradable polymers in aquatic environments remains poorly understood. Furthermore, the large number of polymer blends, copolymers, and polymer composites proposed to achieve functionality only increases the uncertainty of the environmental impact of those materials. In fact, the diversity of biodegradable materials renders making generic, straightforward assessments of their end-of-life behaviour impossible. Although several studies have recently contributed to great advances in predicting the degradation potential and time of polymers in natural environments such as aquatic ecosystems [27–30], such forecasting is a highly complex task owing to the array of conditions—for example, temperature, pressure, pH, salinity, and presence of oxygen and microorganisms—that can differ widely between locations and even be influenced by season.

Great efforts have been made to develop standards that regulate and enable certification for the designation 'biodegradable polymer' in different aquatic ecosystems. As a case in point, the conformity mark 'Vinçotte OK Biodegradable WATER' certifies biodegradation in a natural, freshwater environment (e.g. rivers, lakes, streams, and wetlands) and, as such, makes a substantial contribution to reducing waste in rivers, lakes, and other freshwater bodies. The mark is based on the international standard ISO14 851 [31] to verify that materials achieve at least 90%

Figure 2. Biodegradation process of biodegradable plastics. Reproduced from [26], with permission from Springer Nature. CC BY 4.0.
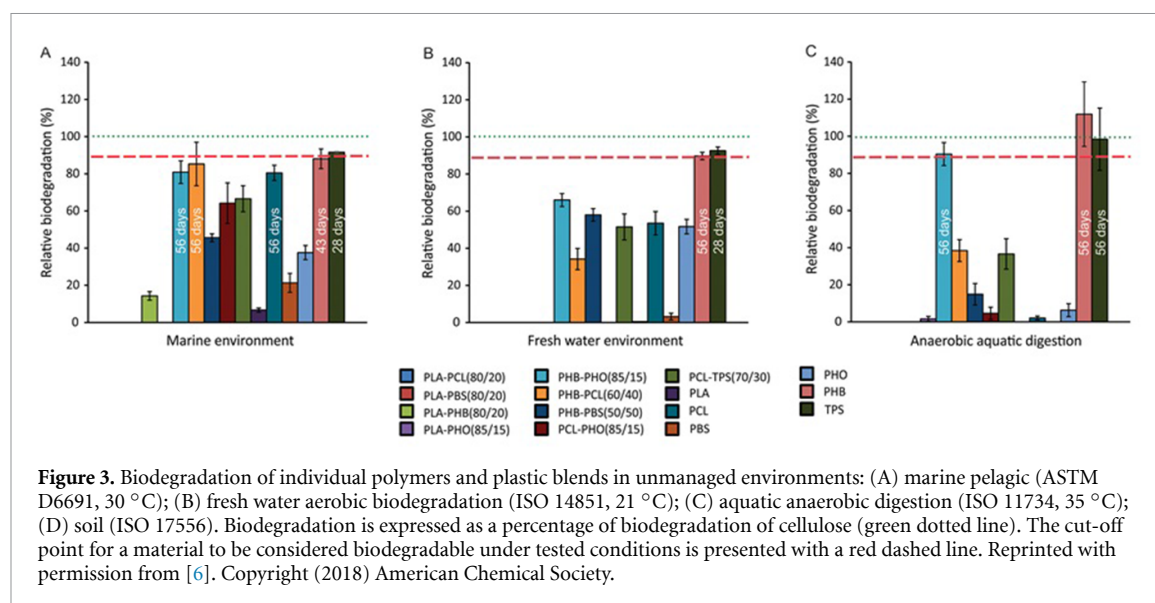
biodegradability in less than 56 d using a mixture of water, inorganic nutrients, and activated sludge. Although the standard tries to solve the problem of determining biodegradability in natural freshwater environments, it uses biomass produced to treat wastewater (i.e. activated sludge) as an inoculation medium instead of using microorganisms present in those environments. For that reason, the standard should be applied to wastewater, as in the case of the specification EN14987 'Plastics—Evaluation of disposability in wastewater treatment plants— Test scheme for final acceptance and specifications' [32]. At the same time, other methods and standards have been developed to assess the biodegradability of plastics in the presence of aerobic wastewater and sewage sludge, as detailed in the literature [33], but those conditions do not represent freshwater habitats. All of those standards and testing methods, in involving the measurement of CO_2 and CH_4 evolution, aim to assess ultimate biodegradability by facilitating exposure to an artificially inoculated medium in a specific temperature range using conditions of static exposure.

As for marine environments, three international standards can be explored. For one, two active international standards, ISO 18 830 [34] and ISO 19 679 [35], refer to aerobic plastic biodegradation at the interface of seawater and sandy marine sediment. They use measurements of the demand for oxygen or CO_2 evolution to test the biodegradation of plastics under laboratory conditions. For another, two active regional testing methods have been developed to assess the biodegradability of polymers in aerobic seawater: ASTM D6691 [36] and D7473 [37]. Whereas ASTM D6691 employs measurements of CO_2 evolution, ASTM D7473 refers to visual evidence and the loss of polymer mass (%) to assess biodegradation and is thus insufficient for establishing biodegradability on its own. By extension, the conformity mark 'Vinçotte OK Biodegradable MARINE' based on ASTM D6691 requires a product 'biodegradable in seawater' to achieve at least 90% biodegradation in no more than 6 months. Last, another testing method published for measuring the aerobic biodegradability

of plastics buried in sandy tidal sediment employing measurements of CO_2 evolution (ASTM D7991 [38]) uses field-collected sediment and seawater. Other test methods, including ASTM D6692-01 [39] and ASTM D7081-05 [40], developed for the marine environment but withdrawn have not been replaced. Because marine biodegradation standards are neither representative nor accessible, researchers have developed economic degradation evaluations based on mass loss, mechanical property loss, and surface erosion evaluation [41–43]. Researchers usually submerge polymer samples in seawater and perform gravimetric and thermal analysis, tensile testing, spectroscopy, and electron microscopy to generate quantitative information about polymer degradation but do not quantify the extent of bioassimilation or biodegradation. The inaccuracy of designating a material as 'marine biodegradable' was recently revealed by Lott *et al* [44], who showed that the biodegradation performance of several biodegradable plastics differed by orders of magnitude depending on climate and habitat. In particular, they performed biodegradation lab tests (20 °C), mesocosm tests (20 °C) with natural sand and seawater, and field tests in the warm–temperate Mediterranean Sea (12 °C–30 °C) and in tropical Southeast Asia (29 °C) under three typical sets of coastal circumstances. Polymers chosen for the test were PHA, polybutylene sebacate, polybutylene sebacate co-terephthalate, and low-density polyethylene (LDPE) as the negative control. As expected, no biodegradation was observed for LDPE in any of the proposed tests; however, all other polymers showed signs of degradation. Even so, the half-life time between them differed significantly, thereby showing that biodegradation is a highly complex process that differs from one marine ecosystem to another.

Using standards of biodegradation in marine (ASTM D6691), freshwater (ISO 14 851), and aquatic anaerobic (ISO 14 853) environments, Narancic *et al* [6] tested the neat polymers PLA, polyhydroxybutyrate (PHB), polyhydroxyoctanoate (PHO), PBS, thermoplastic starch (TPS) and PCL, and some of their blends, of which only PHB and TPS were capable of entirely biodegrading in all aquatic environments (figure 3). The authors also found exciting results when evaluating specific blends. For example, although less than 40% of PHO degraded in the marine environment in the testing time frame of 56 d, the PHB − PHO (85:15) blend showed the same level of biodegradation as PHB under the same conditions. Another interesting case was the blend PHB–PCL (60:40). Although PCL's good biodegradation profile in a marine environment (approx. 80%) was nearly modified after blending with PHB, the situation differed in freshwater and aquatic anaerobic digestion, in which blending PCL with PHB had an antagonistic effect, as shown in figure 3. Another antagonistic biodegradation blend in marine and

Figure 3. Biodegradation of individual polymers and plastic blends in unmanaged environments: (A) marine pelagic (ASTM D6691, 30 °C); (B) fresh water aerobic biodegradation (ISO 14851, 21 °C); (C) aquatic anaerobic digestion (ISO 11734, 35 °C); (D) soil (ISO 17556). Biodegradation is expressed as a percentage of biodegradation of cellulose (green dotted line). The cut-off point for a material to be considered biodegradable under tested conditions is presented with a red dashed line. Reprinted with permission from [6]. Copyright (2018) American Chemical Society.

freshwater environments was PCL–TPS (70:30), as figure 3 also shows.

Along with blending polymers in order to improve their biodegradability, other authors have suggested adding nano- or microfillers to transform polymers or their blends into composites. Amongst them, Shaiju *et al* [45] studied the biodegradation behaviour of PBS–stearate modified with different percentages of magnesium–aluminium-layered double hydroxide (St–Mg–Al-LDH) under marine conditions and observed a significant increase in weight loss for PBS-St–Mg–Al-LDH composites compared with pure PBS. That outcome suggests that incorporating St–Mg–Al-LDH accelerates the degradation of PBS under marine conditions. In another study, Niu *et al* [46] achieved enhanced marine biodegradation for their PLA–cellulose nanofibres composites, and their superior composite degraded approximately 70% after 7 weeks under simulated marine conditions compared with only 15% for pure PLA. Those authors thus concluded that incorporating hydrophilic fillers facilitates the penetration of seawater and promotes the proliferation and growth of microorganisms, which consequently facilitates the polymer's fragmentation into smaller pieces and its bioassimilation.
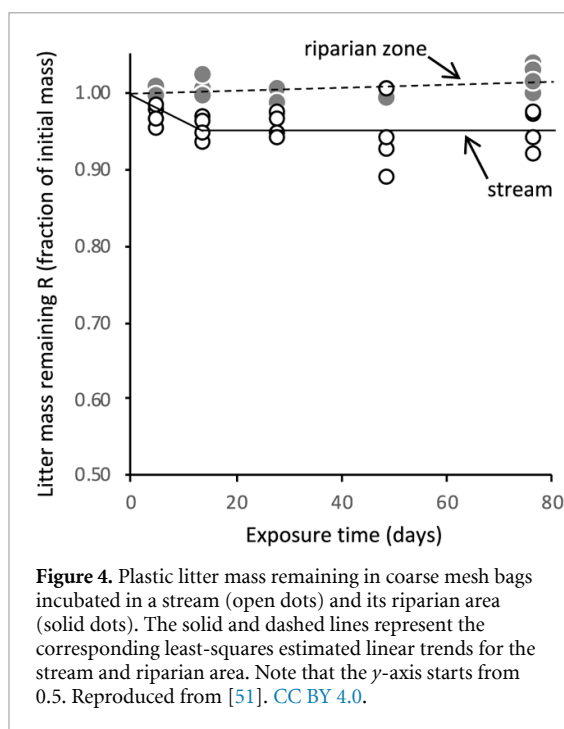
Aside from PHB, other PHAs have been tested and confirmed to be biodegradable in marine environments [47, 48]. Beyond the biodegradability of polymers, Dilkes-Hoffman *et al* [47] studied how the size of PHA sample affects its rate of degradation. Amongst their results, whereas PHA films ⩽0.2 mm thick biodegraded within several months in marine environments, cutlery 1.2 mm thick would take between 2.3 and 5.3 years to completely biodegrade. Thin films thus have an advantage over bulk plastics in their diffusion lengths, which facilitate the penetration of water and microorganisms. For that reason,

standards generally establish maximum thicknesses to be certified biodegradable in a given environment.

PVA is a well-known water-soluble and biodegradable polymer. However, during biodegradation tests in aqueous media, it reached significant biodegradation levels only in the presence of acclimated PVA-degrading microorganisms [49]. In municipal water treatment facilities, PVA can be consumed by the myriad of microorganisms present. Although it is unlikely to be mineralised in isolated environments such as the ocean, researchers have reported the isolation of a PVA-degrading marine bacterium from plastic rope litter found in Tokyo Bay, Japan [50].

Amongst the so-called 'biodegradable' plastics that show poor rates of biodegradation in aquatic ecosystems, PLA stands out as a major bioplastic on the market used primarily as packaging material. According to Narancic *et al*'s [6] results, both PLA alone and other PLA-based plastics can biodegrade in all of the aqueous environments tested (i.e. marine, freshwater, and anaerobic aquatic environments). The best performance was achieved by PLA−PHB (80:20), which was less than 15% biodegradable in the marine environment and thus even less than the PHB content.

Potential sources of PLA in aquatic ecosystems include biodegradable plastic carrier bags usually supplied for free to consumers in supermarkets. Artru and Lecerf [51] studied the degradation rate of such bags certified as biodegradable in the home compost heap (NF T 51–800 standards: AFNOR, 2015; Vinçotte/TÜV AUSTRIA OK compost HOME conformity mark) made of at least 30% of PLA, in both in a stream and a nearby riparian area for an aquatic–terrestrial comparison. Although the material achieved one of the highest biodegradability standards (i.e. 90% mass loss achieved within a year in a home compost heap; Vinçotte/TÜVAUSTRIA, 2013), samples lost at most only 5% of their initial mass after

Figure 4. Plastic litter mass remaining in coarse mesh bags incubated in a stream (open dots) and its riparian area (solid dots). The solid and dashed lines represent the corresponding least-squares estimated linear trends for the stream and riparian area. Note that the *y*-axis starts from 0.5. Reproduced from [51]. CC BY 4.0.

77 d submerged in the stream (figure 4), whereas natural litter, tested for comparison, had wholly disappeared within a year's time.

Microbial communities capable of decomposing leaf litter cannot necessarily decompose plastic. Although the polymer's chemical structure is partly responsible, surface roughness also plays a critical role, for the plastic film's smooth, homogeneous surface may prevent the effective trapping of microorganisms [52]. At the same time, a plastic film's toughness and thinness may prevent the deep penetration of fungal hyphae within the plastic matrix, thereby restricting fungal colonisation to the surface layer.

However, plastic bags are not the only biodegradable plastic-based products on the market that can end up in aquatic ecosystems. Nazareth *et al* [53] experimentally evaluated alterations in the structure and chemical composition of certain biodegradable plastic products marketed in Canada, the United States, and Brazil. Using seawater immersion, they performed 180-d ageing experiments on six samples (i.e. different plastic cups and bags), four of which showed no evidence of degradation. Along with demonstrating the null biodegradability of PLA in seawater, the authors attested to greenwashing practices used by several manufacturers who reported that their products were biodegradable or 100% degradable but according to Fourier transform infrared (FTIR) spectra all had polyethylene (PE) in their composition.

Although PLA is not biodegradable in marine environments, Beltrán-Sanahuja *et al* [54] recently showed that the degradation of a PLA–wood pulp bilayer film under simulated marine conditions was far more pronounced than conventional materials, namely a multilayer material polyamide–polypropylene (PP)-ethylene-vinyl-alcohol and a blend of polyethylene terephthalate (PET) and PE. The authors determined that outcome by combining weight loss (%), spectroscopic, and thermal analyses but did not evaluate whether those changes were dominated by degradation or biodegradation. Given that PLA-degrading microorganisms are uncommon in nature, bioassimilation did not likely occur. It is possible that the degradation was merely fragmentation, thereby indicating microplastics (MPs) with negative consequences for ecosystems.

3.2. Generation of MPs

3.2.1. MPs generated from biodegradable plastics

Because so-called 'biodegradable' polymers need certain environmental conditions in order to biodegrade in the times required by international standards, if those conditions are unavailable, then the material will not mineralise and will thus remain in the ecosystem. Under those circumstances, biodegradable plastic debris disintegrates under the action of ultraviolet radiation, water disturbances, wave impact, physical wear, and the freeze–thaw cycle, as well as the general activities of the biological habitat [55]. Such fragmentation causes the formation of smaller pieces in all shapes and sizes; particles less than 5 mm in length are called MPs [56], whereas ones less than 1 μm are nanoplastics (NPs). Because their generation can precede ultimate biodegradation, it is critical to evaluate the period in which they remain in the environment and the damage that they can cause during that time.

Because the use of biodegradable plastics remains minimal compared with traditional plastics, their use may pose potential unknown risks. Meanwhile, evidence of bio-MPs in aquatic ecosystems is slight. In fact, the only biodegradable polymers detected as MPs to date have been PVA in aquatic ecosystems [57, 58] and storm water retention ponds [59]; PLA in wastewater treatment facilities [60], beach sediment [61] and seawater [62]; and PCL in landfill leachate and leachate treatment works [63]. In all cases, the detected amounts were minimal compared with the amounts of conventional plastics.

Various laboratory tests have shown that MPs can be generated from many biodegradable plastics on the market. The bio-MPs first studied were those generated from PLA, namely in comparison with several essential non-biodegradable plastics also on the market. Lambert and Wagner [64] investigated the formation and size distribution of microscopic plastic particles during the degradation of biodegradable PLA and other conventional plastics (e.g. PE, PET, PS, and PP) using a weathering chamber. All plastics exhibited the release of particles into the surrounding solution, and PS and PLA generated the highest particle concentrations. However, whereas the authors cut 1-cm^2 samples of consumer plastics in

all cases, the initial thicknesses of the samples differed, which could have influenced the degradation and fragmentation profile of the materials [65, 66].

More recently, other biodegradable plastics have been shown to give rise to MPs and NPs. Wei *et al* [67] observed that in artificial seawater, artificial seawater with bottom sediment, and natural seawater with sediment, far more plastic fragments were released from PBAT than from LDPE. Moreover, they demonstrated that the rate of PBAT's formation of MPs increased significantly when a UV-A pre-treatment simulating exposure to sunlight was performed, which did not happen in the case of LDPE. However, because studies of the residence time of bio-MPs in the ecosystem and their toxicity were not performed, it seems that the greater, faster generation of bio-MPs implies greater damage than the slow production of conventional MPs.

Shruti and Kutralam-Muniasamy [68] performed a rapid degradation experiment on PHB films in two types of water—tap water [pH = 8.3] and drinking water [pH = 6.8]), both from Mexico—in laboratory conditions in order to examine the formation of bio-MPs. Microscopic observations and the rapid screening of water samples for MPs using Nile red confirmed the fragmentation of the films in both types of water. Added to that, González-Pleiter *et al* [69] demonstrated that secondary PHB bio-NPs could be produced from PHB bio-MPs by abiotic degradation under environmentally representative conditions (i.e. 28 °C with constant shaking and irradiation simulating solar irradiation in the visible range).

A major challenge in studying MPs is correctly detecting, identifying, and characterising them. Characterising the chemical profile of MPs includes determining their chemical composition, crystallinity and crystal structure, and physical parameters (e.g. density, specific surface area, and wettability). To that end, scanning electron microscopy (SEM) can be used to evaluate the particle surface, whereas dynamic imaging allows determining distributions according to particle size and shape [70, 71].

However, analysing the chemical composition and morphology of MPs and polymers represents a particular challenge for answering questions about the sources and fate of MP samples. In response, Raman microscopy has been used to identify the type, size, and polymer type of MPs. In facilitating the molecular identification and morphological description of MP particles, Raman microscopy offers the advantages afforded by coupling a Raman microspectrometer and image-processing software within the same analysis to obtain physical (e.g. morphological) and chemical (e.g. molecular composition) information from particles. As a result, several hundred particles may be analysed while minimising the intervention of the operator and time needed.
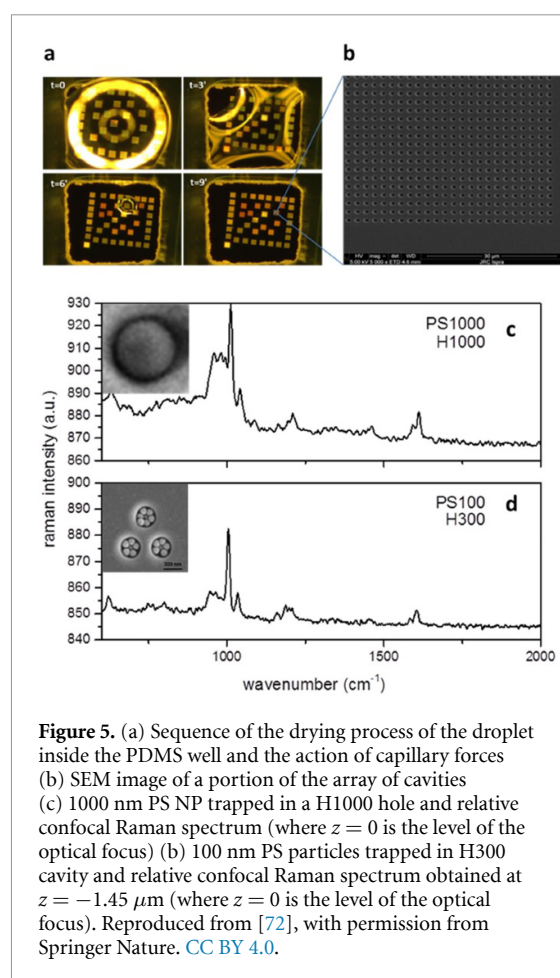

Figure 5. (a) Sequence of the drying process of the droplet inside the PDMS well and the action of capillary forces (b) SEM image of a portion of the array of cavities (c) 1000 nm PS NP trapped in a H1000 hole and relative confocal Raman spectrum (where $z = 0$ is the level of the optical focus) (b) 100 nm PS particles trapped in H300 cavity and relative confocal Raman spectrum obtained at $z = -1.45\ \mu$m (where $z = 0$ is the level of the optical focus). Reproduced from [72], with permission from Springer Nature. CC BY 4.0.

Even so, the characteristic Raman fingerprint of NPs can be masked by laser-induced fluorescence originating from complex biomatrices surrounding the NPs or due to the effects of ageing. Valsesia *et al* [72] recently proposed a novel approach to isolating nano-sized MPs from biological tissue and subsequently enable detection on a chip by way of μ-Raman analysis. After simplifying the biological matrix via enzymatic digestion, they identified the suspension of MPs on nano-engineered surfaces composed of cavity arrays designed to selectively entrap individual sub-micron nanoparticles. Figure 5 shows a sequence of the process of drying droplets inside a polydimethylsiloxane well and the action of capillary forces, together with an SEM image of a portion of the cavity arrays. Images of MPs trapped in holes and relative confocal Raman spectrum are also shown.

3.2.2. Toxic effects on living organisms

The effect of bio-MPs on aquatic ecosystems was not examined until 2015, when Green *et al* [73] demonstrated that, in some cases, bio-MPs can negatively affect the health and behaviour of living organisms in aquatic ecosystems in the same ways as conventional MPs. In using PLA bio-MPs, they set an important precedent for many subsequent studies of the

effects of those MPs. For that reason, other biodegradable polymers have rarely been studied, and only some cases of PHB and Mater-Bi are reported in the literature. In general, authors have compared the effects of bio-MPs, usually generated from PLA, and of the most commonly used conventional plastics on aquatic organisms, both in marine habitats and freshwater ecosystems.

3.2.2.1. Freshwater ecosystems
In the literature on the effects of bio-MPs on living organisms in sediment and the water column, although some studies involved taking samples from natural habitats or experiments directly in natural mesocosms, including in the United States, Italy, and Brazil [74–78], most studies have been conducted in artificial habitats.

In two studies on how bio-MPs affect sediment in freshwater ecosystems, both sets of researchers chose PLA as the biodegradable polymer but studied different aspects of the material. On the one hand, Seeley *et al* [74] demonstrated that bacterial diversity in the sediment of the York River estuary (Gloucester Point, VA, USA) was not affected by the presence of PLA bio-MPs, unlike what happened with conventional MPs, namely PE and polyvinyl chloride (PVC). However, important changes in the nitrification and denitrification processes emerged when PLA bio-MPs were present, possibly facilitated by the polymer organic matter itself. Even so, PLA's biodegradation in water ecosystems in only 16 d is quite unlikely. On the other hand, Klein *et al* [79] studied the effects of PLA bio-MPs on a freshwater oligochaete but in artificial river sediment. They found that PLA bio-MPs mixed into the sediment significantly reduced the survival of *Lumbriculus variegatus*. Nevertheless, they concluded that the chief driver of toxicity were chemicals associated with the plastic product and its previous content, not the polymer itself.

Most studies investigating the effect of bio-MPs on freshwater organisms living in the water column have focused on ones found at the base of the trophic chain. Such work is essential because changes affecting the primary producers and primary consumers may have detrimental effects on the entire ecosystem. For instance, Yokota and Mehlrose [75] examined how PLA bio-MPs from a body wash scrub affected natural phytoplankton communities in a 7-d incubation experiment set in a temperate mesotrophic lake. They found that PLA bio-MPs altered the taxonomic composition of the phytoplankton in the mesocosms, particularly by eliminating cryptophytes and increasing chrysophytes. The authors attributed that outcome to the fact that though cryptophytes lack physical protection for their plasma membranes against the degradation products of bio-MPs, chrysophytes have protective siliceous loricae or plates that may protect them from bio-MPs. González-Pleiter *et al*

[69] also investigated primary producers in freshwater habitats—that is, the cyanobacterium *Anabaena* and the green alga *C. reinhardtii*—and a primary consumer, the crustacean *D. magna*, using secondary NPs (i.e. PHB bio-NPs) produced from PHB bio-MPs by abiotic degradation under environmentally representative conditions. Amongst their observations, secondary PHB bio-NPs induced a significant decrease in cellular growth and altered relevant physiological parameters in all organisms. The growth of *Anabaena* and *C. reinhardtii* decreased by 90% and 95%, respectively, and *D. magna* became significantly immobilised (85%) after 48 h of exposure to PHB bio-NPs. The authors also investigated whether the observed toxicity was exerted by the PHB bio-NPs or by other abiotic degradation products released during the production of the bio-NPs from the bio-MPs. The supernatant obtained after PHB bio-NPs was removed by ultrafiltration and not toxic; thus, the authors concluded that the toxicity derived from the PHB nanoparticles. Along those lines, it should be studied whether the shape of nanoparticles after biodegradation, the polymer itself, and/or the chemicals associated with PHB processing (e.g. plasticisers and additives) are the cause of said toxicity.

Primary consumer *D. magna* was also tested using PLA bio-MPs by Zimmermann *et al* [80], who found that the bio-MPs increased mortality to 60% at 500 mg l^{-1} (i.e. mortality in controls = 5%) and reduced the reproductive output and the mean body length of adult *D. magna*. The authors also found that the effect was induced by the particles, not by possible migrating chemicals (e.g. plasticisers and additives). In other work, Magni *et al* [76] studied another primary consumer, the mussel *Dreissena polymorpha*, in search of sublethal effects caused by Mater-Bi® bio-MPs. They did not observe any significant effects, except for the modulation of the activity of glutathione *S*-transferases after 6 d of exposure. They thus hypothesised that the enzyme's modulation was associated with the release of additives from plastics in the exposure tanks; however, they did not perform any study to test that hypothesis. At the same time, although they reported the absence of bio-MPs in the gut lumen and tissues of exposed mussels, their presence in pseudo-faeces suggests that an efficient elimination mechanism was possibly adopted by the mussels to avoid gulping the micro-debris.

Danio rerio (zebrafish), considered to be a translational experimental model for freshwater fishes, has been chosen by different authors to study the effects of bio-MPs. Amongst them, de Oliveira *et al* [81] evaluated behavioural changes caused by PLA bio-MPs on zebrafish larvae at an early developmental stage. From a statistical study using 216 zebrafish larvae, they concluded that exposure to PLA bio-MPs for 5 d at concentrations of 3 and 9 mg l^{-1} decreased the swimming distance and speed of larvae in an open field test, thereby suggesting effects on animals' locomotive and

exploratory activities. Moreover, anxiety-like behaviour was suggested by the larvae's longer immobility and even permanence in the peripheral zone of the apparatus. PLA bio-MPs had accumulated in the zebrafish larvae and inhibited acetylcholinesterase's activity, thus reinforcing the neurotoxic action in groups exposed to the bio-MPs. Zhang *et al* [82] also studied the effects of PLA bio-MPs on zebrafish larvae but differentiated the toxicities of virgin and degraded MPs to deepen the impact of natural ageing on their toxicity. As a result, they found that the efflux and detoxification of degraded PLA bio-MPs mediated by ABC transporters and P450 enzymes were slower than those of pristine PLA bio-MPs, which increased bioaccumulation and inhibited the skeletal development of zebrafish. Moreover, the high toxicity of degraded PLA bio-MPs was evidenced by the oxidative stress and mitochondrial structural damage, as well as depolarisation, fission inhibition, and apoptosis. Once the fact that PLA bio-MPs cause behavioural changes and biochemical dysfunctions on *Danio rerio* larvae was demonstrated, Chagas *et al* [83] studied the consequences of exposing adult fish to the biopolymer. PLA bio-MPs at concentrations of 2.5 and 5.0 mg l^{-1} accumulated in the liver, brain, gills, and carcass of those fishes. However, unlike what de Oliveira *et al* [81] reported for larvae, no locomotive damage or anxiety-like behaviour was detected in the fish. At the same time, behavioural changes in shoals predictive of co-specific social interaction and an antipredatory defence response deficit were caused by the studied concentrations of PLA bio-MPs, which the authors attributed to cholinergic changes inferred by acetylcholinesterase's increased activity and redox imbalance. A similar redox imbalance was also reported in *Aphylla williamsoni* (dragonfly) larvae exposed to PLA bio-MPs [77]. In another study, Chagas *et al* [77] evaluated the biochemical toxicity of PLA bio-MPs on those insects' larvae as a model system to represent dragonfly species that lay eggs in freshwater environments. They found that although the animals' energy metabolism was not affected by the presence of PLA bio-MPs, larvae exposed to those plastics showed increased nitrite and lipid peroxidation levels that suggested an increase in processes of oxidative stress. Moreover, a redox imbalance caused by the decreased activity of superoxide dismutase and total thiol levels was reported as well. When studying *Danio rerio* adult specimens, Chagas *et al* [83] also demonstrated the anticholinergic activity of PLA bio-MPs on dragonfly larvae by way of decreased AChE activity, the consequences of which included changes in various neurophysiological functions.

Malafaia *et al* [78] were the first, and have so far been the only, researchers to evaluate the biological impact of PLA bio-MPs on an amphibian species (i.e. *Physalaemus cuvieri* tadpoles). The uptake of the biopolymers changed the tadpoles' growth and development features, reduced their lipid reserves,

and increased their production of reactive oxygen and nitric oxide species. On top of that, the authors reported the neurotoxic action of PLA bio-MPs for the two tested concentrations, which they argued was due to increased levels of acetylcholinesterase and butyrylcholinesterase.

All of the above results support the idea that some bio-MPs exert adverse health effects on living organisms in freshwater ecosystems. However, it is also clear that the effect will differ between biodegradable polymers and organisms. Whereas some authors have shown that the toxic effects are due exclusively to chemicals associated with the plastic product and not the polymer itself, others have shown that they are due to the particles alone. Beyond the toxic effects on the health of living organisms in the ecosystem, other important changes, including the invasion of species, can occur due to the presence of those bio-MPs. For example, Senga Green *et al* [84] recently observed a twofold increase in the abundance of New Zealand mud snails (*Potamopyrgus antipodarum*) in the presence of modified regenerated cellulose glitter.

Despite growth in research on the topic, several aspects merit greater attention, as detailed in section 4.

3.2.2.2. Marine ecosystems

Different filter-feeding organisms, including oysters, mussels, ascidians, and worms, that live in marine sediment have been exposed to bio-MPs to determine their toxicity in marine ecosystems. In all cases, the polymer chosen for such tests was PLA. However, studying the effect of other bio-MPs remains to be done—for example, Mater-Bi, which has been certified as biodegradable in the marine environment [85].

The effects of conventional MPs and PLA bio-MPs on marine benthic organisms were explored by Green et al [73] in a mesocosm experiment at increasing concentrations (i.e. 0.02%, 0.2%, and 2.0% of wet sediment weight) for a period of 31 d. They studied not only the health and biological activity of lugworms (Arenicola marina) and nitrogen cycling but also the primary productivity of the sediment that they inhabit. As a result, they found that both conventional and biodegradable MPs in sandy sediment affected the health and behaviour of lugworms and directly or indirectly reduced the primary productivity of their habitats. In fact, all studied concentrations of PLA bio-MPs decreased the feeding activity of lugworms, and high concentrations also reduced microalgae biomass on the sediment's surface. The authors suggested the latter effect could be due to the direct impacts of bio-MPs on the sediment and/or indirect effects due to differences in how the lugworms process the sand when contaminated by bio-MPs. Amongst their other results, the authors found that the pools of pore water ammonium in sediment with PLA bio-MPs were approximately half of

those with conventional MPs or otherwise not contaminated. Carbonyl groups present in PLA are easily hydrated and can therefore adsorb available nutrients, which lowers the amount dissolved in pore water. The authors suggested that the lower concentrations of NH_4 in pore water could have been due to the potential of PLA carbonyl and hydroxyl groups to adsorb cations.

Along with the introduction of PLA bio-MPs in the sediment of marine habitats, their effect when introduced into the sea water column was studied as well. Amongst marine animals, filter feeders are excellent candidates to be studied, especially ones located at the base of the marine trophic chain. Green [86] assessed the impacts of conventional MPs and PLA bio-MPs on European flat oysters' health and biological functioning (Ostrea edulis), as well as on the structure of associated macrofaunal assemblages, by performing an outdoor mesocosm experiment with intact sediment cores. The results showed that whereas effects on the oysters were minimal, high doses of PLA bio-MPs substantially reduced the richness of benthic species. In particular, the abundances of juvenile Littorina sp. (periwinkles) and Idotea balthica (i.e. an isopod) communities and the biomass of Scrobicularia plana (peppery furrow shell clam) were approximately 2.0, 8.0, and 1.5 times greater, respectively, in controls than in mesocosms with the high dose of either type of MP. Green et al [87] also introduced PLA bio-MPs into the water column of marine habitats to study the ecological impacts of PLA bio-MPs on the biodiversity and ecosystem functioning of bivalve-dominated habitats. They found that filtration by O. edulis significantly increased when they were exposed to bio-MPs and that the pore water ammonium and biomass of benthic cyanobacteria decreased, similar to what Green et al [73] found in sediment with lugworms. They additionally reported that the associated infaunal invertebrate assemblages differed, with significantly fewer polychaetes and more oligochaetes in treatments exposed to MPs, which highlights the potential of PLA bio-MPs to impact the functioning and structure of entire habitats. By contrast, filtration by M. edulis was significantly less when they were exposed to the higher concentration of bio-MPs, and no effects occurred on ecosystem functioning or the associated assemblages of invertebrate. That result demonstrates the importance of studying the effect of bio-MPs in different organisms, because, in the case described, two species of bivalves showed completely different behaviours when exposed to the same polluting conditions. The habitats of M. edulis were also studied by Khalid et al [88], who assessed the toxicological effects on the blue mussel of PLA bio-MPs in two concentrations (i.e. 10 and 100 μg l^{-1}) for 8 d. As in Green et al's [87] study, no significant levels of oxidative stress (i.e. activity of catalase, glutathione

S-transferase, and superoxide dismutase), neurotoxicity (i.e. of acetylcholinesterase), or immunotoxicity (i.e. lysosomal membrane stability and activity of acid phosphatase) were detected. In a study on more extended periods, Green et al [89] showed that the repeat exposure of M. edulis to PLA bio-M during a 52-d period in an outdoor mesocosm setting had no adverse effects on the attachment strength of the mussels. However, essential changes in the immunological profiles of their haemolymph were detected, while members of the C1qDC protein family were affected, the antimicrobial peptide myticin increased, and fibrinogen-related proteins were up- or downregulated.

Other filter-feeding organisms tested against bio-MPs have included solitary ascidians. Anderson and Shenkar [90] investigated the effects of MPs generated from single-use cups and plates made of PET and PLA on the biological and ecological features of Microcosmus exasperatus. Both the PET MPs and PLA bio-MPs reduced the organism's rate of fertilisation with no significant differences between materials. Rates of accumulation were also similar for both the biodegradable and conventional plastic particles.

The major published results on bio-MPs' effects in aquatic organisms are summarised in tables 1 (i.e. for freshwater ecosystems) and 2 (i.e. for marine ecosystems). All results demonstrate that bio-MPs may have effects on those organisms similar to those of conventional MPs. However, because the vast majority of such studies have tested PLA only, much remains to be examined.

Various biodegradable plastics on the market (e.g. Mater-Bi®) are certified as biodegradable in marine environments. Although those certifications have been made with reference to discontinued standards and although no information is available regarding their potential ecotoxity, laboratory tests simulating the environmental conditions of the seabed and seashore have shown that 80% of the material biodegrades in approximately 220 d [85]. Those findings suggest that bio-MPs generated from the polymer are not persistent in the ecosystem and therefore do not contribute to concerns about MPs, as underscored by the European Chemical Agency [91]. Evaluations of toxicity have to be performed in conjunction with biodegradability, as well as on a wide range of biodegradable polymers, to clarify the effects of such materials on aquatic ecosystems. Aspects of that topic that should be further investigated and recommended ways of performing such investigations are discussed in section 4.

3.2.3. Pollutant vectors

The negative effects of bio-MPs on aquatic biota can intensify when they coexist with other contaminants. Various chemicals in the environment, primarily heavy metals and organic pollutants, can interact

Table 1. Reported effects of bio-MPs on freshwater organisms.

Polymer	Living organism	Conditions	Effect	Reference
PLA	Sediment microbial community	0.5% by weight of sediment 7 and 16 d	• Bacterial diversity not affected. • Promoted nitrification and denitrification.	[74]
PLA	*Lumbriculus variegatus*	from 1 to 8.4% by weight of sediment 128 d	• Survival reduction. • PLA bio-MPs mixed into the sediment affected more than that layered on the sediment surface. • The main driver for toxicity was chemicals associated with the original plastic product and its previous content.	[79]
PLA	Phytoplankton	67 mg L^{-1} 7 d	• Eliminated cryptophytes and increased chrysophytes.	[75]
PHB	• *Anabaena* sp. • *Chlamydomonas reinhardtii* Dangeard • *Daphnia magna*	48 h with secondary PHB-nanoplastics, released from 50 mg ml^{-1} PHB bio-MPs	• Decreased the growth of both *Anabaena* sp. and *C. reinhardtii* by 90 and 95%, respectively. • Effective immobilization (85%) of *D. magna* was induced after 48 h of exposure.	[69]
PLA	*Daphnia magna*	10, 50, 100 and 500 mg l^{-1} 21 d	• Reduced the reproductive output. • Increased the mortality in a concentration-dependent manner to 60% at 500 mg l^{-1} (the mortality in the controls was 5%). • Reduced mean body length of adult *D. magna*.	[80]
Mater-bi	*Dreissena polymorpha*	1 mg l^{-1} in semi-static conditions 14 d	• Not significant sub-lethal effects, except for the glutathione-S-transferase activity modulation after 6 d of exposure.	[76]
PLA	*Danio rerio* (larvae)	3–9 mg l^{-1} 5 d	• Decreased swimming distance and speed of larvae in the open field test (effects on animals' locomotor and exploration activities). • Caused anxiety-like behavior. • Larvae's acetylcholinesterase activity was inhibited (accumulative potential and neurotoxic action).	[81]
PLA and degraded PLA	*Danio rerio* (larvae)	0.1, 1, 10 and 25 mg l^{-1} 7 d	• The efflux and detoxification of degraded PLA were slower than those of pristine PLA. • Higher bioaccumulation of degraded PLA and skeletal development inhibition of zebrafish. • Oxidative stress–triggered mitochondrial structural damage, depolarization, fission inhibition, and apoptosis.	[82]
PLA	*Danio rerio* (adult)	2.5–5 mg l^{-1} 30 d	• Accumulation in the liver, brain, gills, and carcass. • No locomotor damages or anxiety-like behavior. • Behavioral changes (in shoal) is predictive of co-specific social interaction and antipredatory defensive response deficit. • Cholinergic changes are inferred by increased acetylcholinesterase activity and REDOX imbalance.	[83]

(Continued.)

Table 1. (Continued.)

Polymer	Living organism	Conditions	Effect	Reference
PLA	*Aphylla williamsoni* (larvae)	6 mg l^{-1} 48 h	• Not differences in animals' energy metabolism. • Increased nitrite and lipid peroxidation levels due to an increase in oxidative stress processes. • REDOX imbalanced, caused by a decrease in superoxide dismutase activity and total thiol levels. • Anticholinergic activity (demonstrated by a decreased AChE activity).	[77]
PLA	*Physalaemus cuvieri* tadpoles	0.76 and 15.02 mg l^{-1} 14 d	• Changed growth and development features. • Reduced lipid reserves. • Increased reactive oxygen and nitric oxide species production. • Neurotoxic action (increased acetylcholinesterase and butyrylcholinesterase).	[78]

with bio-MPs, sorb onto them, and thus act as vectors of hazardous contaminants. Such organic pollutants generally include polycyclic aromatic hydrocarbons (PAHs), perfluoroalkyl substances (PFASs), pesticides, and pharmaceuticals [92], while such heavy metals, referring to metals and metalloids with high atomic weights, include Cu, Pb, Hg, Cd, and Cr [92].

Sorption can occur due to several simultaneous mechanisms, including hydrophobic interactions, hydrogen bonding, and/or electrostatic interactions between sorbent and sorbate [93]. In hydrophobic interactions, which generally occur between two nonpolar substances, hydrophobic contaminants are adsorbed to the surface of nonpolar MPs, as is the case with many conventional plastics but not most biodegradable polymers. However, bio-MPs usually have nanoscale pores through which contaminants can enter and remain trapped. That sorption mechanism is called *pore filling*. Depending on the polymer type in hydrogen bonding (i.e. depending on when proton donor and acceptor groups are present), van der Waals forces and π–π interactions can be involved in the sorption of chemical pollutants. For example, carboxylate oxygens in PBS and PLA may form hydrogen bonds with polar pollutants that are more substantial than the hydrophobic and π–π interactions between pollutants and non-degradable MPs [94]. A schematic representation of the six mentioned sorption mechanisms appears in figure 6.

The number of mechanisms that take place and the strength of sorption both depend on bio-MPs' physical and chemical properties, pollutants, and the medium where sorption occurs [95]. While MPs' characteristics can affect the sorption of chemical pollutants in multiple ways, their polarity, crystallinity, and functional groups are determined by the type of polymer. For example, it has been demonstrated that the proportion of rubbery domains, accessible to organic contaminants thanks to their large free volume, is a major physicochemical property affecting the sorption capacity of MPs [96–99]. As a consequence, both the crystallinity and the relationship between the temperature at which the test is conducted relative to the polymer's glass transition temperature are pivotal to the sorption capacity of the bio-MPs. By contrast, the properties of pollutants (e.g. sorbates) and the characteristics of sorption environments also influence the sorption behaviour of MPs [92]. Environmental conditions can affect the ability to adsorb by changing the surface properties of MPs. For example, the pH of the water can generate or modify the charges on the surface of the MPs and thus the electrostatic interaction with the sorbate. Dissolved organic matter can complex with sorbates in aquatic environments, thereby resulting in competitive or synergistic adsorption on the surface of the sorbents.

To explore the sorption behaviour of pollutants in relation to MPs, kinetic and isothermal experiments can be performed. The experimental data obtained from sorption kinetics experiments can be fitted by applying various kinetic models, including pseudo-first-order, pseudo-second-order, intraparticle diffusion, and Boyd models. By comparison, the adsorption isotherm model is a method typically used to describe adsorption behaviour and its mechanism. Two of the most-used models to fit isothermal data are the Langmuir and Freundlich models [100]. Table 3 summarises sorption properties of various chemical pollutants on bio-MPs. Exposed data were obtained from the best-fitting model.

As with the study of MPs' toxic effects on living organisms, there are far fewer reports in the literature for bio-MPs than for conventional MPs. Furthermore, when the effect of MPs is compared with that of bio-MPs, critical parameters such as
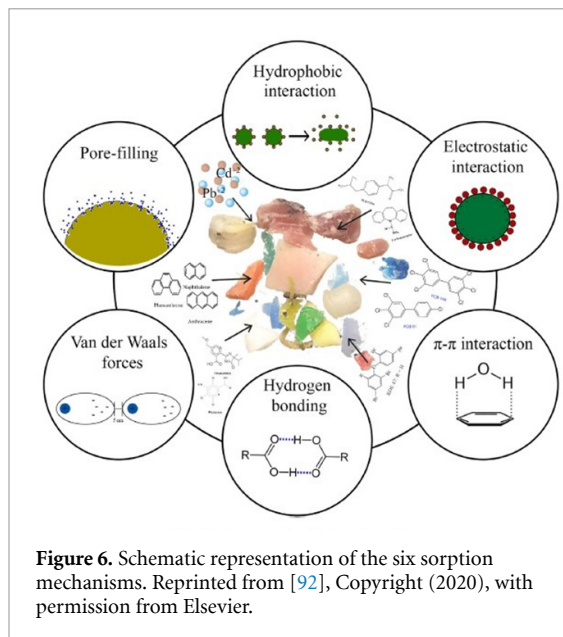
Table 2. Reported effects of bio-MPs on marine organisms.

Polymer	Living organism	Conditions	Effect	Reference
PLA	*Arenicola marina*	0.02, 0.2 and 2% of wet sediment weight 31 d	• Feeding activity decreased at allconcentrations. • Reduction of microalgae biomass on the sediment surface at high concentrations. • Lower concentrations of NH_4 in porewater.	[73]
PLA	*Ostrea edulis* and the structure of associated macrofaunal assemblages	0.8 and 80 mg l^{-1} in the water column repeatedly for 60 d	• Effects on the oysters were minimal. • Benthic assemblage structures differed. • Benthic species richness decreased. • The total number of organisms was $\sim$1.2 and 1.5 times greater in control mesocosms than those exposed to high doses of microplastics.	[86]
PLA	European flat oysters (*Ostrea edulis*) Blue mussels (*Mytilus edulis*)	0.0025 and 0.025 mg l^{-1} in the water column repeatedly for 60 d	• Filtration by *M. edulis* was significantly less when exposed to the higher concentration. • No effects on *M. edulis* ecosystem functioning or the associated invertebrate assemblages. • Filtration by *O. edulis* significantly increased when exposed to both studied concentrations. • Porewater ammonium and biomass of benthic cyanobacteria decreased in *O. edulis* ecosystem. • The associated infaunal invertebrate assemblages in *O. edulis* ecosystem differed, with significantly fewer polychaetes and more oligochaetes in treatments exposed to microplastics.	[87]
PLA	Blue mussels (*Mytilus edulis*)	0.01 and 0.1 mg l^{-1} for 8 d	• No effects on oxidative stress (catalase, glutathione-S-transferase, and superoxide dismutase activities). • No effects on neurotoxicity (acetylcholinesterase). • No effects on immunotoxicity (lysosomal membrane stability and acid phosphatase activity).	[88]
PLA	Blue mussels (*Mytilus edulis*)	0.025 mg l^{-1} in the water column repeatedly for 52 d	• No adverse effects on attachment strength. • Changes in the immunological profiles of mussels hemolymph (C1qDC genes affected, increase in the antimicrobial peptide myticin, fibrinogen-related proteins up- or down-regulated).	[89]
PLA	*Microcosmus exasperates*	1 or 10 daily particles for 28 d	• Reduced the fertilization rate. • Accumulation rates in adults comparable to PET MPs.	[90]

the viscoelastic state of the polymers (i.e. rubber or glassy) were not maintained at the temperature tested. Another notable trend is that PE has generally been used as an example of conventional MPs. Although a highly common polymer, PE cannot strongly interact with many contaminants due to its simple chemical structure. Other conventional polymers that are also widely used, including PET, PVC, and PS, afford far more possibilities for interacting with pollutants

thanks to the presence of carbon rings in their chemical structure. However, they are also far less used as models for comparison with bio-MPs. As for polymers that are biodegradable in water, although their MPs may absorb pollutants, their low residence time [6, 85] prevents them from acting as vectors.

A problem with past studies performed on the topic is that the tests have been performed at room temperature (24 °C–30 °C), which is not

Figure 6. Schematic representation of the six sorption mechanisms. Reprinted from [92], Copyright (2020), with permission from Elsevier.

the temperature of many natural aquatic environments. As a result, the representativeness of the results obtained is compromised, as discussed in greater depth in section 4.

Of all organic pollutants, PAHs rank amongst the most widespread contaminants present in all kinds of environments, including aquatic ecosystems [96]. Sixteen PAHs have been listed as priority pollutants by the U.S. Environmental Protection Agency due to their severe toxicity (i.e. carcinogenicity and teratogenicity) to humans [97]. Therefore, MPs' role in aquatic ecotoxicology as vectors of those toxic substances has become a key topic of study in the scientific community. In three studies evaluating the sorption of PAHs into bio-MPs and comparing it with that of different conventional MPs [98–100], rubbery domains emerged as a highly relevant factor of sorption capacity. Large amounts of free volume in rubbery domains and their flexibility make them highly accessible for hydrophobic organic contaminants such as PAHs. In all cases, sorption did not depend on the biodegradable character of the MPs but on their rubber fraction. Although bio-MPs can indeed act as vectors—in some cases, with even greater sorption capacities than some conventional MPs—if they can biodegrade in aquatic ecosystems, then their residence time before being mineralised will be short, and they will be unable to transport pollutants long distances, as discussed in section 4.

Another cause for global concern is the presence of antibiotics in aquatic ecosystems [101, 102]. It has been observed that for some antibiotics such as tetracycline (TC) and ciprofloxacin (CIP), PLA bio-MPs could have a higher sorption capacity than PVC MPs [103]. However, in such work, the tested MPs have had different sizes, and both the difference in particle size and the molecular weight used can significantly influence the sorption capacity of MPs. As a result,

they should not be modified to purely study the effect of the polymer used on the pollutant's sorption, as further examined in section 4. Another characteristic that can influence sorption capacity is the ageing of the bio-MPs. It has been reported that PLA-bio-MPs' physicochemical properties changed due to the effect of UV light exposure, which generally simulates the ageing process [103]. In particular, essential factors affecting the sorption rate and capacity of contaminants, including surface porosity, charge, and hydrophilicity, changed during the ageing processes. As a result, the adsorption capacity of TC and CIP on PLA bio-MPs increased by 1.18–2.19 times.

Beyond the effects of ageing caused by UV radiation, the effect of biodegradation on the sorption capacity of bio-MPs relative to pollutants has also been investigated. The presence of biofilm on PLA bio-MPs increased their adsorption capacity oxytetracycline (OTC) by 20.15%, primarily due to OTC's complexing with N–H functional group existing in biofilms [104]. After biofilms were extracted, the degraded PLA bio-MPs had an adsorption capacity approximately two times higher for OTC than for virgin PLA, which was attributed to the biofilm-related alteration of physiochemical properties (i.e. increased surface-to-volume ratio, more negative surface charges, and more oxygen-containing functional groups generated). The mechanisms of OTC's adsorption on PLA bio-MPs during the initial degradation process proposed by the authors are schematised in figure 7. Concerning the desorption of antibiotics, biofilm PLA bio-MPs have been shown to possess a greater possibility of pollutant desorption, which suggests that the problem of PLA bio-MPs worsens over time. After all, once the biofilm is formed, its potential to spread antibiotics increases.

The problem of biofilm formation is serious for another reason as well: it can potentially facilitate the spread of antibiotic resistance [105]. Sun *et al* [106] have shown that microorganisms in PHA bio-MPs biofilm can host antibiotic-resistant genes (ARGs) and that their profiles differed significantly from those of non-biodegradable PET MPs. Not only that but the abundance of multidrug resistance genes on PET MPs was statistically higher than on PHA bio-MPs. As their initial investigation confirmed, both conventional MPs and bio-MPs can host ARGs, and therefore their role in the spread of those pollutants should be extensively studied, as detailed in section 4. One of the most important pathways for MPs' entry and transport in aquatic environments is wastewater treatment plant (WWTP) effluents [107]. Because those environments are hotspots for bacterial pathogens and ARGs, characterising bacterial communities on MPs present in those effluents is essential. When Martínez-Campos *et al* [108] studied bacterial communities in the early stage of biofilm formation on PLA, PHB, and PCL bio-MPs deployed in two WWTP effluents, they found a significantly higher diversity of

Table 3. Sorption properties of various chemical pollutants on bio-MPs.

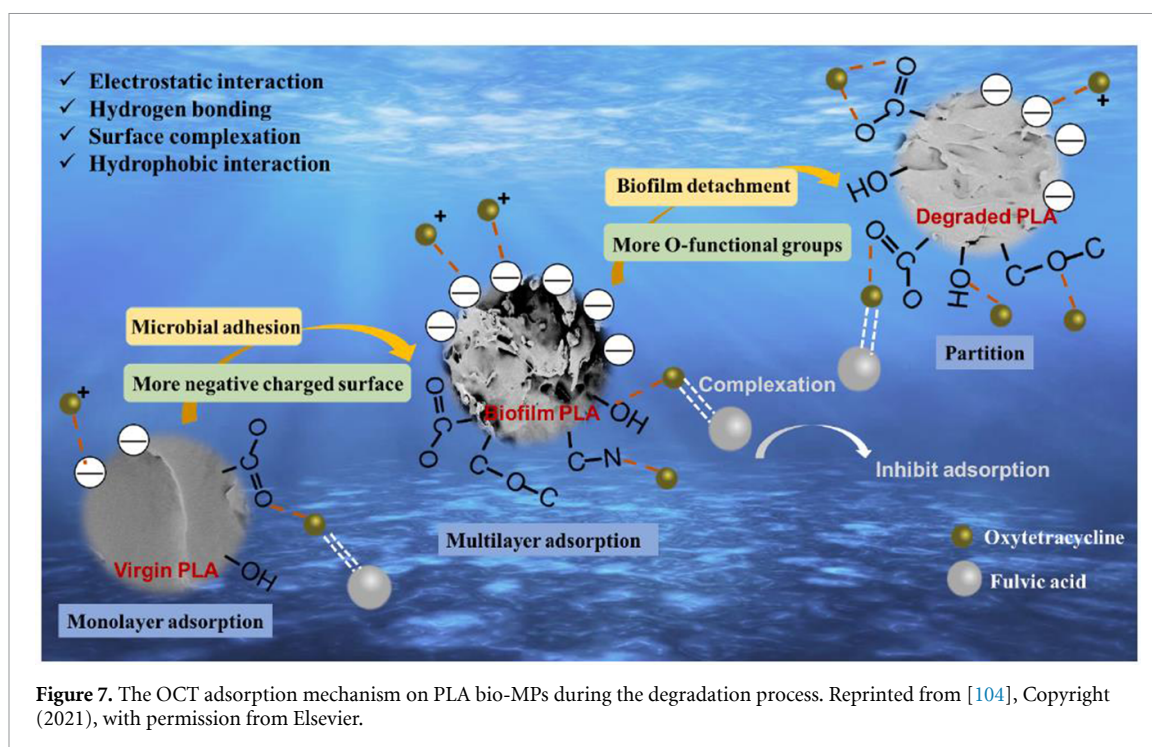| Compound		Polymer	Bio-MPs size	Experimental conditions	Sorption capacity (mg/g)	Sorption coefficient (L/kg)	Reference
PAHs	Phenanthrene	PBAT	50–200 μm	10 mg bio-MPs + 20 ml background electrolyte solution (30% sea salt) + PHEN solution in methanol $(1.0\text{–}3.5) \times 10^{-3}$ mmol l^{-1} pH = 8, 150 rpm at room temperature for 3 d	—	5.48×10^4	[98]
		PBS	150–200 μm	A certain amount of bio-MP + 40 ml 0.01 M CaCl$_2$ solution + sorbate solutions (100–1000 mg l^{-1} for phenanthrene, 20–200 mg/L for pyrene, 2–20 mg l^{-1} for 1-nitronaphthalene, 2–20 mg l^{-1} for 1-naphthylamine and 1–10 mg l^{-1} for atrazine) pH = 6.5, 180 rpm at 25 °C for 5 d	—	2.5×10^4	[99]
		PCL			—	3.18×10^4	
	Pyrene	PBS			—	12.1×10^4	
		PCL			—	14.2×10^4	
	1-nitronaphthalene	PBS			—	7.12×10^2	
		PCL			—	9.31×10^2	
	1-naphthylamine	PBS			—	5.07×10^2	
		PCL			—	5.18×10^2	
	Atrazine	PBS			—	68.8	
		PCL			—	58.1	
Pharmaceuticals	Tetracycline	PLA	250–550 μm	20 mg of bio-MPs + 50 ml of antibiotic solution (0.5–10 mg l^{-1}) 190 rpm at 24.8 °C for 48 h	2.51	7.92×10^4	[102]
	Ciprofloxacin	PLA			3.19	7.12×10^4	
	Oxytetracycline	PLA	75–150 μm	0.03 g of bio-MPs + 30 ml antibiotic solution (1–12 mg l^{-1}) pH = 5.5, 150 rpm at room temperature for 48 h	0.581	2.24×10^5	[104]
	Triclosan	PHB	1222 ± 104 μm	10 mg of bio-MPs + 10 ml of triclosan solution (5–25 mg l^{-1}) 150 rpm at 30 °C till equilibrium	9.442	—	[112]
		PLA	3 mm	A certain amount of bio-MP + triclosan solution (0.7 mg ml^{-1}) 140 rpm at 25 °C for 4 h	<10	—	[113]
Pesticides	Fipronil	PBS	75–150 μm	10 mg of bio-MPs + 10 ml solution 0.01 M CaCl$_2$ + fipronil (0–300 mg l^{-1}) Shaken at 25 °C for 5 d	0.72	3.26×10^3	[94]
		PLA			0.23	0.42×10^3	
	4-CP	PLA	3 mm	20 mg of bio-MPs + 30 ml synthetic matrix or freshwater + pesticide (0–100 μg l^{-1}) 150 rpm at 25 °C for 48 h	0.0327	2.52×10^4	[115]
	2,4-DCP	PLA			0.0455	1.52×10^4	
	2,4,6-TCP	PLA			0.0822	1.18×10^4	
	PCP	PLA			0.0627	9.40×10^4	
	Triadimefon	PBS	75–150 μm	10 mg of bio-MPs + 10 ml solution 0.01 M CaCl$_2$ + fungicide (30–300 μg l^{-1}) pH = 7.0, 200 rpm at 25 °C for 72 h	0.104	1.00×10^3	[118]
	Difenoconazole	PBS			0.193	28.8×10^3	

Figure 7. The OCT adsorption mechanism on PLA bio-MPs during the degradation process. Reprinted from [104], Copyright (2021), with permission from Elsevier.

bacteria on all bio-MPs' surfaces than free-living bacteria in the WWTP effluents. In particular, they detected a significant abundance of genera with potential pathogenic members (i.e. *Pseudomonas, Comamonas, Aeromonas*, and *Vibrio*), thereby suggesting that biodegradable MPs might be vectors of pathogenic bacteria in aquatic environments [109, 110].

Other pharmaceutical organic pollutants in aquatic ecosystems are antimicrobials. Of them, triclosan (TCS) is known to be a persistent endocrine disruptor that can bioaccumulate in different aquatic organisms [111]. Tong *et al* [112] have shown that the sorption of TCS in bio-MPs of PHB was far higher than in the MW of PE and that TCS could be easily desorbed from PHB and polyethylene under physiological conditions. Their results highlight the problems of PHB bio-MPs, even if the similarity between their laboratory conditions and reality is questionable. In other work Verdú *et al* [113] evaluated the potential of different conventional MPs and PLA bio-MPs to serve as vectors of TCS. As a result, they found that PLA bio-MPs sorbed less TCS due to its glassy state at the testing temperature.

PFASs are another harmful pollutant found in many aquatic ecosystems. Even so, Ateia *et al* [114] alone have examined their sorption in bio-MPs, in a study showing that the PLA bio-MPs had a higher sorption capacity than several conventional MPs (e.g. PE, PP, PET, PS, ABS, and PMMA). In lieu of any explanation from the authors, the presence of rubber domains can at least be ruled out, for while PLA was in the glassy state at the testing temperature, the other MPs tested (e.g. PE and PP) were in the rubbery state.

Thus, the cause of that outcome needs to be studied in greater depth.

Although not necessarily common pollutants in aquatic ecosystems, pesticides in agricultural soils and their sorption in both conventional and biodegradable MPs can be potential problems for aquatic ecosystems. As initial research on bio-MPs' action as pesticide vectors in aquatic ecosystems has shown, the sorption capacity of bio-MPs is higher or lower than that of several conventional plastics depending on the type of pesticide. For fipronil, PLA and PBS bio-MPs had higher sorption rates than many conventional MPs (e.g. PE, PP, PVC, and PS) [94], whereas for chlorinated phenols, PLA bio-MPs' sorption capacity was higher than that of PE MPs but less than that of PP MPs [115]. The influence of environmental factors on bio-MPs' absorption capacity when it comes to pesticides has also been examined, albeit the results were mixed. In one case, the presence of natural organic matter lowered PLA bio-MPs' sorption capacity [94], whereas in another the organic matter had no effect on the capacity of PBS bio-MPs to sorb pesticides [94]. Again, the great diversity of factors that influence the behaviour of bio-MPs highlights the need for more studies on the subject, as detailed in section 4.

As mentioned above, bio-MPs' can also adsorb heavy metals from the environment. To our knowledge, however, only two works have explored the absorption of heavy metals by bio-MPs in aquatic environments. Liao and Yang [116] conducted a chromium (Cr) adsorption–desorption study with four conventional MPs (i.e. PE, PP, PVC, and PS) as well

as PLA bio-MPs. Amongst their results, whereas the absorbed concentration of Cr in PLA bio-MPs was the lowest, Cr(VI) bioaccessibilities for PLA bio-MPs peaked in a human model. As the authors explained, PLA's biodegradability allows surface decomposition caused by digestive enzymes and gastric acid in the human gut and thus increases the release rate of Cr adhered to PLA bio-MPs.

By contrast, Richard *et al* [117] investigated how biofilm affects the accumulation of metals on PLA bio-MPs and LDPE MPs suspended in an urbanised estuary (San Francisco Bay, CA, USA). They found that biofilm could significantly predict all metals. In particular, LDPE MPs and PLA bio-MPs accumulated metals similarly for nearly all metals measured (i.e. Ba, Cs, Ga, Fe, Ni, Rb, Al, K, U, Cu, Pb, Co, Mn, and Mg). To be sure, however, bio-MPs' possible action as vectors of heavy metals should be explored in far greater depth, as discussed in section 4.

4. Research gaps

The global trend of replacing conventional plastics with biodegradable ones makes studying the potential contamination by bio-MPs vital. For such studies, it is crucial to recognise that international standards certifying a plastic as biodegradable require its mineralisation in a given time and in a specific environment. As detailed in the previous sections, the scientific community has recently begun to study how polymers classified as biodegradable in any habitat may affect aquatic ecosystems and compared their effects to the effects of conventional plastics. However, there remain great unknowns regarding those materials and essential points that have not yet been considered. In what follows, we present some critical concerns that need to be studied in greater depth to ascertain the risks that biodegradable plastics pose to aquatic ecosystems:

- **More biodegradable polymers need to be studied in depth.** Most studies regarding the toxicity of bio-MPs or the aquatic biodegradation of biodegradable plastics have examined PLA. Although the most widely used so-called 'biodegradable' plastic on the market, PLA is already known to only biodegrade at certain temperatures and in the presence of certain microorganisms. Meanwhile, biodegradable polymers in several environments, including TPS, PCL, PHA, and cellulose, have been poorly studied. In that context, expanding the number of biodegradable polymers evaluated in different aquatic ecosystems is vital to determining their real impact.

- **The standards used to regulate which materials are biodegradable in different aquatic ecosystems (i.e. freshwater and marine) are deficient.** Current international standards and norms as well as regional test methods cannot realistically predict

the biodegradability of so-called 'biodegradable' plastics in wastewater, inland waters (i.e. rivers, streams, and lakes), or marine environments. As shown in this review, the same plastic can take varying amounts of times to biodegrade depending on the characteristics of the ecosystem where they are found. In particular, certifying biodegradation in the marine environment is complicated because it depends not only on the depth at which the plastic is found but also on the specific characteristics of the sea and the damage that can be generated in the organisms of that ecosystem [44]. It is therefore necessary to improve standards based on realistic environmental conditions.

- **The formation of MPs and NPs from biodegradable plastics in natural conditions has been poorly studied.** Understanding how any biodegradable product disintegrates and degrades in a given environment is vital to understanding its environmental impact. The formation of MPs and NPs from biodegradable plastics in natural conditions has not been thoroughly evaluated. As mentioned, however, only four investigations have been conducted to demonstrate that bio-MPs are generated during the natural degradation of certain biodegradable plastics in water [64, 67–69]. In all other cases, the bio-MPs were generated mechanically from macroscopic samples, or else commercial pellets were used. More studies on the possibility of generating MPs from plastics that are biodegradable in any environment using natural conditions of aquatic ecosystems are therefore needed.

- **The residence time of bio-MPs in aquatic environments has not been studied.** Beyond studying the possibility of generating MP and NP particles from biodegradable plastic waste, it is essential to evaluate their permanence in aquatic ecosystems and the toxic effects that can be generated during that time. However, in comparing the effects of bio-MPs and conventional MPs, the factor of time has usually been ignored. As a result, although it is well-known that conventional MPs can last in aquatic ecosystems for hundreds of years without degrading [119], what happens with bio-MPs remains unclear. Bio-MPs do not contribute to concerns with MPs if they are not persistent in aquatic ecosystems, as stressed by the European Chemical Agency [91] in its proposal for a restriction on intentionally added MPs. However, in our opinion, the information needed understand whether those materials are hazardous to aquatic ecosystems remains too limited for any decision on restrictions to be made.

- **The effect of biofilm formation on the biodegradation of bio-MPs has not been explored.** As described in this review, the formation of biofilm on the surface of bio-MPs can be hazardous, either due to possibly harbouring highly contaminating pathogens or altering particles' surface properties

and thereby increasing their ability to absorb different pollutants. That capacity is formed under natural conditions in which the microorganisms responsible are present. Given their influence on the sorption and desorption capacity of pollutants, it is critical to study the growth rate of those biofilms as a function of the physicochemical characteristics of the surface of polymers. That is, the influence of biofilm formation on bio-MPs' biodegradation time in natural ecosystems needs to be evaluated in depth in order to illuminate their dangerous potential.

- **MP toxicity should be evaluated under environmentally realistic conditions.** Both in studying the toxicity of bio-MPs and their ability to act as a vector of pollutants, MPs used and the concentrations involved have to be consistent with their real-world counterparts in real-world ecosystems. The vast majority of studies have used concentrations of bio-MPs far exceeding those found in the environment. Beyond that, real bio-MPs are not spherical pure polymer pellets, unlike the ones used in many works in the literature. Employing natural MPs in research is critical for obtaining environmentally meaningful results; however, many researchers use commercial virgin MPs, which are smooth, micron-sized spheres starkly unlike what is obtained by the natural degradation of plastic waste. Furthermore, those commercial MPs could have been synthesised via emulsion-aided methods using exogenous surfactants, thereby altering the surface characteristics (e.g. hydrophilicity). Further still, most studies have been conducted with pure polymer MPs, even though real bio-MPs have other constituents such as plasticisers, stabilisers, pigments, fillers, extenders, and solvents. The effect of those additives on ecosystems has to be evaluated to understand whether the true threat is the polymer itself or some chemical associated with its processing.

- **When comparing the effect of different MPs, no more than one parameter should be varied**. In comparing, for example, the absorption capacity of bio-MPs with conventional MPs or their effect on the health of a given organism, particles of different morphology, roughness, or surface area should not be compared, because those factors may condition the result. For instance, it has been widely demonstrated that for biodegradable plastics, the rate of biodegradation increases with the available surface until a maximum rate is reached [47, 65, 66]. At the same time, sorption is a surface mechanism, meaning that different surface areas will have different sorption capacities [120]. However, recent studies have insisted on comparing the sorption or biodegradability properties of particles of different materials with different sizes and shapes [53, 103]. Meanwhile, other authors have drawn conclusions regarding the possibility of generating MPs and

bio-MPs from experiments comparing plastics of different origins and thicknesses [64]. Such discrepancies make the results impossible to analyse because it cannot be determined whether the effect studied is due to the polymer itself or a difference in the surface area-to-volume ratio.

- **The possibility that bio-MPs harbour antibiotic-resistant genes or act as vectors of heavy metals has rarely been studied.** Beyond the risk to aquatic ecosystems, pollutants harbouring antibiotic-resistant genes or acting as vectors of heavy metals pose a great risk to human health [121, 122]. Although the interaction between those contaminants and conventional MPs has been explored, very few results of that relationship with bio-MPs have been reported. Amongst them, the ARG profiles on bio-MPs differed significantly from those on conventional MPs [106] amid the presence of potential pathogenic members on the bio-MPs [108]. Added to that, bio-MPs showed similar or lower sorption capacities than conventional MPs when it came to heavy metals [116, 117]. However, the sole authors who explored bioaccessibilities found that Cr(VI) was more bioaccessible for PLA bio-MPs in a human model than for many conventional MPs [116]. Those results show the urgent need to continue deepening knowledge on that topic.

- **The study of how plastics, MPs, and NPs affect organisms needs to be systematised by defining model organisms, and the study of the scope of the trophic chain needs to be systematised.** Despite recent studies on the mentioned topics, further standardised testing protocols are required to guide experiments and improve the comparability of the results obtained [123]. Although a great variety of genera have been studied for conventional MPs, studies on bio-MPs have been limited and highlight the need to conduct studies delving into the entire trophic chain and its mobility dynamics. Some studies have shown varied effects concerning the size and the effect of accumulation in the trophic chain. Even so, it remains necessary to improve tests and analytical methods for determining bio-MPs and the effects on animals, water, soil, and plants.

- **Analytical techniques for identifying bio-MPs' effects on living organisms need to be systematised.** In the case of conventional MPs, exploratory information obtained by using microscopy and FTIR determinations of their presence in the digestive system of aquatic organisms (e.g. mussels, fish, and dolphins) is extensive. However, in the case of bio-MPs, such information is scarce. It is therefore necessary to systematise studies that associate the accumulation of MPs and bio-MPs with physiological effects, including decreased growth, decreased reproduction, altered intestinal flora, physiological dysfunctions, and metabolic

dysfunctions. For example, variables such as the dose of bio-MPs administered, the shape and size of the MPs, and the time of exposure of the organism to the MP need to be agreed upon to establish model assays. By contrast, despite examples of biomarkers for determining liver alterations in experimental animals (e.g. mice) using triglycerides, total cholesterol, pyruvate, liver mRNA, and intestinal flora, there are no such examples of aquatic animals. It is therefore necessary to define biomarkers for the different levels of the food chain in aquatic ecosystems to provide specific data on the effects. Other studies that should be systematised are ones on the trophic chain that use specific techniques at each level.

5. Conclusions and future perspectives

To be sure, the accumulation of plastics on aquatic ecosystems is one of the most severe environmental problems today. Far from decreasing, however, the global production of plastics and their consumption increase day after day and only exacerbate the situation. Although biodegradable plastics could be at least a partial solution to that problem, many considerations first need to be taken into account. The first is that not all so-called 'biodegradable' plastics are indeed biodegradable in aquatic ecosystems. Each biodegradable plastic has specific biodegradation conditions, and only a few biodegrade in aquatic media. Whereas starch and PHB biodegrade in aquatic ecosystems in the time required by international standards, many other plastics included in the biodegradable category do not.

It is necessary to establish transparent, genuinely representative degradation standards in each aquatic ecosystem, differentiated as marine or freshwater. Otherwise, one may assume that PHB is perfectly safe for aquatic ecosystems due to its biodegradable character. However, as a recent study showed, secondary NPs of PHB had toxic effects in aquatic organisms. Biodegradability, defined in terms of the time that a material takes to mineralise, does not guarantee the absence of toxicity, which explains why standards to certify biodegradability in a given environment include tests of ecotoxicity. Aquatic environments are extremely complex, and for that reason, even content appropriate today for the norms that regulate biodegradable plastics in different aquatic ecosystems is being reconsidered.

By contrast, though the rest of biodegradable polymers do not biodegrade in aquatic ecosystems, their implementation can nevertheless exert highly positive effects if waste management is done efficiently. For example, if all PLA is destined for industrial compost, then very little will reach oceans, and the same will happen with PBAT if used in agricultural applications, where it can biodegrade according to standards. However, because those bioplastics

are increasingly being implemented and have a high probability of ending up in aquatic environments, it is essential to perform studies on the generation of bio-MPs and their associated toxicity in order to disseminate the enormous importance of the proper final destination in the population.

Last, the negative effects that bio-MPs can produce in aquatic environments could become a problem for human health. Despite evidence of the combined effects and trophic transfer of MPs and associated chemical contaminants for conventional MPs, no such studies have addressed bio-MPs. However, as in the case of conventional MPs, bio-MPs bioaccumulate in aquatic organisms (e.g. fish) and cause toxic effects, it is imperative to study the potential problems in human health arising from the trophic transfer of bio-MPs in aquatic ecosystems.

As shown throughout this review, the problem of so-called 'biodegradable' plastics in aquatic ecosystems is severe but underexamined. As mentioned, in most work in the literature regarding biodegradable plastics' effects on aquatic ecosystems, PLA, despite not biodegrading in any aquatic habitat according to standards, is used as a model. In other words, it is critical to broaden the spectrum of bioplastics studied, to define standards for the way in which the studies should be conducted, to adequately simulate the natural environment, and to increase the number and quantity of species on which toxicity studies are performed in order to increase the certainty of statistical results.

Data availability statement

No new data were created or analysed in this study.

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