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REVIEW

Bioplastics and marine invertebrates: assessing immunological and environmental impacts in the transition from petrochemical to sustainable polymers

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Abstract

Nowadays, bioplastics are widely considered as a viable alternative to traditional petrochemical-based plastics. Given the significant projected growth in bioplastic production in the coming years, there is an urgent need to assess whether the environmental challenges associated with conventional plastics are being transferred to bioplastics. This review integrates and discusses the most recent and important findings to analyze and consolidate the existing literature on this novel and urgent environmental concern, exploring the relationships among plastics, bioplastics, the chemicals they contain, and marine invertebrates exposed to them in natural environments.

Key Words: animal response; mussels; marine invertebrates; immunity; bioplastics; biopolymers

Bio-based plastics

The continuously increasing interest in bioplastics primarily stems from the desire to find solutions to the significant environmental pollution caused by petroleum-based conventional plastics, which affects the environment worldwide (Otero *et al*., 2015; Mínguez-Alarcón *et al*., 2016; Raza *et al*., 2018; Shruti and Kutralam-Muniasamy, 2019; Miller *et al*., 2020; Manfra *et al*., 2021; Banaderakhshan *et al*., 2022). Bio-based polymers find application across a widening array of sectors that impact daily life (see Figure 1), leading to a rapid expansion in the bioplastics sector. In recent years, the production of bio-based polymers has increased up to 2.22 million tons, constituting approximately 1% of the total production volume of bio-based plastics derived from oil (Naser *et al*., 2021). Projections suggest that growth rates could surge, potentially reaching around 30% by 2025 (Coppola *et al*., 2021).

Recent political strategies have also fueled the shift towards bioplastics. Since January 2018, the European Commission's adoption of the "European Strategy for Plastics in a Circular Economy" has initiated a regulatory process targeting single-use plastic materials, aiming to promote more sustainable production, consumption, and disposal models. This regulation was established on July 2, 2019, with the enactment of Directive (EU) 2019/904, commonly known as the "Single-Use Plastic Directive," implemented in Italy through Legislative Decree No. 196/2021 ("Gazzetta Ufficiale" No. 285 del 30 November 2021 - Italy). Additionally, the European Green Deal, introduced by the European Commission in 2019, aims to support the EU's transition to a green economy, striving for climate neutrality by 2050. The objectives of the Single-Use Plastic Directive and the Green Deal align closely with the principles of the Circular Economy, emphasizing waste prevention, reuse, and recycling of plastic materials, along with the adoption of alternative, biologically sourced raw materials over petrochemical ones (Johansen *et al*., 2022). Further, advances in the sector and the development of new types of biomaterials for petroleum plastic substitution align with the objectives of the United Nations Development Program's 2030 Agenda for Sustainable Development Goals (SDGs) (UNEP, 2016).

Despite being considered a promising alternative to conventional plastics, bioplastics still come with drawbacks. The primary disadvantage relates to their high production costs, which are currently up to 10-fold higher than those of conventional polypropylene (PP) and polyethylene (PE) (Kourmentza *et al*., 2017; Shen *et al*., 2020;

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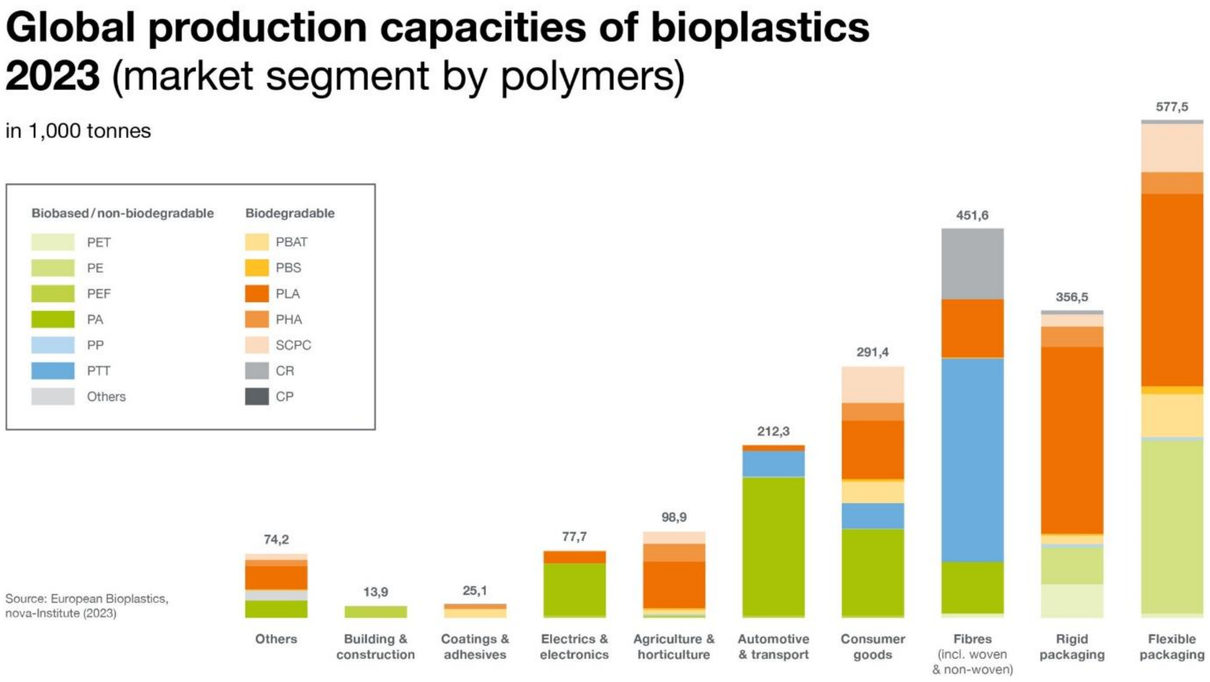

Fig. 1 Global production capacities of bioplastics in 2023, by market segment (European Bioplastics, 2023)

Singh *et al*., 2019, 2020). Additionally, there are indirect negative effects stemming from bioplastic production, notably the reliance on intensive monocultures (e.g., sugar, starch, oil, or natural rubber), leading to significant water consumption, fertilizer use, and enhanced eutrophication (Kabasci, 2014; Pellis *et al*., 2021). European Bioplastics recently indicated that the production of bio-based polymers in 2022 required 0.8 million hectares of land, with global production projected to increase to 6.3 million tons by 2027, which corresponds to the exploitation of 2.9 million hectares, representing approximately 0.06% of the global rural fields in 2027 (European Bioplastics, 2023). Another notable disadvantage of bioplastics is their inferior mechanical properties compared to conventional plastics (Emadian *et al*., 2017). Generally, plastics consist of polymers with repetitive units called monomers, which can have linear, branched, or crosslinked structures. While there are various categorizations for polymers, it is crucial to understand that, for industrial applications, polymers alone may not fulfill all the desired functions. Therefore, organic or inorganic compounds known as additives-such as plasticizers, dyes, light stabilizers, and pro-oxidants-are incorporated into the composition of plastics. These additives can impart new properties to plastics, resulting in the overall concept of plastics being a combination of multiple additive polymers. However, these substances, during the breakdown processes of bioplastics, are more readily dispersed into the environment (Fan *et al*., 2022) along with consequences on the living forms (Coltelli *et al*., 2008; Khan *et al*., 2017; Aznar *et al*., 2019; Campani *et al*., 2020; Zimmermann *et al*., 2019, 2020; Uribe-Echeverría and Beiras, 2022). Despite these concerning aspects, various types of bioplastics are currently available on the global market (Table 1) (Shruti and Kutralam-Muniasamy, 2019). Given the limited knowledge of the potential environmental impact of these bioplastics at the end of their lifecycle, it is essential to explore these aspects further.

In response, to the significant environmental concern caused by plastic materials derived from petroleum, the industry has begun exploring alternative solutions, including the development of new sustainable materials primarily based on biologically or chemically synthesized polymers from renewable sources (Xie, 2021; Döhler *et al*., 2022; Xie *et al*., 2023). These materials, such as polyhydroxyalkanoates (PHAs), polylactic acid (PLA), alginate, chitin, chitosan, keratin, which consists mainly of polymers directly made from biological renewable resources (Xie, 2021), are commonly known as "bioplastics," and they function like traditional plastics but are considered environmentally sustainable throughout their lifecycle and at the disposal stage (Atiwesh *et al*., 2021). The environmentally correct disposal of solid waste is a crucial area of study due to its potential to mitigate environmental, social, and public health issues (Hisatugo and Marçal Júnior, 2007; Cruz and Paulino, 2013). According to Siracusa *et al*. (2008), the lifecycle of bioplastics mirrors that of biomass, with benefits involving the conservation of fossil resources, water, and the production of CO_2 (Siracusa *et al*., 2008; V O Sousa and Do Nascimento, 2017).

Table 1 General characteristics of commonly available bioplastics (Shruti and Kutralam-Muniasamy, 2019)

Bioplastics	**Group**	**Consumer products**
Polylactic acid (PLA)	Bio-based homo-polymer	Films, bottles, medical devices and plates metal and paint coatings
Polyhydroxy alkanoates (PHA)	Bio-based polymer	Bags, bottles, disposable items, personal hygiene, diapers, films, coatings on paper, encapsulation of fertilizers, food packaging
Polybutylene succinate (PBS)	Fossil based co-polymer	Food packaging, coffee capsules, disposables, agriculture fibres, nonwovens industrial/automotive, mulch film, plant pots, hygiene products, fishing nets and lines, wood-plastic composites, composites with natural fibres
Polyethylene succinate (PES) Polybutylene	Fossil based co-polymer	
Polybutylene succinate adipate (PBSA)	Fossil based co-polymer	
Polyglycolic acid (PGA)	Fossil based homo-polymer	Subcutaneous sutures, intracutaneous closures, abdominal and thoracic surgeries, implantable medical devices
Polycaprolactone (PCL)	Fossil based homo-polymer	Food packaging, medical devices

According to the raw material from which they originate and their biodegradability, bioplastics can be divided into 4 different categories (Figure 2).

Indeed not all bio-based materials are biodegradable, nor are all biodegradable plastics necessarily bio-based (Naser *et al*., 2021). Therefore, it is more accurate to recognize that bioplastics can exhibit a high degree of biodegradability, though not always entirely, in natural environments and/or in “specific controlled conditions” according to standardized testing methods (Niaounakis, 2019; Briassoulis *et al*., 2021; Naser *et al*., 2021). Biodegradable plastics undergo biological degradation by microorganisms, a process involving the reduction of the molar masses of the macromolecules that make up these substances. This process is distinct from, though often confused with, “ultimate biodegradation,” which refers to the complete breakdown of organic compounds into simple molecules such as carbon dioxide/methane, nitrate/ammonium, and water (Vert *et al*., 2012).

The biodegradation of bioplastics relies on specific conditions, which are typically ensured within specialized disposal facilities where the polymers are appropriately managed at the end of their lifecycle. These biopolymers can degrade due to the presence of ester groups that undergo enzymatic hydrolysis. Enzymatic processes can occur under specific conditions, whether biotic or abiotic, which may be replicated in laboratory settings or specialized disposal facilities. These conditions include factors like oxygen availability, temperature, humidity levels, and the presence of specific degrading microorganisms. Additionally, the efficiency of these processes is influenced by the complexity, chemical structure, and crystallinity of the biopolymers themselves (Emadian *et al*., 2017; Shen *et al*., 2020).

However, if these materials are dispersed in the natural environment, they may not encounter the optimal conditions necessary to facilitate the degradation process. It is important to note that information regarding degradation processes and their impact on the natural environment, habitats, and organisms remains scarce and fragmented and scientific literature is still limited.

Bio-based bioplastics

PHA and PLA

Among the most prevalent bio-based and biodegradable materials the manufacturing industry has shown significant interest on PHAs and PLA, (Manfra *et al*., 2021). Consequently, numerous studies have focused on the characterization of these polymers. PHAs are straight-chain aliphatic polyesters obtained by the bacterial fermentation of organic substrates (Raza *et al*., 2018). PHAs constitute a class of polymers composed of repeating monomeric units biosynthesized by the enzyme PHA synthase (Sudesh *et al*., 2000). Their synthesis is influenced by factors such as the microorganism, growth conditions, and the type of organic substrate used as a starting material. Additionally, PHAs can be modified through combination with other biopolymers, enzymes, organic and inorganic materials, and other additives aimed at enhancing their characteristics, mechanical properties, and biocompatibility (Chan

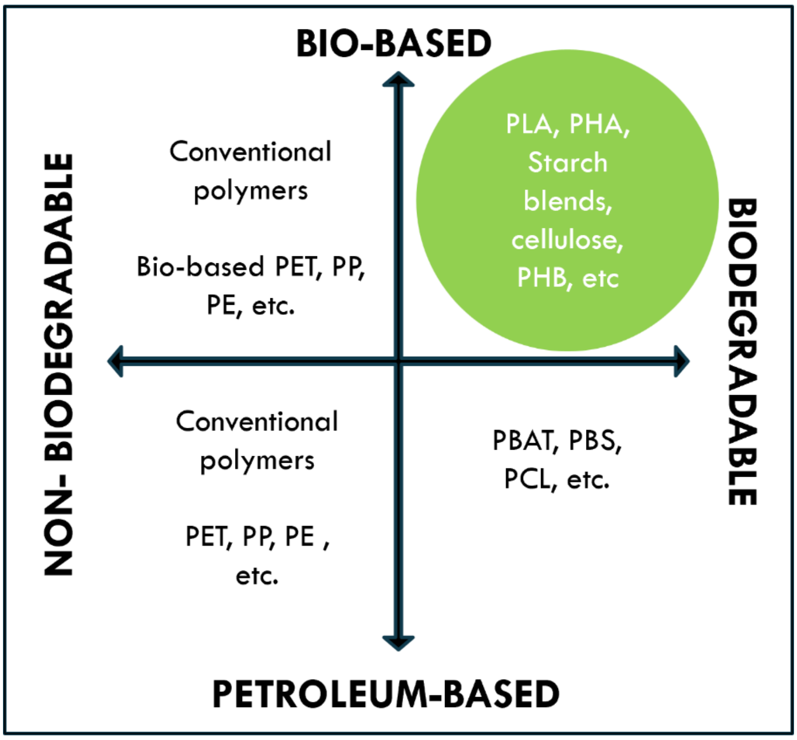

Fig. 2 Categorization of plastic (adapted from: European Bioplastics)

et al., 2018). One common form of PHAs is the co-polymer of (PHB) with polyhydroxyvalerate (PHV), a low molecular weight straight-chain carboxylic acid that can undergo degradation. This co-polymer exhibits improved processability and facilitates recycling processes. Moreover, enhancements in the biodegradation process have been observed in mixtures blended with up to 50% wood flour by weight (Chan *et al*., 2018; Hubbe *et al*., 2020). PHAs, in homo- or hetero-polymer forms, pure or mixed, find applications in many different fields (Table 1), such as packaging, medicine and pharmacology, agriculture, the food industry, as raw materials for the synthesis of enantiomerically pure chemicals, and in paint production (Rehm and Steinbü, 1999).

PLA is a naturally occurring, biocompatible, and biodegradable thermoplastic polyester, possessing stiffness and clarity similar to polystyrene (PS) or polyethylene-terephthalate (PET) (LimLoong-Tak *et al*., 2022). Lactic acid monomer units are sourced mainly from agricultural substrates (starch, glucose, lactose, maltose, corn, and potato) and obtained by fermentation of microorganisms, notably those of the genus *Lactobacillus*. Poly-L- and poly-D-lactide (PLLA and PDLA) are two isomeric forms of the PLA polymer. When used as unmodified, these forms have certain limitations in practical applications; indeed, they result in brittleness and poor resistance to oxygen exposure, making them less suitable for applications requiring durability and mechanical strength, particularly in oxygen-environments (Nandy *et al*., 2022). Additionally, another characteristic affecting the use of PLLA and PDLA is their slow degradation rate at room temperature. Due to their chemical properties, the hydrolysis process of ester linkage by water occurs very slowly (Ilyas *et al*., 2022). As a result, PDLLA, an amorphous form of the polymer, is frequently employed in industrial processes. By adjusting the ratio through the addition of PLLA, manufacturers can more effectively regulate the crystallization, morphology, and hydrolysis behavior of the polymers (la Carrubba *et al*., 2008; Carfì Pavia *et al*., 2013; Hong *et al*., 2021). This approach is particularly prevalent in the biomedical sector, where PDLLA has been extensively used since the 1960s, both in its pure form and in blends, for applications such as sustained drug and protein release and various surgical procedures (LimLoong-Tak *et al*., 2022). Today, PLA has broad applications across numerous industries. It serves as a versatile material suitable for packaging, disposable tableware, clothing, bottle production, injection molding, extrusion coatings, and more (Gupta and Kumar, 2007; LimLoong-Tak *et al*., 2022). In agricultural applications, PLA is utilized in mulching by the utilization of films that guarantee the gradual release of pesticides and fertilizers (Gupta and Kumar, 2007). This widespread adoption highlights PLA's versatility and its ability to meet diverse needs across different sectors.

Fate in marine environment

Given the wide utilization of products containing PHAs and PLA biopolymers, it is important to delve

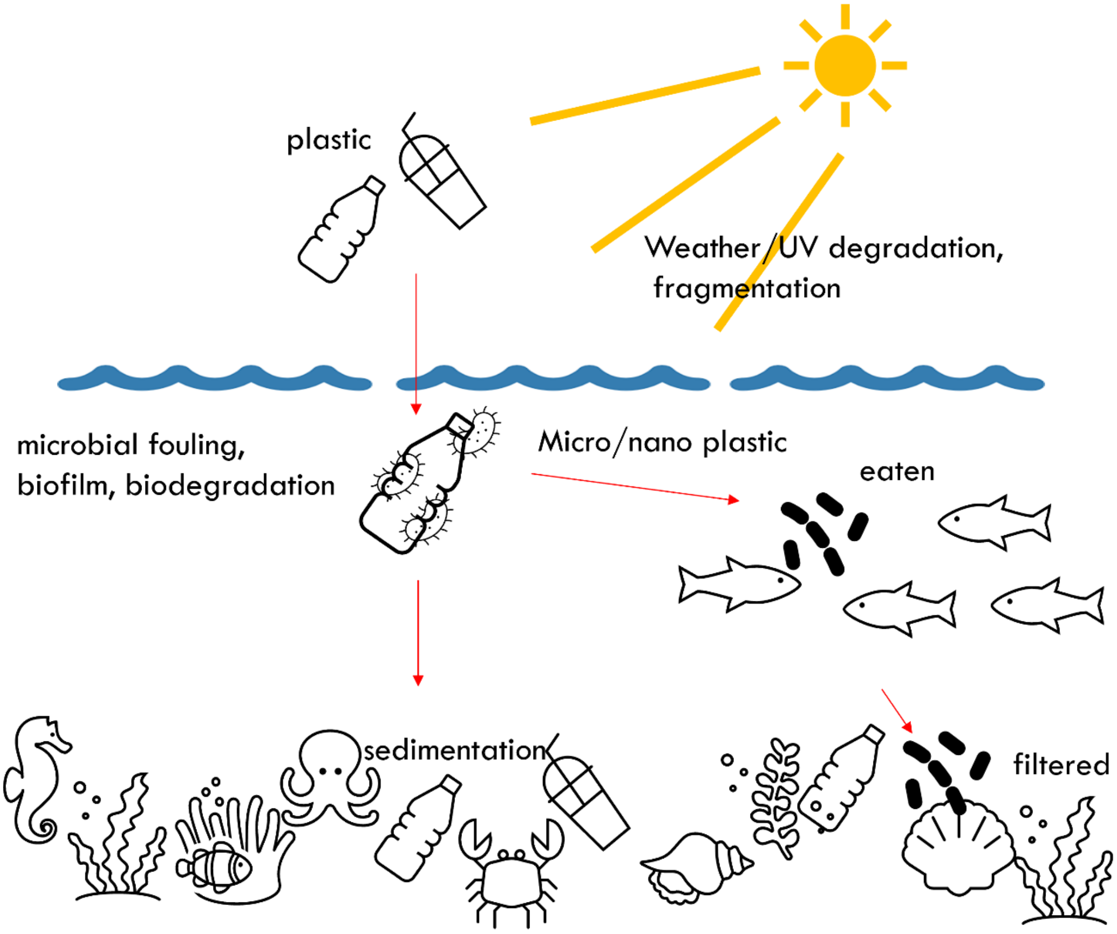

Fig. 3 Schematic representation of plastic deterioration by abiotic and biotic factors in the marine environment (adapted from Shilpa *et al*., 2022)

deeper into their biodegradation processes; however, these processes tend to occur at a slower pace in natural environments, lowering their potential benefits to ecosystems and limiting their advantages in bioplastic waste management (Shen *et al*., 2020; Fan *et al*., 2022). Studies indicate that PLA experiences minimal mass loss even after 400 days of marine immersion (Bagheri *et al*., 2017). Conversely, a study on the degradation of PHAs in marine environments conducted by Deroiné *et al*. (2015) revealed complete degradation of a 200 µm thick PHB film after 9 months of immersion (Deroiné *et al*., 2015). Consequently, although the average lifetimes of PHA bioplastics in the environment are notably shorter than those of conventional petroleum-derived plastics (Ward and Reddy, 2020), it remains crucial to assess the impact of these biomaterials on organisms and marine ecosystems before their complete biodegradation. Microbial communities adhering to the surfaces of polymeric materials play a pivotal role in degradation processes. In marine environments (as depicted in Figures 3 and 4), plastic swiftly becomes colonized by microbial species, pioneers in substrate colonization, leading to the formation of a biofilm attached to the plastic, commonly referred to as the "plastisphere" (Zettler *et al*., 2013; Syranidou *et al*., 2017).

Figure 4 illustrates the process of bacterial colonization and biofilm formation on materials in aquatic environments. Indeed, microbial communities adhere to these surfaces secreting extracellular polysaccharide substances (Carrasco-Acosta *et al*., 2022; Shilpa *et al*., 2022). In a second step, other organisms, starting from unicellular algae and protozoa and going up to algae and benthic invertebrates colonize the surface (Dang and Lovell, 2016; Syranidou *et al*., 2017). It is crucial that the biota associated with plastic debris, whether bio-based or conventional, participate in the fragmentation of the MP; indeed, it can alter the surface topography, creating fractures that cause the fragmentation of the polymer into even smaller particles down to micro and nano dimensions (Syranidou *et al*., 2017). The last step of biodegradation is mineralization, where the biomass assimilates useful components from the polymeric substrate and releases CO_2 and water (Figures 3 and 4) (Syranidou *et al*., 2017; Vinay Kumar *et al*., 2021).

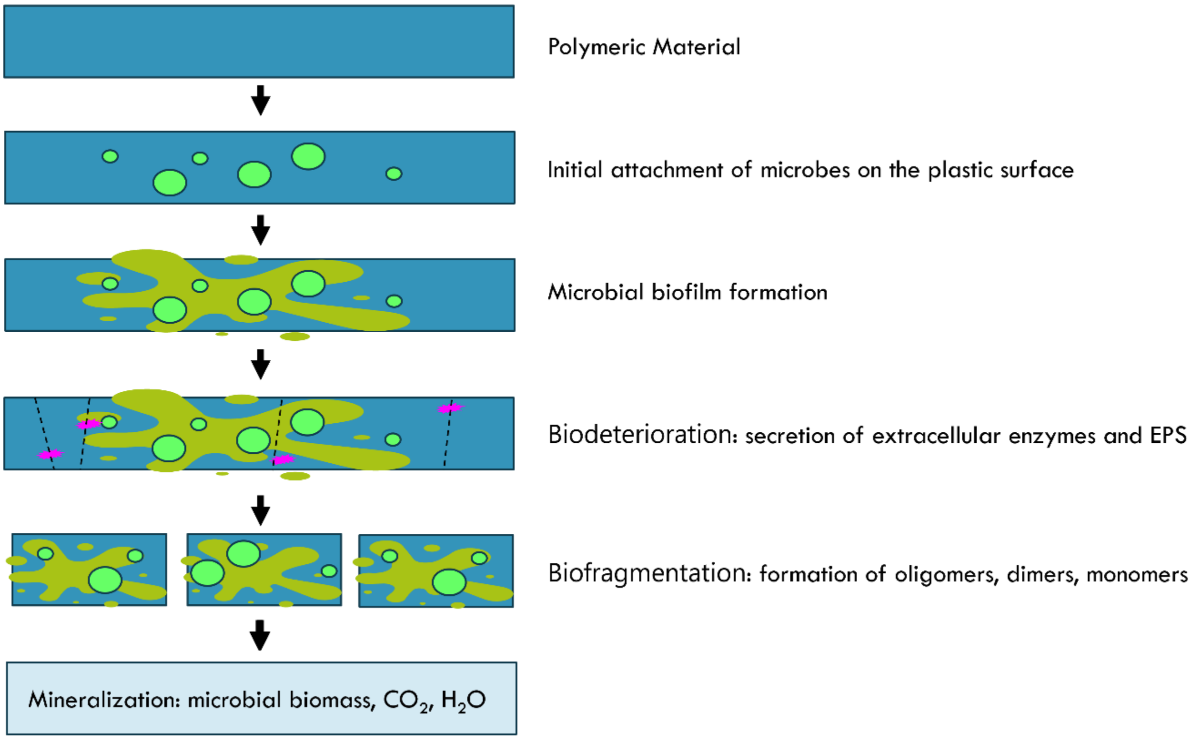

Fig. 4 Schematic illustration of plastic fragmentation caused by biofouling. This diagram, originally presented for conventional plastics, is also applicable to describing the processes that lead to the fragmentation of bioplastics (adapted from Kumar *et al*., 2020)

Plastic additives

As mentioned above to enhance macroscopic characteristics of biopolymers such as stability, color, and texture, polymers are seldom utilized in their pure form. Instead, chemical products are mixed as additives and, consequently, they are released into the environment during fragmentation and biodegradation processes (Emadian *et al*., 2017). Enhanced susceptibility to photodegradation and thermo-oxidation can, indeed, promote the biodegradation of materials. However, it is essential to take into account the influence of additives on the plastic's composition and investigate their fate during plastic degradation (Yousif and Haddad, 2013; Gewert *et al*., 2015). The longevity of plastic materials can be partially regulated by incorporating additives such as pro-oxidants. These additives are triggered by photosensitivity or are thermally activated to initiate abiotic degradation of polymer bonds. Depending on the composition and concentration of these additives, the timing of activation can be managed. The resulting chemical alterations in the polymeric structure render the material accessible to environmental microbes, thereby accelerating its biodegradation process (Fontanella *et al*., 2010). Of course, their destiny requires clarification as the buildup of additives could pose risks. Essentially, additives facilitate a hastened mechanical (abiotic) decomposition of plastic. As mentioned earlier, this renders the polymer susceptible to microbial degradation, which is a desirable trait for most biodegradable plastics. However, it becomes problematic when it leads to the creation of MPs and NPs that are not fully biodegradable. These MP fragments, particularly if they have started accumulating in the environment, present consequences that are not yet fully understood (Kjeldsen *et al*., 2019). Several studies have examined the characteristics and rate of degradation induced by the abiotic pre-treatment of oxo-biodegradable materials. Most of these studies were conducted under elevated incubation temperatures and accelerated conditions, simulating sunlight exposure using UV lighting to trigger mechanical deterioration (Vogt and Kleppe, 2009; Jakubowicz *et al*., 2011; Corti *et al*., 2012; Yashchuk *et al*., 2012; Contat-Rodrigo, 2013; Portillo *et al*., 2016; Eyheraguibel *et al*., 2018). In particular, it has been shown that one of the main factors controlling degradation is the type of pro-oxidant, rather than the actual type of polymer (Fontanella *et al*., 2010), or that the exposure time is more important than the intensity of the radiation, and that higher temperatures enhance the rate of degradation (Yashchuk *et al*., 2012; Portillo *et al*., 2016). Further results reveal that, once activated, chemical breakdown by the oxidants will continue even in scarce light conditions or in the absence of light. This is an important property as disposed materials may well end up buried (Vogt and Kleppe, 2009). The additives currently used are usually metal salt mixtures. One study testing the thermoand photo-degradation of low-density polyethylene found that iron stearate has more desirable properties than cobalt stearate and manganese stearate as additives (Jeon and Kim, 2014). Most of the metals used in additives occur naturally in small amounts, but the accumulation of certain additives can potentially become toxic. For example, it has been demonstrated that high cobalt concentration decreased the rate of subsequent bacterial degradation because of cobalt toxicity on microorganisms (Fontanella *et al*., 2010).

The environmental consequences of polymer residues need comprehensive examination and clarification within established standards. This is essential to prevent product degradation from causing adverse environmental effects (Portillo *et al*., 2016).

Biological sieve model: mussels as study model to investigate pollutant effects on marine invertebrates

Bioplastics subjected to erosion can readily decrease in size, transitioning into MPs and NPs, posing environmental consequences akin to conventional petroleum plastics (Arpia *et al*., 2021). This results in frequent occurrences of bioaccumulation and biomagnification events, causing harm not only at the individual organism level but at the ecosystem level as well. They introduce various environmental chemical contaminants and increase the risks of pathogenic microorganisms moving into food webs (Mülhaupt, 2013; Shruti and Kutralam-Muniasamy, 2019, Manfra *et al*., 2021; Manzoor *et al*., 2022). As MPs tend to migrate deeper due to the biofouling phenomenon (Eich *et al*., 2015), a dislocation in the vertical distribution of species involved in bioplastics ingestion has been noted, which could pose a challenge to the stability of ecosystems (Rodríguez *et al*., 2023).

Thus, a critical issue is the assessment of potential harm to living organisms caused by bioplastics, accomplished through the use of sentinel organisms known as bioindicators. As reported by Michalak and Chojnacka (2014), a desirable sentinel organism possesses the following characteristics: (1) the ability to accumulate high levels of pollutants without succumbing to death; (2) a sessile lifestyle, ensuring it accurately reflects local pollution levels; (3) abundance and wide distribution for repetitive sampling and comparison; (4) longevity to facilitate comparisons across various age groups; (5) presence of suitable target tissues or cells for further research at the microscopic level; (6) ease of sampling and cultivation in laboratory settings; (7) ability to survive in water; (8) an important position in the food chain; and (9) a well-defined dose-effect relationship (Michalak and Chojnacka, 2014).

Bivalves, particularly species belonging to mussels, are commonly utilized as environmental bioindicators and are important actors in monitoring activity (Beyer *et al*., 2017b; Caricato *et al*., 2019; Gornati *et al*., 2019). They are readily available, easy to sample, and can be maintained and manipulated in the laboratory. Moreover, they exhibit high tolerance for pollutants and extreme environmental conditions, and their reactivity to anthropogenic stressors is well-documented (Beyer *et al*., 2017b; Caricato *et al*., 2019; Gornati *et al*., 2019). These model organisms are also consumed by humans, making them significant vectors for the transfer of pathogens and pollutants within the food chain. Indeed, Europe produces over 300,000 tons per year for human consumption (Beyer *et al*., 2017a; Caricato *et al*., 2019; Figueras *et al*., 2019; Gornati *et al*., 2019; Gedik and Eryaşar, 2020; Han and Dong, 2020; Cappello *et al*., 2021).

When exposed to MPs, they can bioaccumulate in soft tissues and organs (hemolymph, digestive gland, foot, and mantle) at different developmental stages (Capolupo *et al*., 2018; Pittura *et al*., 2018), activating different pathways and responses. (Rochman *et al*., 2015; Foley *et al*., 2018; Zhang *et al*., 2020; Hoellein *et al*., 2021). The feeding and respiratory processes produce a water flow through the siphons, where the edge of the mantle serves as the organism's initial filter, preventing particles larger than 5mm from entering. Plastic particles that pass this initial barrier are processed by the gills, allowing only those smaller than 300 µm to pass through. Particles that pass through the gills, along with food and organic substances, move into the digestive tract, where they are mixed with mucus and transported to the digestive gland. Since plastic particles are resistant to digestive enzymes, larger particles are eliminated as pseudofeces (< 25 µm), while smaller particles may accumulate in internal organs such as the gills, hepatopancreas, adductor muscle, and move through the hemolymph. This accumulation may lead to cell and tissue damage, or particles may be stored inside lysosomes, leading to morphological and functional alterations (Ringwood, 2021). Histopathological analyses and lysosomal assays conducted through *in vivo* exposure tests are therefore essential evaluations for comprehensively assessing the potential effects of plastic ingestion on mussels (Sendra *et al*., 2020). But if, on the one hand, *in vitro* tests allow for rapid immunobiological screening, on the other, *in vivo* tests allow for the evaluation of chronic and long-term effects. The latter, indeed, considers not only the hemocytic and humoral response but also whole organism impact. Through *in vivo* tests, it has been possible to observe that particles can enter via feeding and respiration routes, whether they are food or pollutants, which can accumulate and concentrate inside the organisms. On these basis Ringwood described the bivalves as a "Biological Sieve Model" (Figure 5), enabling the characterization of exposure and risks associated with pollutants, including plastic, over time and space (Ringwood, 2021).

The immunological biomarkers

The goals set out in the majority of studies on polymers of fossil origin aimed to clarify whether they may behave detrimentally after degrading and generating fragments (with dimensional distribution in the order of micro and nanoparticles) that could interact with natural environments, causing harmful impacts on marine organisms. The effects of several

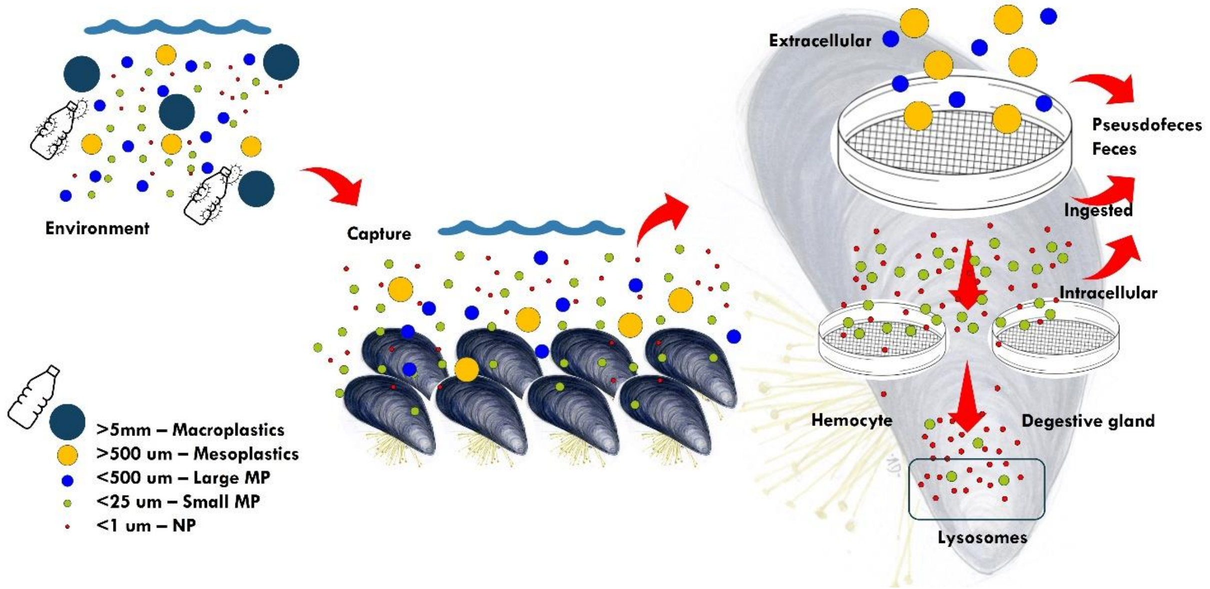

Fig. 5 Schematic representation of the “Biological Sieve Model” (adapted from Ringwood, 2021)

pollutants, including heavy metals, organotin compounds used as biocides in anti-fouling paints, microplastics (MPs) and nanoplastics (NPs) from conventional plastics were studied in numerous studies through the investigation of different biomarkers, including immunological ones: histological abnormalities, altered lysosomal integrity, nuclear abnormalities and genotoxicity, up-regulation of genes associated with immune response and apoptosis, as well as measurements of clearance, respiration, and byssus production (Von Moos *et al*., 2012; Avio *et al*., 2015b; Paul-Pont *et al*., 2016; Détrée and Gallardo-Escárate, 2017; Ribeiro *et al*., 2017; Salerno *et al*., 2021), but also the quantity of free circulating hemocytes present in hemolymph, spreading activity, phagocytic activity, and granulocytoma formation as well as the activity of several immunological enzymes in the hepatopancreas and hemolymph (Cooper *et al*., 1995; Falleiros *et al*., 2003; Von Moos *et al*., 2012; Trapani *et al*., 2016; Parisi *et al*., 2021; Dara *et al*., 2022; La Corte *et al*., 2023). These markers are considered useful tools in these studies, firstly, because bivalve hemocytes are ready to react to exogenous stressors due to their functional cell-mediated immune responses (Paillard *et al*., 2004; Gagnaire *et al*., 2006). Enzymes produced in the digestive gland are known to participate in the hemocyte response, assisting, modulating, and accelerating their immunological processes. The hepatopancreas is a source of different immunity molecules (Smith, 2016) involved in various defensive processes, including pathogen clearance, antigen processing, and infection-induced metabolic changes (Alday-Sanz *et al*., 2002). Hydrolase enzymes, normally involved in detoxification, inflammatory and digestive processes, the phenoloxidase cascade, and reactive oxygen species (ROS) scavenging, are recognized as immune parameters that can be used as markers to observe responses to environmental stress (Parisi *et al*., 2021). Lysozyme-like activity is a highly phylogenetically conserved humoral response and has been studied in different tissues and organs, such as the mucosa, hepatopancreas, and hemolymph of many vertebrate and invertebrate species. It is the first humoral protection of organisms against pathogens, with a bactericidal hydrolytic enzyme that hydrolyzes specific bonds of the peptidoglycan present in the cell walls of Gram-negative bacteria, causing the rupture of bacterial walls and consequently destabilizing the membrane (Li *et al*., 2008; Cammarata *et al*., 2019; Dara *et al*., 2022; La Corte *et al*., 2023, 2024; Bisanti *et al*., 2024).

Below we discuss some recent studies supporting the utilization of immunological markers to assess the effects of different plastic particles on mussels.

Détrée and Gallardo-Escárate (2018) subjected mussels to a single exposure, a recovery period, and a second exposure to PET microbeads. Their results evidenced that long-term exposure, 18 days, to MPs caused disruption of mussel global homeostasis, inducing stress and, consequently, the secretion of stress and immune-related proteins, resulting in a decrease in energy allocation for growth. During the recovery period after exposure, the activation of apoptotic mechanisms and the up-regulation of immune receptors and stress-related enzyme and proteins (Gluthatione peroxidase, GPx and Heat Shock protein 70, HSP70) were observed. These responses suggest the establishment of compensatory mechanisms to recover homeostasis (Détrée and Gallardo-Escárate, 2018).

Park and colleagues (2024), in their comparative analysis of different MPs, found that shapes, sizes, and concentrations are the drivers influencing bioaccumulation, altering global DNA methylation levels and inducing microbiome alterations in Mediterranean mussels. These changes were accompanied by inflammatory and immune reactions. Chronic exposure, indeed, activated epigenetic responses, reflected in global DNA methylation, and altered immune responses mediated by toll-like receptors. Their study evidenced that small fiber-type MPs of different shapes and sizes were associated with multiple risk factors (Park *et al*., 2024).

Cole and colleagues (2020), by using biomarkers of oxidative stress response, lysosomal stability, and genotoxic damage measured the toxicity of polystyrene MPs, polyamide microfibers, and polystyrene NPs on mussels after acute or chronic exposures in the hemolymph, digestive gland, and gills. MP and microfiber accumulation was observed in the digestive glands, with significantly higher plastic concentrations related to long exposure. NPs had a significant effect on hyalinocyte - granulocyte ratios, indicative of an activated and stimulated immune response. SOD activity significantly increased following short exposure, returning to basal levels after long exposure. No evidence of lysosomal destabilization or genotoxic damage was caused by the materials confronted. The results of this study demonstrate the importance of particle dimensions in terms of their toxicity (Cole *et al*., 2020).

In addition, Auguste and colleagues (2020), used the rich toolbox of mussel immunity for evaluating the effects of different types of NPs, including amino-modified nanopolystyrene (PS-NH_2) as a model of NPs. The effects of multiple exposures to PS-NH_2 on *M. galloprovincialis* were investigated through multiple immune responses. Functional parameters were measured in hemocytes, serum, and whole hemolymph samples. In hemocytes, the gene transcription level involved in proliferation, apoptosis processes, and immune response was measured by qPCR. An initial exposure to PS-NH_2 significantly affected mitochondrial and lysosomal parameters in hemocytes, serum lysozyme activity, and the transcription of proliferation/apoptosis markers; it induced a significant upregulation of extrapallial protein precursor, downregulating lysozyme and mytilin B. However, it did not alter hemocyte bactericidal activity. Following the second exposure, a change in hemocyte subpopulations was recorded, accompanied by the restoration of basal functional parameters and proliferation/apoptotic markers. Furthermore, the bacterial activity of the molecules in the hemolymph, as well as the transcription of immune-related genes coding for secreted proteins, were significantly increased. The results of this study indicate that mussels may shift immune parameters as compensatory mechanisms to regulate and maintain immune homeostasis, even when stimulated by a second encounter with PS-NH_2 (Auguste *et al*., 2020)

Considering the above studies, we recognize the validity of the physiological and immunological approach to assessing the effects of MPs and NPs of different materials, shapes, and dimensions on marine bivalves. In this perspective, it is certainly a valid approach, and we support its use in analyzing the effects caused by bio-based materials available in commerce or dispersed in the environment on marine biota. Indeed, not all bio-based materials are biodegradable, or biodegradable in a very short timeframe, and certainly interact with living species, causing various and plausibly detrimental effects.

The state of the art of animal response to bioplastics

Bivalve bioplastic response

Preliminary results indicate that bioplastics about immune-stimulation also exhibit similar behaviors to fossil-based plastics (Rochman *et al*., 2015; Foley *et al*., 2018; Zhang *et al*., 2020; Hoellein *et al*., 2021). Capolupo and colleagues (2023) reported that plastics are able to affect egg fertilization and larvae motility, and also impact adult mussel physiology after exposure to different bioplastic leachates. All lysosomal parameters were affected, and serum lysozyme activity was inhibited (Capolupo *et al*., 2023). Using biochemical markers in the blue mussel *M. edulis*, Magara *et al*. (2019) conducted an exposure and incubation treatment study, comparing the toxicity of a bio-based MP polymer, PHB (10-90 µm), to PE (10-90 µm), a conventional plastic material. The research revealed significant decreases in catalase (CAT) activity in the digestive gland of organisms exposed to PE compared to control organisms. Conversely, organisms exposed to PHB showed significant decreases in CAT and superoxide dismutase (SOD) activity in the gills and digestive gland. Between the two exposure treatments, differences were observed only in gill CAT activity, which was lower in the PHB treatment. The biochemical responses, SOD activities, CAT, GPx, glutathione S-transferase (GST), and glutathione reductase (GR), were generally comparable between the two treatments. The digestive glands and gills of *M. edulis* have been demonstrated to be organs which are affected by bioplastic materials (Magara *et al*., 2019). In a study comparing the effects of PLA and HDPE, Green *et al*. (2019) examined the tenacity of *M. edulis* to the substrate and the hemolymph proteome following an extended exposure. After exposure, mussels produced a lower quantity of byssus than the control samples and showed decreased tenacity compared to unexposed mussels, although PLA-exposed animals demonstrated higher tenacity than HDPE-exposed ones despite producing fewer byssal threads. Further, exposure to MPs induced the overexpression of 19% of hemolymph proteins, correlating with the activation of detoxification mechanisms and immune system functions (Green *et al*., 2019). Zhong et al (2024) demonstrated that in mussels PLA MPs polylactic acid exerts the similar toxic effects to traditional petroleum-based polystyrene MPs. To test the ecological risk of bio-based PLA, *Mytilus coruscus* specimen were exposed to different concentrations of PLA and PS (10^2, 10^4, and 10^6 particles/L) for a period of 14 days. The exposure induced an increase in oxidative stress enzyme activities and immune response demonstrating that mussels were under homeostatic compromission. Indeed, enzyme activities of nerve conduction and energy metabolism were significantly affected and normal physiological activities in respiration, ingestion and assimilation were also suppressed in association with enzyme changes. These remarkable changes in biomarkers in the gill tissues and haemolymph obviously affect the normal physiological activities of the organism evidence that PLA MPs has detrimental effect akin the PS MPs (Zhong et al., 2024). Even on *M. coruscus* a study by Zhong, Huang, et al., (2024) demonstrated the detrimental effect of microplastic made of PLA and plastic additives. The effect of PLA MPs and organophosphate flame retardants (OPFRs) tris(1-chloro-2-propyl) phosphate (TCPP) were investigated examining oxidative stress enzyme (catalase, superoxide dismutase, malondialdehyde), immune responses acid (phosphatase, alkaline phosphatase, lysozyme), neurotoxicity (acetylcholinesterase), energy metabolism (lactate dehydrogenase, succinate dehydrogenase, hexokinase), and physiological indices (absorption efficiency, excretion rate, respiration rate, condition index) after 14 days exposure. The results shown that PLA MPs and TCPP could be responsible of detrimental effects on the organisms (Zhong, Huang, *et al*., 2024).

In a 60-day-long investigation involving the mollusk *Ostrea edulis*, the European flat oyster, Green *et al*. (2016) compared the toxicity of PLA versus HDPE. The results reported that oysters exposed to high levels (80 mg/L) of PLA exhibited increased respiration rates compared to organisms exposed to HDPE, although there were no statistically significant differences between the two polymers in terms of filtration rates and shell growth (Green, 2016). Dara *et al*. (data yet unpublished) found that bio-MPs and bio-NPs can stimulate the immune system, initiating the removal of foreign particles through cellular responses. Their findings showed that, in *in vitro* assays, bioplastics trigger the immune system, activating pathways for the elimination of non-self-particles through the cellular responses of phagocytosis and encapsulation. *In vivo* experiments with exposure to environmentally coherent concentrations showed that exposure to PHA and PLA microparticles (1-50 µm range) and nanoparticle PHB present in the surrounding environment affected the biomarkers analyzed, having a potentially detrimental effect on organisms after 72 h of exposure. Histological analysis of the digestive gland revealed alterations of the microscopic structure of the digestive tubules, with the main alteration caused by HDPE and PLA MPs.

Not only filter feeding

Despite the limited number of studies involving bivalves, bio-polymeric materials, and immunological biomarkers, a number of other organisms and markers have been used to assess the toxicity of bioplastic compounds. The animal models used in these studies were worms, crustaceans, ascidians, and also vertebrates like Osteichthyes and reptiles (Venâncio *et al*., 2022). Examining the macrofauna associated with oyster, it was observed that both PLA and HDPE altered its composition, structure, and abundance, leading to a significant reduction in the number of amphipods and juvenile isopods (Green, 2016). In a study conducted by Green *et al*. (2015), the annelid species *Arenicola marina* was utilized as a model organism to investigate the effects of exposure to HDPE, PVC, and PLA MPs. Interestingly, all three types of plastic caused comparable effects on feeding and bioturbation activity, as well as on sediment primary productivity and nitrogen cycling, regardless of the dosage. However, PVC exhibited a strong impact on oxygen consumption, which serves as a bioindicator of metabolic rates. A similar response pattern was observed for lugworms bioturbation and feeding activity, which were significantly higher than the controls at high doses of PVC (2% weight/total sediment volume) (Green *et al*., 2015). Amelia *et al*. (2020) investigated the uptake and elimination rates of PLA microbeads by the marine copepod *Nitokra lacustris* over a 24-hour ingestion period and a 72-hour egestion period. Using fluorescence microscopy, they observed that more than 70% of the PLA particles were consumed by the copepods. However, during the subsequent 72-hour depuration phase, around 20% of the ingested particles were retained and not expelled through fecal pellets. Despite no mortality being observed during acute exposure, the findings suggest that PLA MPs can be ingested and accumulate in zooplankton, raising concerns about the potential implications of this accumulation and its transfer through the food web (Amelia *et al*., 2020). Ingestion data were also reported by Hodgson (2020), who reported the ingestion of biodegradable/compostable plastic by Arthropoda, specifically Amphipoda species such as *Orchestia gammarellus* and *Orchestia mediterranea*, based on field and laboratory experiments (Hodgson *et al*., 2018). Among vertebrates Müller and colleagues (2012) observed bioplastics in the digestive system of Green turtles (*Chelonia mydas*) and Loggerhead turtles (*Caretta caretta*) by analyzing fluids. The presence of these polymers, originating from biodegradable shopping bags, suggests that their breakdown within the digestive system is not sufficiently rapid to guarantee harmlessness and eliminate mortality risks in both Green and Loggerhead turtles. This underscores the need for further research to thoroughly understand the environmental decomposition of biodegradable polymers, particularly in marine environments (Müller *et al*., 2012).

The additives effects

As previously mentioned, additives are included in products and, consequently, they are also released into the environment during plastic breakdown (Emadian *et al*., 2017). Various studies have investigated the chemicals extracted to simulate the effects of chemicals leaching from bioplastics dispersed in the environment. Research involving *Lumbriculus variegatus*, a species of annelid worm, indicated that biodegradable PLA MPs mixed into sediments have a more pronounced impact compared to MPs layered on the sediment surface. These biodegradable PLA MPs typically result in greater toxic effects than kaolin, which served as a particle control. Severe adverse effects were observed in experiments where worms were exposed to chemicals extracted from the PLA MPs, shedding light on the chemicals added to or absorbed by biodegradable MPs and their degradation products (Klein *et al*., 2021).

Uribe-Echeverría and Beiras (2022) conducted a study investigating the impact of PLA and PHB on sea-urchin larvae. They observed a notable reduction in larval growth due to a leachate, indicating slight toxicity. These results align with the findings reported by Beiras *et al*. (2021). Chemicals with surfactant, antimicrobial, and lubricant properties, along with flame retardants and plasticizers, were identified through GC-MS analysis. These compounds detected in PHB may be responsible for the toxic effects observed on sea-urchin larvae in this study. In the same study by Uribe-Echeverría and Beiras (2022), chemical analysis of PLA did not reveal the presence of any compounds considered toxic in the literature (Uribe-Echeverría and Beiras, 2022). However, various additives commonly used to enhance their low thermal and oxygen permeation resistance have been reported to cause nonspecific toxicity, as documented in previous studies. Thus, it appears that the toxicity of PLA materials varies depending on their specific chemical composition. Despite being generally regarded as safe, certain PLA products may contain chemicals with potentially harmful health effects (Coltelli *et al*., 2008; Khan *et al*., 2017; Aznar *et al*., 2019; Zimmermann *et al*., 2019, 2020). Anderson and Shenkar (2021) carried out a study using the ascidians *Microcosmus exasperates* and *Herdmania momus* (Chordata, Stolidobranchia) to assess the impact of PLA (derived from single-use plates and drinking cups) on fertilization and bioaccumulation, observing a decrease in the fertilization process and bioaccumulation rate comparable to the bioaccumulation of PET particles used as a control. Jang *et al*. (2022) investigated the impact of PLA on *Clarias gariepinus* (Chordata, Siluriformes). The authors observed disruptions in microbiota homeostasis, characterized by increased levels of *Vibrio fischeri* in the intestine, indicating dysbiosis. Additionally, they found higher levels of metals, specifically copper (0.050 mg/L) and lead (0.060 mg/L), transported through ingested PLA taken with nutrition (Jang *et al*., 2022).

Conclusion

This review aimed to consolidate and enhance the existing literature on a novel and urgent environmental concern: the emergence of a new type of pollutant that has not yet been thoroughly explored. This pollutant, bioplastics, has the potential to harm organisms. While there have been numerous studies on the effects of petrochemical-based MPs and NPs, research on bioplastics, although still limited, is steadily increasing. The data presented in the papers discussed clearly show that bioplastics elicit effects similar to those of traditional plastics, and these effects may manifest across various levels of biological organization. Not only can the plastic bio-polymers themselves cause harm, but the chemical additives used to enhance material performance are also damaging. Given the projected rapid growth in the production of bio-based biodegradable bioplastics, especially those made with PHAs and PLA biopolymers, there is a real risk that the pollution problem posed by petroleum-based plastics could simply shift to bio-based plastics. Additionally, it is important to note that bioplastics degrade much faster into MPs and NPs compared to conventional plastics, exacerbating their potential harmful effects. Therefore, it is crucial to focus on assessing the safety of bioplastics using both chemical and biological approaches, and to support the production of sustainable bioplastics. Despite their petroleum-free origin, the findings suggest that biodegradable plastics may not necessarily be a superior alternative to conventional plastics, as their toxicological impact varies depending on a number of factors. Further, this review presents different studies supporting the utilization of zoological models and different markers, including the immunological ones, to assess the effects of new materials distributed on the market and foresee the possible detrimental effects on living species, from the bottom up to apex species which occupy the highest trophic level, including humans. On the other hand, our ongoing research continues to study the impact that bioplastics have on organisms, particularly when coupled with the wide range of emerging contaminants coexist in the marine environment other contaminants abundantly released into the environment, which can be adsorbed by bioplastic and vehiculated into the biological compartment of an ecosystem. Thus, their potential toxicity and environmental impact require effective toxicological assessment to investigate their potential adverse effects individually or in combination.

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