A critical review on the evaluation of toxicity and ecological risk assessment of plastics in the marine environment

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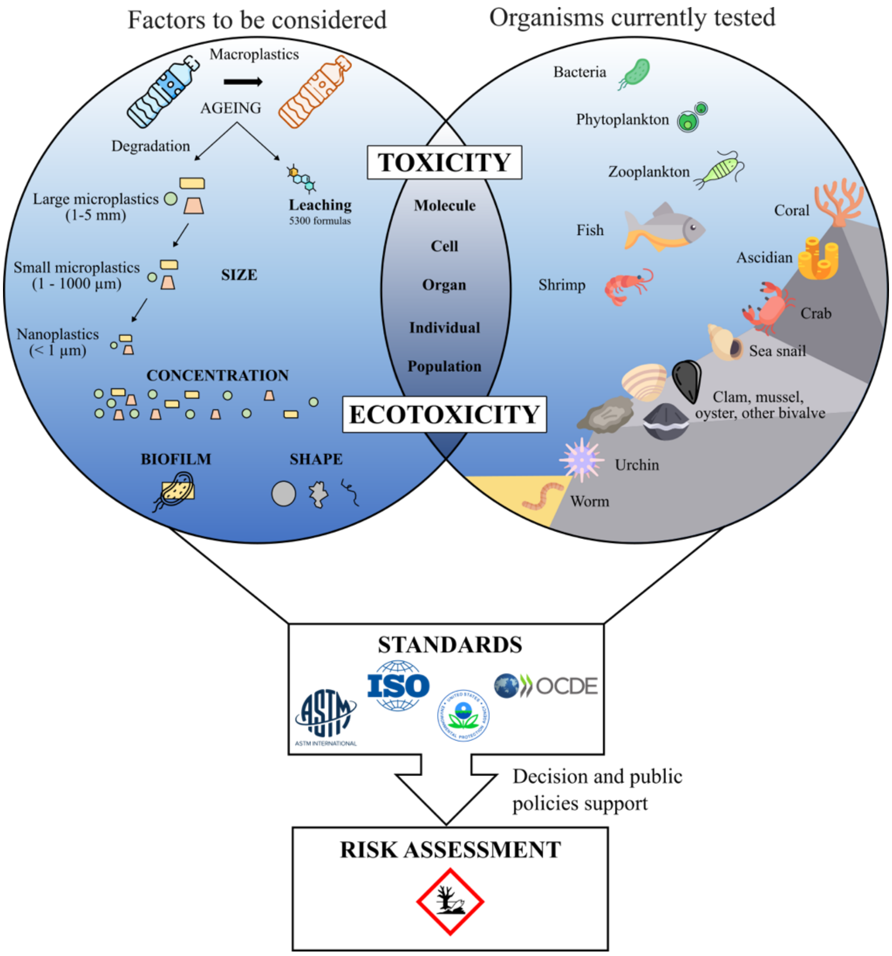
Highlights :

- Recurrent toxic effects of plastic debris seen from molecular to population levels
- Tested conditions (concentration, type, size, shape) lack environmental relevancy
- Environmental studies on plastic debris are scarce
- Actual toxicity standards are not adapted to plastic

Graphical abstract



Abstract:

The increasing production of plastics together with the insufficient waste management has led to massive pollution by plastic debris in the marine environment. Contrary to other known pollutants, plastic has the potential to induce three types of toxic effects: physical (e.g intestinal injuries), chemical (e.g leaching of toxic additives) and biological (e.g transfer of pathogenic microorganisms). This critical review questions our capability to give an effective ecological risk assessment, based on an ever-growing number of scientific articles in the last two decades acknowledging toxic effects at all levels of biological integration, from the molecular to the population level. Numerous biases in terms of concentration, size, shape, composition and microbial colonization revealed how toxicity and ecotoxicity tests are still not adapted to this peculiar pollutant. Suggestions to improve the relevance of plastic toxicity studies and standards are disclosed with a view to support future appropriate legislation.

Keywords: plastic debris, microplastics, nanoplastics, ecotoxicity, standards, quality assessment

1. Introduction :

Plastic refers to a man-made material composed of polymers to which additives are supplemented to confer specific properties to the material [1]. It is used in a wide variety of sectors, from packaging to electronics but also through construction, farming or transport [2]. This ubiquity is based on its low production costs and great variety of properties (e.g., lightweight, resilience, resistance to corrosion, ease of processing), explaining its use for a wide range of applications. Therefore, the plastic production followed an exponential increase since the 1950s. It almost doubled in the last twenty years, going from 234 to 460 millions of tons/year [3].

The increase of plastic use leads to a significant waste production and thus to an important pollution all around the world [4], and especially in the oceans which are the final receptacle of mismanaged land-based wastes [5]. Through different natural processes (light, heat, mechanical impact or biodegradation), plastics are fragmented in microplastics (MPs) (<5mm) that are subcategorized in 3 size classes: large microplastics (LMPs) (1-5 mm), small microplastics (SMPs) (1-1000µm) and nanoplastics (NPs) (< 1 µm) [6]. MPs are, in terms of number, the most dominant size-class of plastics in the oceans [7]. In fact, according to a mathematical model, there are more MPs in the oceans than stars in the Milky Way [8]. The roots of the plastic issue lies in the dissonance between its single-use and one of its key features: durability. Its omnipresence is a growing concern for the entire marine ecosystem and represents physical, chemical and biological threats. The mechanical hazard corresponds to, for example, an obstruction or injury of feeding organs [1]. Plastic also induces chemical toxicity through the release of additives or the sorption of environmental hydrophobic pollutants [9]. Possible transfer from pathogenic strains from the microbial life living on plastics (so-called plastisphere) to an organism upon ingestion constitutes a biological threat [10,11]. The research interest on the toxic impacts of plastic has intensified in the last decade. Toxicity, defined as the potential for biological, chemical or physical stressors to affect an organism [12], is more studied on plastics than ecotoxicity, referring to the potential effects of stressors on an ecosystem, probably due to the higher level of complexity in the evaluation [13]. This research effort is, however, necessary for an effective ecological risk assessment (ERA), which supports public policies [14]. ERA is defined as the assessment of the severity (nature and magnitude) and the probability of effects to nonhuman organisms, populations and ecosystems) [15]. Contrary to other pollutants, no concentration threshold is indicated for the current seawater quality assessment, enlightening the lack of efficient standards to evaluate plastic toxicity. Indeed, the actual standards are mostly adapted to chemical toxicity that require dissolvable products, which is not compatible to plastic. We provided here some recommendations towards a better environmental relevance for future toxicity tests. Because standards are crucial for public policies and regulatory organizations, their limits and key points for their improvement are also disclosed.

The objective is not to produce an exhaustive list of toxic effects observed, since other reviews already treated this aspect [16,17]. In this review, we give a balanced critical overview of the literature on plastic toxicity in the marine environment. To ensure a base level of quality assurance, only peer-reviewed articles were selected. From the 87 articles reviewed, we selected 50 articles for this analysis. The selection criteria were a minimum of 20 citations (median of 86 citations, except for articles published after 2022) together with recent publication (96% were published in the last decade). We used common databases (ISI Web of Knowledge, Elsevier and Google Scholar) with search terms including: plastic, microplastic, synthetic polymers, toxicity, marine organisms. The following information was retrieved: species, type of plastic, size, shape, concentration, single and/or multiple exposure, duration of the test, endpoints and observed effects. The endpoints were classified in different levels of biological integration according to [18]. Even though a consequent literature study was performed, the studies retrieved might not be fully representative of the entirety of the published articles.

2. Evidence of microplastic toxicity on marine organisms at the molecular, cellular, organ, individual and population levels.

A compilation of the effects of MPs toxicity on marine organisms at the molecular, cellular, organ, individual and population levels is summarized in Figure 1. For a more detailed description of effects in relation to the species and corresponding references, see SI Table 1. The most studied effects were first at the population (54 tests), individual (44 tests) and molecular (39 tests) levels, followed by tests at the cellular (22 tests) and organ levels (13 tests).

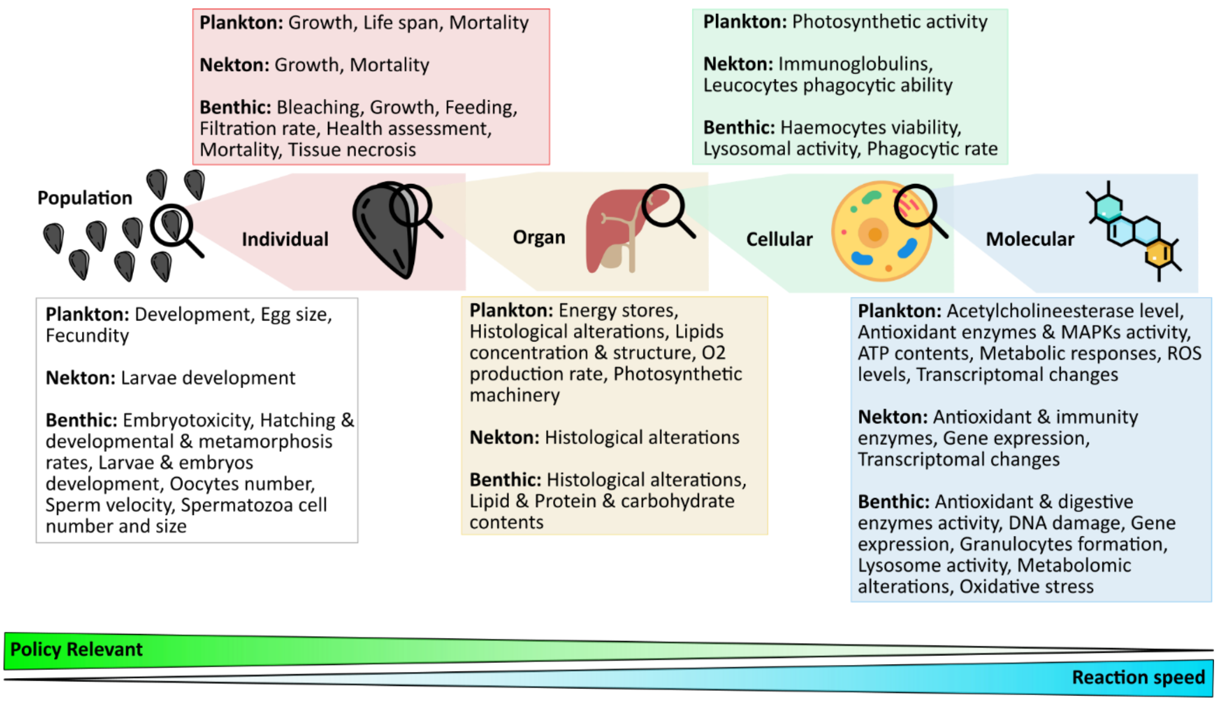


Figure 1: Compilation of the observed effects of plastic toxicity on marine organisms described at the molecular, cellular, tissue, individual and population levels in the plankton, nekton and benthic species.

2.1. Toxic effects at the molecular level

The evaluation of toxicity at the molecular level aims to decipher subtle impacts of plastic pollution on organisms through stress mechanisms involving gene expression, enzymatic activities, oxidative stress and metabolomic alterations. For instance, impact of MPs exposure on gene expression was observed on several marine organisms, from bacteria, with a decrease in transcription of genes associated with carbon fixation or cell wall transport [19], to fish, for genes related to lipid, steroid oxidation and inflammatory response [20–24]. Enzymatic activities were also modified in many species, from plankton (antioxidant and neurotransmitter enzymes) [25–28] to bivalves (antioxidant and digestive enzymes, lysozyme) [29,30] and fish (antioxidant and immunity enzymes) [21,22]. Oxidative stress was observed on plankton [25,26,28,31], worms [32], and bivalves with an increase of ROS content and broken DNA strands [33–35]. Metabolomic alterations after MPs exposure were also identified in microalgae (glycerophospholipids, carbohydrates, amino acids and ATP content), bivalves (hemolymph proteome) [36] and fish (lipids, serum composition) [23,24].

2.2. Toxic effects at the cellular level

A large number of endpoints are available on cells, the smallest unit of life, encompassing the membrane stability, phagocytic response, hemocytes viability and mitochondrial metabolism. In the literature, MPs exposure led to the modification of not only the cell content of plankton (lipids and pigments) [28,37,38] and bivalves (lipids, proteins and carbohydrates) [39] but also the cell structure of diatom (thylakoid and lipid structure) [38], worms (lipid droplets, secretory vesicles) [32] and bivalves (lysosomal membrane stability) [35]. In many cases, immune cells were also affected, such as fish's leucocytes, immunoglobulin production and phagocytosis activity [22]. In addition, hemocytes' viability and granulocytes' number in bivalves were negatively impacted [35,40]. Cell functioning was impacted for planktonic organisms [19,31,38] and zooxanthellae corals [41] through a reduction of photosynthetic efficiency. At last, microplastics also modifies the mitochondrial metabolism of mussels [42].

2.3. Toxic effects on tissues

Scientific articles at the tissue level focused on the effects of MPs on the histopathology, energy reserves and metabolism demand. After MPs exposure, histopathological alterations were observed on microcrustacean juveniles (eradication of the basal lamina and epithelial layer) [27], and on fish (histological alterations) [22,43]. Toxic effects on tissue functions were also observed on bivalves (epithelial deteriorations, hemolymph infiltrations in gills, reduction of cilia) [40,44].

2.4. Toxic effects at the individual level

Toxic effect at the individual level has been classically evaluated by health assessment, survival and growth of individuals. Impacts of MPs exposure on health were observed on several organisms, from bleaching and tissue necrosis for corals [41,45] to feeding disruption for worms [32] and bivalves [40]. Survival of plankton [27,46,47] and fish at different developmental stages [21,48] were affected, with a large increase in mortality. The growths of many species were also impacted, from plankton [19,25,28,31,37] to fish [48] and benthic organisms such as ascidians [49], sea snails [50] and corals [41,51,52].

2.5. Toxic effects at the population level

Toxic effects at the population level are more ecologically relevant, classically used for decision making and support to public policy. Behavioral changes were observed on corals (polyp activity and prey capture rate) [51,52] and mollusks (number and tenacity of byssal threads) [36]. In addition, swimming activity was impacted for microalgae [28], microcrustacean [27] and bivalve larvae [53]. Population recruitment of copepods and rotifers was shown to be troubled [25,26] and benthic organisms such as bivalves [53–57] and sea urchins [49,58–62] also displayed several signs of alteration of their fecundity (low hatching rate, sperm velocity or fertilization rate, small gamete number or diameter) and larval development (larval malformation, low larval growth or metamorphosis rate) after MPs exposure. The severity of these effects at the reproductive level is of main concern, since reproduction ensures the continuity of species and prevents their disappearance. Impacts on fertility, fecundity, recruitment and offspring development of a species can have consequences at the population level [18,55], but also for other species with which they interact and for the ecosystem.

3. Ecotoxicity of plastics

Evaluating *in situ* effects of plastics on organisms is challenging due to the tampering of the marine environment with numerous chemical and trash [63], but also the existence of other sources of stressors (e.g. ocean warming and acidification, habitat degradation, diseases). Therefore, the origin of the toxicity assessed might not be directly linked to plastics, even if they are present in the organisms according to their size.

3.1. Ecotoxicity of macroplastics

Compared to MPs, fewer laboratory experiments studied the physical impact of macroplastics [51,52]. Since macroplastics are usually afflicting big size animals, the experiment set up is more complex and it is challenging to produce a comparable natural physical control with same sizes [64]. Moreover, as regulations on manipulations of living beings in laboratory are more and more restrictive, setting up experiments is laborious. Field studies demonstrated an evident impact of macroplastics on the marine wildlife. Significant effects linked to entanglement have been described since 1997 for birds, turtles and marine mammals [65]. With the increase of plastic pollution, the number of marine species of these three last animal groups with known entanglement increased from 20.5% in 1997 to 30% in 2015 [66]. Physical impact included also smothering, which can induce deleterious effects on marine vegetation [67] and corals [68], through shading effect or crushing due to weight. Corals were up to 89% more prone to disease when in contact with plastic waste (< 50mm) [69]. Ingestion of macroplastics was also a rising concern, with a clear increasing of ingestion percentage from 33% in 1997 [65] to 44% in 2015 [66] for bird, turtle and mammal species. Even though direct mortality was probably not the most relevant outcome of ingestion, it leaded to a partial blockage or damage of the digestive tract that contributed to poor nutrition and dehydration [70]. Evidence of fibrosis was disclosed in a recent field studies on seabirds [71]. Interestingly, other natural particles such as pumices did not exert similar effects.

3.2. Ecotoxicity of MPs

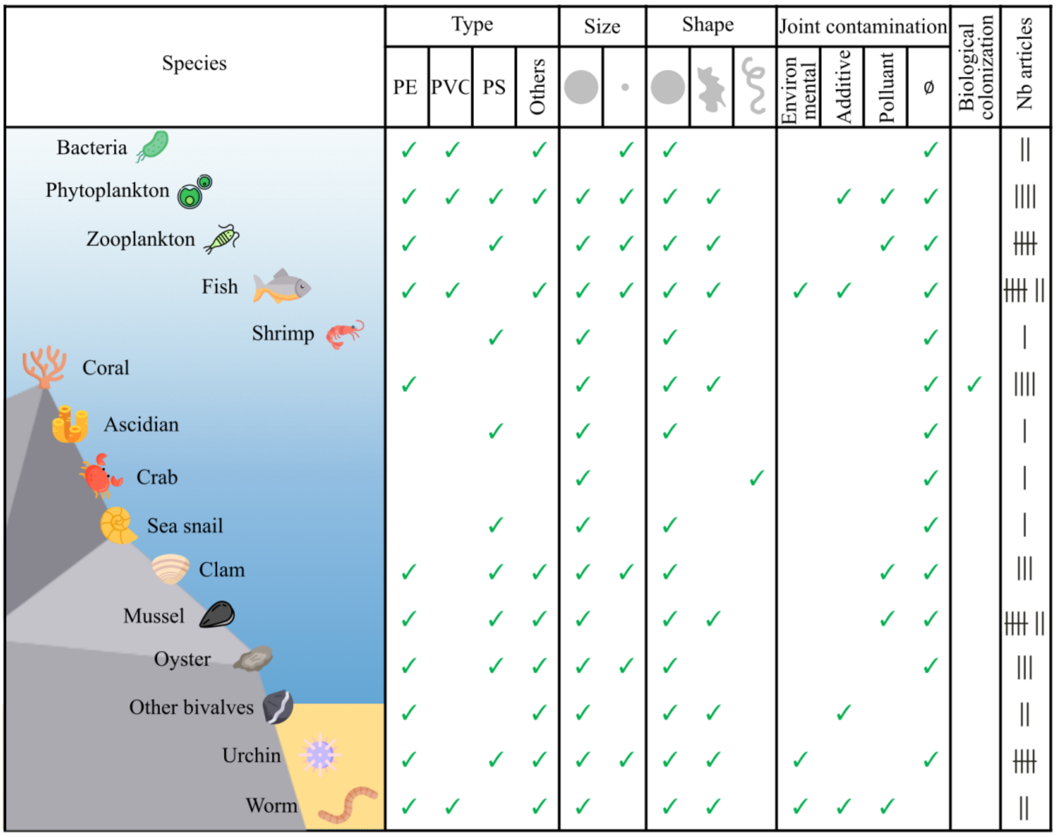
A few experiments mimicked the impact of MPs on the biodiversity and ecosystem functioning, mainly on bivalve and lugworm habitats. Those experiments in controlled mesocosm conditions resulted in a higher filtration rate for oysters (*Ostrea edulis*) but a lower filtration rate for mussels (*Mytilus edulis*) when exposed to Polyethylene (PE) and Polylactic acid (PLA) [72,73]. While for mussels, only the filtration differed from the control, for oysters the primary productivity of microphytobenthos (lower cyanobacteria biomass), the porewater nutrients (increase of ammonium) and the invertebrates and macrofaunal assemblages were impacted. Likewise, in a similar experiment set up with lugworms (*Arenicola marina*), the microphytobenthos was altered upon exposure of PE, PLA and Polyvinyl chloride (PVC) [74]. In addition, an increase in O_2 consumption by the lugworm and the bioturbation was reported, with a dose-dependent reduction in number of surface casts [74].

3.3. Transfer along the trophic chain

The ingestion of plastics by marine biota has been demonstrated in laboratory experiments [26,61] and also in the environment [75]. The residence time of MPs in the gut was closely linked to the size, shape [76], roughness [20], and of course the species [77]. Despite the evidence of MPs being ingested, a question subsists: do MPs manage to rise along the food web? A semi-systematic review underlined that MPs did not biomagnify along the marine food web and that there is currently no risk to human health when considering the current literature [78]. However, few articles showed that NPs were transferred from preys to predators. For instance, trophic transfer from mussels to crabs has been demonstrated experimentally [79]. NPs were observed in the stomach, hepatopancreas, gills but also in the ovary of mussels. The number of NPs in crabs hemolymph increased just after ingesting the contaminated mussels. Another study showed that NPs could be transferred from algae exposed to polystyrene (PS) NPs to herbivores (*Daphnia magna*) and fish (Crucian carp), thus causing behavioral changes such as slower movement and less hunting but also disturbance in the lipid metabolism for the top consumer [80]. Even though a trophic transfer is present, no biomagnification of SMPs has been observed [78]. For example, the effect of SMPs exposure on beach hopers found no behavioral change [81].

4. Plastic characteristics (concentration, duration of exposure, size, shape, chemical composition and biological colonization) as crucial factors for comparable toxicity tests.

Plastic characteristics used in the current literature were gathered and summarized in Figure 2, in order to evaluate the relevancy of actual toxicity studies. For a more detailed description of these characteristics, see SI Table 1.



Species	Type				Size		Shape			Joint contamination				Biological colonization	Nb articles
	PE	PVC	PS	Others						Environmental	Additive	Polluant	ø		
Bacteria	✓	✓		✓		✓	✓						✓		II
Phytoplankton	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓		IIII
Zooplankton	✓		✓		✓	✓	✓	✓				✓	✓		卌
Fish	✓	✓		✓	✓	✓	✓	✓		✓	✓		✓		卌 II
Shrimp			✓		✓		✓						✓		I
Coral	✓				✓		✓	✓					✓	✓	IIII
Ascidian			✓		✓		✓						✓		I
Crab					✓				✓				✓		I
Sea snail			✓		✓		✓						✓		I
Clam	✓		✓	✓	✓	✓	✓					✓	✓		III
Mussel	✓		✓	✓	✓		✓	✓				✓	✓		卌 II
Oyster	✓		✓	✓	✓	✓	✓						✓		III
Other bivalves	✓			✓	✓		✓	✓			✓				II
Urchin	✓		✓	✓	✓	✓	✓	✓		✓			✓		卌
Worm	✓	✓		✓	✓		✓	✓		✓	✓	✓			II

Figure 2: Compilation of the MPs' characteristics in toxicity experiments: chemical composition, size, shape, and biological colonization (= plastisphere). PE = Polyethylene, PVC = Polyvinyl Chloride, PS = Polystyrene, = Small Microplastic (1-1000µm) = Nanoplastic (<1µm), = Regular shape, = Irregular shape, = Fibers (for more detailed information, see SI Table 1)

4.1. Plastic concentrations used in the toxicity tests

A comparison of MPs concentrations used in the literature enlightened that toxicity tests are generally far to be representative of environmental concentrations, which themselves are heterogenous in function of the location, meteorological parameters and time (Fig. 3). Most studies (94%) used concentrations 10 to 10^{14} times higher than the highest concentration measured in surface seawaters (150 particles/L, > 0.75µm) [82], although this concentration can be mitigated by sampling biases. Quantification of MPs were generally performed by using manta nets with 333 µm mesh size [83–85] , thus missing the non-negligible portion of small MPs and NPs. Sampling were mostly performed at the sea surface or sub-surface, leaving the deeper part of oceans poorly attended [86]. Other environmental factors such as the proximity of the coast or water currents present in the ocean were shown to induce a high variability of MPs and NPs concentration [84,87]. Methodological developments were necessary to assess small MPs and NPs invisible by eyes that need further field studies both in the water column and benthic environments [88].The mean and median concentrations used in these studies were equal to 4×10^{8} and 10^{6} particles/L for SMPs, the latter being 10^{3} higher than the highest concentration recovered in the environment. For NPs the mean value was equal to 3×10^{14} particles/L and the median to 10^{12} particles/L. It must be noticed that the concentration of MPs reported in the marine environment varies significantly depending on the geographical location and it has generally been estimated to MPs larger than 333 µm (*i.e.*, manta net mesh size), which underestimates the real concentration of MPs. Indeed, the environmental MPs concentration measured with a 100 µm manta net is 2.5 times higher than using a classical 333 µm net, and 10-fold greater than a 500 µm net [89]. Another study underlined that SMPs that are poorly identified by classical manta sampling may represent similar weight but contain 10^{2} to 10^{5} more particles/L than LMPs [90]. Moreover, in surface waters, 86% of MPs were < 100 µm in the North Sea [91]. Therefore, some high concentrations in those articles may be more environmentally realistic than firstly thought. Another drawback for an effective comparison with environmental concentration is the unit of measure. Indeed, the unit of measure used in most toxicity studies is mg/L, which is convenient for the preparation of MPs solution by weighting. However, environmental concentrations units are, in majority, expressed as number of particles per m^2 for surface waters, per m^3 or per L in the water column, or per kg for sediment. Amongst the selected experimental studies, only a few expressed concentrations in particles/L [30,45,50–52,61,92]. To make these studies comparable, we propose that authors also provide information on the number of particles per liter or per gram of sediment, which can bring more information than only weighting that is very size dependent. Using the measure in weight per unit of volume may have severe drawback. Indeed, we calculated that a solution with 1 mg/L of perfectly spherical MPs with a diameter of 500 µm will contain 15.3 particles/L whereas a solution with the same concentration with a diameter of 1 µm will contain 1.91 x 10^9 MPs/L, thus increasing greatly the bioaccessibility. A formula : $MPs/L = \frac{\left(\mathrm{weight}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right)\times 3\right)}{4\pi\times radius(\mu m)^3\times 10^{-12}\times density(g.cm^3)\times 10^3}$, has been elaborated to link the number of plastic particles to their weight per unit of volume, assuming that particles were all spherical. Because of possible biases of this assumption, we propose that authors provide information both in the number of particles (using laser granulometry for instance) and weight per unit of volume when running toxicity tests on MPs.

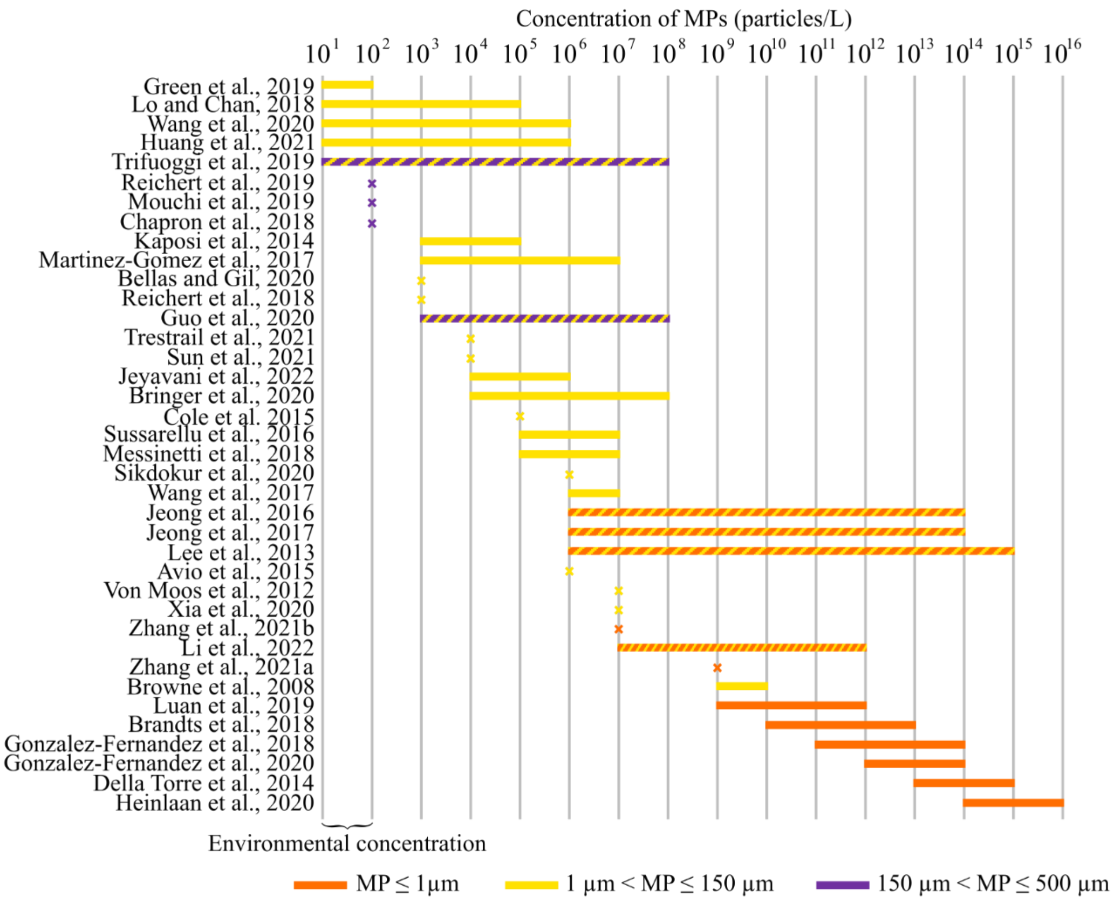


Figure 3: Range of MPs concentration (particle/L) used in the reviewed articles. When needed, an approximation of number of particle/L was calculated from data initially expressed mg/L (see conversion formula in the text).

4.2. Duration of exposure

Another critical parameter in toxicity tests is the duration of exposure. We distinguish between acute tests, which are short-term tests with usually, high concentrations of pollutants, and chronic tests, which are long-term tests with relatively lower concentrations [93]. We included an intermediate term “subchronic”. These terms are closely related to the life span of the species tested and were adapted from [94]. For bacteria and algae, a toxicity test was considered chronic when the experiment lasted a complete life cycle. Subchronic was between half and a full life cycle, whereas acute was determined for toxicity tests with a duration of less than half of a life cycle. However, for every other organism with longer life expectancies, we adapted the duration from the standard ASTM E2455-22 for freshwater mussels which determines an acute, subchronic and chronic toxicity tests with duration of <7days, between 7 and 28 days and >28 days, respectively.

We observed an almost even repartition of the duration of the experiment in the literature, with a slight dominance of acute toxicity tests. Indeed, 40% concerned acute toxicity tests, 27% mid-term toxicity tests and 33% chronic tests (Fig. 4). The median of the minimum and maximum concentrations (only in MPs/L) used in the different toxicity tests was calculated in function of the duration. Acute toxicity tests used higher concentrations (median min and max: 10^5-10^8 MPs/L) than mid-term (median min and max: 10^5-10^6 MPs/L) that were higher than chronic toxicity test (median min and max: 10^3-10^6 MPs/L).

Acute tests allow to determine the lethal dose (LD50) or the effect concentration (NOEC and LOEC) with small set-ups and a high number of replicates. Moreover, various parameters (e.g. concentration, size, shape) can be tested at low costs. Even though, chronic experiments are limited concerning the beforementioned assets, they are more representative of environmental conditions where organisms are continuously exposed to a relatively low plastic concentration. Both of these tests' duration are needed and can be complementary. Indeed, with the vast quantity of different plastic types and additives acute toxicity experiments fit perfectly to assess quickly the impact of a wide variety of plastic. After this first categorization a more focused chronic study could be performed to analyze in depth the impact of previously determined plastics.

We recommend that preference should be given to a combination of acute and chronic toxicity tests that consider several life stages and sensitivity of the organisms. The size also plays a decisive role on the chosen concentrations since a higher bioaccessibility is generally associated with smaller size (see section 4.1)

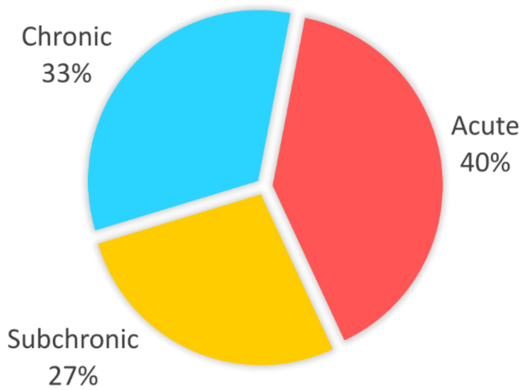


Figure 4: Repartition of experiments' duration in the reviewed articles

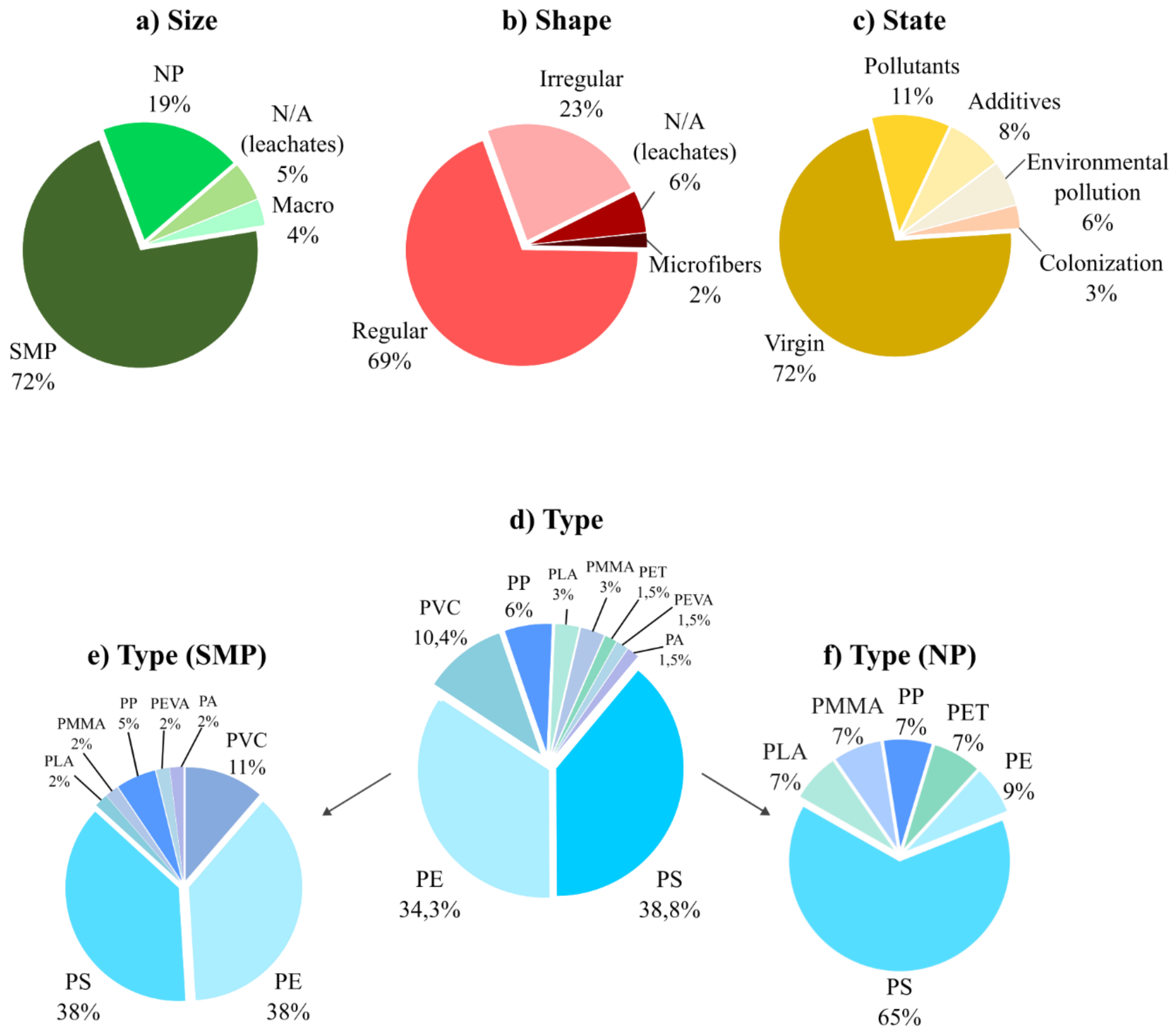


Figure 5: Characteristic of the plastic used in the reviewed articles: size (a), shape (b), presence of additives and adsorbed pollutants (c) or polymer composition (d). Chart (e) and (f) decomposes the polymer composition in function of size class. PE = Polyethylene, PLA = Polylactic acid, PMMA = Polymethylmethacrylate, PP = Polypropylene, PS = Polystyrene, PVC = Polyvinyl chloride; B-Plastic sizes used in experimental studies. SMP = Small microplastic (1-1000 µm), NP = Nanoplastic (1-1000 nm) and N/A = leachates (dissolved).

4.3. Range of plastic sizes used in toxicity tests

SMPs represent the majority of the tested microplastics, as they were used in 72% of the selected of studies for this review (Fig 5a). Nanoplastics (NPs) were used in 19% of the selected articles, whereas only 3 studies used leachates and 2 others used macroplastics.

Several studies enlightened the importance of plastic size in relation to ingestion rate, transit and the resulting potential toxicity on organisms. For example, the increase of abnormal larvae of oysters (*Crassostrea gigas*) was much greater with 4-13 µm compared to 25 µm size SMPs [53]. The impact on protein content in sediment-dwelling bivalves was also significantly higher for large SMPs (125-500µm) compared to smaller SMPs (6 and 25 µm) [39]. The same tendency was observed in NPs, which were shown to be differentially ingested at a dispersed (< 1 µm) or aggregated (> 100 µm) state in mussels (*M. edulis*) and oysters (*Crassostrea virginica*) [95]. NPs with a size of 26 nm induced toxicity for the bacteria *Vibrio fischeri*, whereas no effect was observed with NPs of 100 nm size [46]. Likewise, 50 nm-size NPs increased the mortality of copepods but did not affect their fecundity, whereas 6 µm-size SMPs had no impact on their mortality but had an effect on their fecundity [26,47]. These results enlightened the crucial role played by the size of the plastic debris in relation to the size of the organisms that would greatly influence the toxicity outcomes. It must be noted that the decrease in particle size did not result in an increase of toxicity. In fact, the opposite was observed in a literature review, where a higher concentration of smaller particles was required to induce an effect [96]. We recommend to fill the gap of knowledge on NPs in further toxicity tests since they are the most abundant type of plastic in the marine environment in terms of particle numbers [89,90] and also because the smaller the size, the greater is the potential for uptake by organisms. As they are mostly derived from the degradation processes of MPs, we also recommend to use in priority NPs obtained from MPs by grinding rather than commercial particles [97]. The presence of NPs together with its eco-corona is also recommended in toxicity tests in order to fit with natural conditions [98].

4.4. Plastics shape used in toxicity tests

Distinction was generally made between primary MPs, purposefully manufactured in small size, and secondary MPs that result from the weathering and breakdown of larger plastic items. Primary MPs usually possess a spherical or cylindrical shape (*i.e.*, regular shape), whereas secondary MPs present various irregular shapes [1]. The majority of the reviewed articles used MPs of uniform shape for toxicity tests (Fig. 5b). However, spherical primary MPs represent a negligible part of the total MP pollution all over the world [99–102]. Those results highlight that the use of uniform shape is not the most representative of the environmental MP pollution. The shape influences the ingestion of MPs depending on the species [77], which is probably linked to prey selectivity. The shape also influences the toxicity: irregular fragments were shown to induce higher toxic effects on *Daphnia magna* [103–105]. In addition, secondary MPs tended to provoke more intestinal injuries than primary ones [20]. The shape plays a role in plastic toxicity [106] and since the environmental shapes of plastics are mostly fibers or irregular ones, we recommend using those shapes in relation with the model species used (what is preferentially ingested) and the experiment goal. For example, true-to-life MP and NP resulting from the cryogrinding degradation of plastic goods is gaining interest [107,108].

4.5. Polymer composition of plastics used in toxicity tests

The mostly used polymer types in toxicity tests were PS, PE and PVC, with 39%, 34% and 10% of the reviewed articles, respectively (Fig. 5d). A similar repartition of polymer composition was observed for SMPs in toxicity tests (Fig. 5e). However, in the NPs toxicity tests, there was an important predominance of PS, because standardized PS nanospheres are commercially available with a great variety of sizes and functionality (Fig. 5f). PE (including low and high density) is the most commercially produced polymer and constitutes the major source of plastic pollution on Earth [2]. PVC occupies an important fraction of the toxicity studies because standardized microbeads are commercially available, although its presence in the marine environment is low compared to other plastics [86]. This review analysis indicates that there is a gap between the polymer types used in the toxicity studies and their respective representativeness in the environment. For example, PP has only been used in 6% of the selected toxicity tests, whereas it is the second most abundant polymer at the sea surface [86]. Another concern is the lack of studies using polyesters (PES), polyamides and acrylics, which are among the most abundant polymers in the water column and in sediments [86]. This lack of studies is probably because those polymers are a complex material to study. Indeed, fibers are difficult to obtain and were poorly quantified in the environment, even if a recent study started to tackle this issue [107].

It is noteworthy that the proportion of polymer types within the plastic litters sampled in the environment was rather stable. Even if local disparities exist, notably in coastal zones, the effects of the watershed and local activities (such as industries, tourism, wastewater treatment plants or water currents closed to the sampling areas) were of major importance in the observed plastic pollution. By instance, we emphasize here the need to broaden the scope of polymer types used in toxicity tests, and especially for PP, PES, Polyamide and acrylics that suffer from a severe lack of studies compared to their omnipresence in the environment.

Heterogeneous results were observed when comparing the toxicity of different plastic types [22,37], or the same effect was observed, regardless of the polymer composition [37,59]. The mortality of *Vibrio fischeri* was only linked to the presence of additives [46] , whereas a material specific toxicity was observed for *Daphnia magna* [108]. Those results indicate that plastic toxicity is closely linked to its chemical composition as a whole, i.e. polymer and additive.

4.6. Plastic additives and adsorbed pollutants as part of plastic toxicity

Most of selected articles (>72%) used pristine MPs and do not take into account the possible adsorption of pollutants (*e.g.*, PCBs, organochloride pesticides, PAHs, heavy metals, biotoxins) [109,110] (Fig. 5c). This is probably because reproducing an environmental pollution is complicated since no homogeneous concentrations of pollutants are present in the environment. Some authors underlined that a pre-incubation of pristine plastics in the natural environment before the tests would be a more realistic situation, because it would take into account the possible leaching of plastic additives together with the possible adsorption of environmental pollutants on plastics [43]. Another option would be to test the toxicity of plastic collected in the natural environment, even if such approach would need a large number of samples to counterbalance the variation due to local environmental conditions and to the various history of the plastics [48,58]. The studies evaluating the impact of plastic additives were performed in laboratory conditions, through plastic leaching [19,56]. Other studies tested the impact of adsorbed pollutants by adding one selected product (hydrocarbon, pesticide or metal) together with plastics [37]. It is difficult to consider that these laboratory experiments fully mimic the wide range of combination between plastic additives and adsorbed pollutants encountered in the environment. In any case, the part of hydrophobic organic chemicals hold by MPs could be negligible compared to the part brought by natural particles which are much more numerous in nature [111] leaving this question under debate and calling for further *in situ* exploration.

4.6.1. Toxic impact of plastic coupled with additives

Plastics are generally produced with a range of chemical additives such as plasticizers, flame retardants, antioxidants and other stabilizers, pro-oxidants, surfactants, inorganic fillers or pigments, thus resulting in more than 5300 grades of synthetic polymers for plastics in commerce [112],[113]. Opposite effects were observed when MPs were co-exposed with additives. Triclosan had a significant impact on feeding and survival of lugworms (*A. marina*) when coupled with PVC particles, as compared to the additive alone. However, the effects of polybrominated diphenyl ethers (PBDE-47) were similar whether PVC particles were present or not [32]. Scallops (*Chlamys farreri*) displayed a significant decrease of their phagocytic rate when PS microparticles were added to decabromodiphenyl ether (BDE-209) [44]. On the other hand, the toxicity of triphenyl phosphate was decreased when coupled with PS particles [24].

The leaching of additives from plastic is linked to several factors ranging from the polymer type, texture, and strength of its bond with the additives, to the physicochemical properties of the additives themselves as well as the exposure media/surrounding environment characteristics. Laboratory analyses on leaching additives suffer from methodological differences (e.g. leaching period, initial state of plastics, temperature or presence of light) hindering comparisons between the studies [114]. Moreover, the exact composition of plastic is usually not accessible and since a wide variety of additives are used, the comprehensive analysis of leachates is challenging [114]. Many additives were already recognized as endocrine disruptors [115] or “harmful for aquatic organism” or “causing long-term adverse effect in the aquatic environment” [116]. Their ubiquitous presence in marine waters [9] could indicate a desorption into the environment. Nevertheless, those compounds have many sources, *e.g.* Polychlorinated Biphenyls (PCBs) are used for dielectric or adhesives substances [117] and Polycyclic Aromatic Hydrocarbons (PAHs) can be introduced via urban runoff of oil spillage [118]. Even though, the leaching of additives from plastics was proven and resulted in toxicity [19,56], its overall impact on the marine ecosystem is yet to be determined. The “coho salmon case” is an exemplary demonstration that linked chemical signatures of tires in urban runoff and freshwater samples and abnormal mortalities of *Oncorhynchus kisutch* over decades in western North America [119].

4.6.2. Toxic impact of plastic coupled with environmental pollution

Few studies assessed the toxicity after pre-incubation of plastics in a marine environment, in order to evaluate the possible effects of the release of additives in the environment or the possible effects of adsorption of various and unknown pollutants on plastics. They showed higher toxicity for pristine MPs. Indeed, glassfish (*Ambassis dussumieri*) exposed to virgin and environmentally polluted MPs lead to the same growth decrease in mass, length, and body depth, but survival probability was lower for virgin rather than environmentally pre-incubated MPs [48]. Pristine plastic also led to more severe histopathological alterations in European seabass (*Dicentrarchus labrax*) than environmentally pre-incubated plastics for the first two month, even though it became similar after three months of exposure [43]. Higher toxicological effect (abnormal larvae development) was also found when comparing pristine to environmentally pre-exposed plastics for sea urchins (*Lytechinus variegatus*) [58]. These studies concluded that the leaching of additives might be a factor leading to a higher toxicity of the pristine compared to environmentally pre-incubated MPs.

4.6.3. Toxic impact of plastic particles coupled with chemical pollutant

Another set of studies evaluated the impact of other chemical contaminants (hydrocarbons, pesticides, metals) added before (test of adsorption on plastics) or during the plastic exposure (co-exposition). The sorption of pollutants on plastic particles has been well documented, and the use of plastic waste was even suggested as a potential sustainable approach in remediating environmental pollution [109].

The combination of PS and PE MPs with pyrene resulted in an increased frequency of micronuclei in hemolymph cells of mussels (*Mytilus galloprovincialis*) [35]. An increase of toxicity, by addition of chlorpyrifos with PE MPs, was found on copepods (*Acartia tonsa*), when compared to the exposition of solely the pollutant [120]. Co-exposure of PS MPs and tetrabromobisphenol A on two microalgae was shown to be more toxic than single exposure, suggesting a synergistic effect [28].

Although adsorbed pollutants on plastic sometimes increased its toxic effect on marine organisms, decreased toxicity was also observed in other experiments. The combination of PVC together with phenanthrene and nonylphenol polluted sand was less toxic for lugworms (*Arenicola marina*) than solely exposed to the polluted sand [32]. Another study showed that mercury pre-sorbed on PE particles was poorly transferred on clams (*Ruditapes* *philippinarum*) compared to mercury alone [40]. In addition, the phenanthrene stress induced on diatoms was minimized by the addition of MPs [37] and several types of MPs decreased sulfamethoxazole (SMX) toxicity on the microalgae *Skeletonema costatum* [31]. However, two studies suffered from methodological limits. Due to lugworms' diet (sand), a higher desorption effect from polluted sand rather than MPs did not imply a negligible vector role of MPs [32]. Moreover, the particle size was too big to be ingested by microalgae and since plastics act as sponge for pollutants, they could have reduced the pollutant accessibility [37]. The laboratory concentrations of pyrene and phenanthrene adsorbed on MPs were environmentally relevant for plastics located on beaches [121]. However, when comparing with plastics recovered in marine waters, only phenanthrene is representative of concentration recovered in the environment [122]. However, representativeness towards environmental concentrations is unknown for these studies [40,120] since the quantity of pollutants pre-sorbed on plastics was not measured. The impact of pollutants adsorbed on plastics compared to the contamination through other media is challenging due to the unit difference: weight/L for environmental concentrations and weight/g for surface plastic concentration.

These contradictory results prevent from making any clear conclusions on the impact of adsorbed pollutants on plastics and further analysis are needed to better understand the potential impact of the combination between chemical pollutants and plastics. Nevertheless, the hypotheses under which MPs act as vectors for chemicals has been severely questioned. Indeed, the bioaccumulation flux of hydrophobic organic pollutants from ingested MPs was found negligible compared to its bioaccumulation through preys [111].

4.7. Taking into account the biofilms growing on plastics in toxicity tests

A growing body of literature described the microorganisms living on plastic debris (so-called plastisphere), including putative animal or human pathogens [123]. The plastisphere is involved in the plastic debris buoyancy, which influence its bioavailability and its palatability. When a MP together with its biofilm is ingested, a transfer of microorganisms to the host microbiome has been described for several species [124,125]. To date, only a few toxicological studies used a pre-incubation step of plastic pieces in the marine environment [51,52], which would be more realistic considering the omnipresence of microorganisms on their surface [123]. Moreover, several studies indicated that the plastisphere eased up the ingestion of MPs for some organisms. For example, copepods (*Eucalanus pileatus* and *Schizopera* sp.) did not consume any pristine MP particles but were differentially attracted by MPs covered by a biofilm [126], [127]. Copepods chemically selected their food using long-range (particle capture) and short-range (particle ingestion) chemoreceptors at their mouth, thus explaining their ability to detect the nutritional values of the biofilm covering the MPs [126]. Similarly, example of oysters (*C. virginica*) ingested ten times more MPs with biofilm than pristine ones, in accordance to their preferential ingestion of organic compared to inorganic materials [128], [129]. Predators such as fish may also ingest MPs accidentally when attacking the plastic-fouling organisms [130]. The role of the plastisphere in plastic debris bioavailability and overall toxicity might also be overlooked when considering its importance in contaminants sorption kinetics on plastics [131]. Indeed, the adsorption of persistent organic pollutants (POPs), heavy metals and other contaminants were enhanced through the presence of a plastisphere on plastic [132,133].

We recommend to consider the role of the plastisphere in further toxicity analysis for more realistic experimental conditions, by incubating any plastic debris for at least one month in natural conditions. This time period has been shown to be sufficient for the development of a mature biofilm in the natural environment [134]. In addition, a characterization of the plastisphere is important in order to understand the role of the (at least) dominant species.

5. Evaluation of toxicity risk assessment

5.1. Regional, national and international initiatives to face plastic pollution

In the last decade, increasing international initiatives, law and policies denoted a growing political and societal concern on plastic litters in the environment [135], the last initiative being from the G20, G7 and UNEA process, supporting the set-up of an international treaty, under negotiation [136]. Numerous bans of single-use plastics (mainly plastic bags) entered in force in all the continents. Contrary to usual norm pattern dynamic, it emerges from the South to the North [137]. Africa is the continent where the largest number of countries (36 countries) instituted a prohibition of production and use of plastic bags [138]. In Asia, 4 countries, including India and China, introduced a ban on single-use plastic bags with in particular Bangladesh which implemented a ban since 2002. Several countries in Oceania imposed a national ban of plastic bags and only local bans have been enforced in Australia [139]. A list of single-use plastic items were banned in the European Union markets since 2021 (bags, cotton bud sticks, cutlery, plates, straws, stirrers, cups, beverage containers made of expanded polystyrene, exfoliating rinse-off cosmetic products, and all products made of oxo-degradable plastics) [140]. Recently, France aims to achieve the end of the marketing of single-use plastic packaging by 2040 [141]. In North America, a recent national ban is planned to be enforced gradually in Canada (2023-2025) for 6 single-use plastics (check out bags, cutlery, flexible straw, food service ware, ring carrier, stir stick and straw) [142]. In the United States, several states and cities instituted bans, however 11 states enforced countermeasures prohibiting local regulation on plastics bags [139]. Columbia, Chile, Panama, Bahamas, Haiti, Belize are the only countries of Central and South America that implemented national bans. In addition, several local bans were established in Argentina (Mendoza, Buenos Aires) and Brazil (Sao Paolo, Belo Horizonte, Rio de Janeiro) [139]. It is noteworthy that the majority of bans were limited to thin plastic bags (from <20µm to <100µm, depending on the country), meaning thicker plastic bags are still allowed [135]. Overall, these initiatives are used as a precautionary principle, based on (i) the overwhelming presence of single-use plastics in the environment, (ii) their ingestion by animals all along the trophic chain and (iii) their potential toxic effect observed on various animals under laboratory conditions.

Considering the difficulties of testing the large variety of composition of the targeted plastic items, none of these initiatives were based on relevant evaluation of ecological risk assessment (ERA). For example, in the case of plastic bags that have been banned in several countries, the exposition of marine animals has been proven because of their dispersion all over the world's Oceans [143,144]. Even though scientific articles analyzing plastic bags toxicity were published [56,145–147], no thorough ERA has been conducted. Most of the impact of plastic bags have been proven for digestive tract obstruction and entanglement on large mammals, such as turtles, sharks or seals and whales [148–151]. This contributed to growing media coverage and public awareness. Another study showed an increase of cold corals polyp activity but decreased prey capture rates after partial covering of living polyps (~50%) by plastic bags that acted as physical barriers for food supply [52]. Further studies are still needed to test more indirect toxicological effects, given the large variety of chemical composition of plastic bags that are generally based on PE but with a large variety of additives [152]. The toxic impact of plastic bags additives was analyzed through leachates.

However, the different leaching procedures (e.g., leaching time, T°C, agitation speed, light, shape and state of oxidation of plastic) make laborious comparison between the few articles available. As previously explained, there is a very large number of plastic composition and it is very difficult to tests them all. The clear labelling and listing plastic additive content would greatly facilitate the establishment of a relevant strategy for of ERA. Additionally, the reduction of the number of plastic additives, for example by removing in priority the substances supposedly the more potentially toxic, will allow to significantly reduce the multitude of possible formulation and facilitate ERA processes.

Finally, most of the current legislation leave the door open to biosourced and/or biodegradable plastic bags, except for oxodegradable plastics that have been banned in Europe [140]. Despite the fact that several studies underlined the limits of current standards to mimic the fate of so-called "biodegradable plastics" in environmental conditions [153], most toxicity tests on biodegradable plastics only concerned the polymers alone and do not yet take into account the toxicity of additives and degradation by-products [154].

Considering the large variety of composition of plastics and widespread dispersion in the Oceans, a more holistic view of plastic pollution is emerging by diverse stakeholders at the regional, national and international levels. There is an urgent need for further studies on accurate ERA measurements to support the current and future government measures and to increase their scope by being more realistic on the potential impact of plastic litters in the marine environment.

5.2. Plastic marine litters in the seawater quality assessment

In the last few years, plastic litter was selected as a criterion for water quality assessment in several countries. This was the case for the Canadian Water Quality Guidelines in 1999 for the Protection of Aquatic Life [155], the European amendment in 2019 to the Marine Strategy Framework Directive [156] and the United States amendment « Beaches Environmental Assessment and Coastal Health Act » in 2020 (not mandatory) to improve the quality of coastal recreation waters [157]. Contrary to other chemical pollutants, none of these guidelines gave threshold and they focused only on macroplastics, not on MPs. Considering the size range among MPs may lead to a large variability of behavior and toxicity, it may be relevant to consider specific sizes ranges that remains to be clarified for toxicity/ecotoxicity as done for air particles. Other guidelines on water quality assessment omit plastic, as the Australian and New Zealand Guidelines for Fresh and Marine Water Quality or the ASEAN Marine Water Quality Management Guidelines and Monitoring Manual [158]. Adding plastic in the water quality assessment with a specific monitoring is a step further to better evaluate plastic pollution. Data on the temporal and spatial dynamics of MP concentration are needed for ERA. Another critical aspect of an effective ERA is missing: the development and standardization of toxicity studies [159]. Unfortunately, this coincides with the vast majority of European projects concerning marine litter being focused on "Monitoring" whereas "Risk Assessment" projects were underrepresented [160]. We listed below three main aspects that should be taken into consideration for further improvement to include plastics in seawater quality assessment:

- *Plastic: a peculiar pollutant.* As explained above, plastic encompasses 3 levels of toxicity: physical, chemical and biological, making plastic a peculiar pollutant that should be assessed accordingly. Indeed, the existing frameworks for assessing environmental risks of pollutants, which are used in regulatory contexts worldwide, are yet to be applied to marine MPs. Such a generic ERA is composed of an exposure assessment, an effect assessment and a risk characterization and objectively determines the risk of a contaminant to marine ecosystems [161].

- *Regulation on chemical toxicity***.** The presence of harmful chemicals on commercial products is regulated by the "Registration, Evaluation and authorization of chemicals" (REACH) in the European market [162], by the "Toxic substances control act" (TSCA) in the US [163] and by the Canadian Environmental Protection Act (CEPA) in Canada [164]. Additives such as bisphenol-A or phthalates have been banned in EU and North America through these regulations ([165]). Concerning plastics, the TSCA excluded completely all polymers because "they do not present an unreasonable risk of injury for human health or the environment" [166]. On the other hand, REACH covers, in theory, monomers and polymers. However, there are in practice no requirements for their registration and evaluation "… until those that need to be registered due to the risks posed to human health or the environment can be selected in a practicable and cost-efficient way on the basis of sound technical and valid scientific criteria" [167]. The CEPA covers also in theory polymers, however without any standardized toxicity tests there is no possibility to determine the toxicity of a plastic.

- *Limits of actual toxicity standards.* In order to assess risks with the goal of setting risk reduction targets in a global approach of decision support, ERA tools such as standardized bioassays are essential. Numerous standardized toxicity tests already exist for the marine environment: EPA (1004.0 to 1008.0, 2019.0), ISO (5430:2023, 10253, 11348, 14380, 14669, 16712, 17244, 19820, 20666), OECD (203, 210, 210), ASTM (E1367-03, E1611-21, E1562-22, E2122-22, E729-23, E1191-03A(2023)e1, E724-21, E1218-21, E1022, E1192). These standards focus on chemical toxicity, but do not consider a physical or biological pollution. New standards are needed for an effective ERA of the physical effects of plastics, by using different sizes and concentrations. Very few initiatives have been putted also in standardizing the biological effect of plastic pollution, including the transport of invasive or pathogen species.
- *Evaluation of chemical toxicity.* Even though chemical toxicity of plastics could be assessed using already available standards, another adjustment is still needed: the standardization of leaching of additives. No standard exists on the leaching time, presence or absence of light/UV radiation, temperature. Other key methodological points are the plastic size class that should be introduced in the leachate and at which state (pristine or pre-weathered), as well as their specific shape (using of pre-grinding to reduce the specific surface difference, for example) or state of polymer oxidation. A special care to the laboratory equipment is needed in order to reduce cross contamination of additives [168]. Glassware is strongly recommended for leachates formation.
- *Evaluation of physical toxicity.* The ideal way to observe MP physical toxicity would be through chronic experiments and using either irregular sized MPs or fibers which are the most recovered in the environment. Moreover, plastics should undergo a bacterial colonization of at least several weeks in the marine environment [123] and plastic sizes should be coherent with the species tested in terms of bioavailability and ingestion rate. In addition of a negative control, a "particulate control" with a natural particle such as smectites, diatomites or kaoline mimicking mineral particle in the environmental water is recommended. The objective is to decipher specific physical injuries related to plastic.

6. Conclusion

The omnipresence of MPs in marine waters makes a vast range of biota susceptible to MPs exposure, with a variety of adverse effects at different trophic levels of the marine food web and from molecules to population levels. Gaps concerning the quantification of exposure to large and small MPs as well as NPs in the water column and in benthic environments still needs to be addressed for relevant ERA. Moreover, methods to evaluate the hazardous effects of NPs and the potential difficulties of their identification in organs and tissues are still under development. In addition, knowledge about toxic effects suffers from non-negligible methodological biases that limit an effective ecological risk assessment of plastic in the marine environment. To tighten the gap between the environment and laboratory experiments, we mentioned that special cares are needed in further studies by considering the plastic type, size, shape, state of oxidation, concentration and colonization by marine microorganisms to better fit to environmental conditions and gaining into exhaustivity and therefore complexity. Public policies including seawater quality assessment concerning plastics are still in their infancy. The lack of scientific knowledge on the chemical, but also physical and biological aspects associated with plastic pollution, hinders the development of new standards that are more representative of the fate of plastics in the marine environmental conditions. With the development and analysis of growing datasets on acute and chronic exposure across discrete organisms in various environments, we will be able to transition from baseline and monitoring to an effective ecological risk assessment of plastic pollution in the marine environment. These goals are critical, as we move forward towards a sustainable future of improved human and ocean health.

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