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The hidden threat of plastic leachates: A critical review on their impacts on aquatic organisms

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ABSTRACT

Plastic products are made from the essential polymer mixed with a complex blend of substances including catalyst remnants, polymerization solvents, and a wide range of other additives deliberately added to enhance the desirable characteristics of the final product. Additives include bisphenols, phthalates, flame retardants, and further emerging and legacy contaminants. With a few exceptions, additives are not chemically bound to the polymer, and potentially migrate within the material reaching its surface, then possibly leach out to the environment. Leachates are mixtures of additives, some of which belong to the list of emerging contaminants, i.e. substances that show the potential to pose risks to the environment and human health, while are not yet regulated. The review discusses the state of the art and gaps concerning the hidden threat of plastic leachates. The focus is on reports addressing the biological impacts of plastic leachates as a whole mixture. Degradation of plastics, including the weathering-driven fragmentation, and the release of additives, are analysed together with the techniques currently employed for chemically screening leachates. Because marine plastic litter is a major concern, the review mainly focuses on the effects of plastic leachates on marine flora and fauna. Moreover, it also addresses impacts on freshwater organisms. Finally, research needs and perspectives are examined, to promote better focused investigations, that may support developing different plastic materials and new regulations.

1. Introduction

Quantities of solid waste are increasing globally. Plastics are a major fraction of this waste, and the foremost category of litter reported in the ocean (Napper and Thompson, 2019). It is estimated to be between 50% and 90% (Agamuthu et al., 2019) and sometimes up to 95 % of the total waste found on shorelines, sea surface and seafloor (Galgani et al., 2015). Most plastic materials are made of organic polymers synthetically produced using feedstocks deriving from natural gas processing or crude oil refining. Generally, they are the ultimate product of convenience because of their properties ranging from lightweight to high strength and durability; thus, they are ubiquitous, versatile and play several roles for societal benefits (Andrady and Neal, 2009). On the other hand, the increasing occurrence of plastic in the environment has become one of the most concerning issues among scientists and policy makers. The rate of plastic production has exponentially risen worldwide during the past decades, passing from 1.5 million tons in 1951 to around 335 million tons in 2016 (PlasticsEurope, 2017). As a consequence of increasing plastic discharge, nearly eight million tons of plastics are estimated to flow into the oceans every year from either land- or sea-based sources (Gallo et al., 2018).0

Plastics are classifiable based on their chemical structure, polarity and application. Globally, polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) are the classes of synthetic polymers most frequently used in highest-volume industrial applications, and are therefore widely detected and worldwide distributed in marine and coastal environments (Hidalgo-Ruz et al., 2012). Physico-chemical forces such as wave action, photodegradation and other weathering processes lead to the progressive fragmentation of oceanic plastics, which might thus be categorised into macroplastics (>20 mm diameter), mesoplastics (5-20 mm diameter), microplastics (MPs, <5 mm diameter) down to nanoplastics (NPs, from 1 μm to 1 nm) (Franzellitti et al, 2019; GESAMP, 2016). To these, native plastic items directly commercialized as micro- or nano-sized particles for industrial purposes (known as primary micro/nanoplastics) must be added (Ribeiro et al., 2019).

Macro/mesoplastics seem to prevail in tonnage, while MPs are the most numerically abundant. Sediment is generally considered to be a final sink for MPs. Items denser than seawater will natu-

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rally sink and end up in sediments. However, the density of MPs originally lighter than seawater may increase under the effect of biofilm formation, ingestion/egestion by animals, or aggregation with marine organisms (Ioakeimidis et al., 2019). A recent study in the Beibu Gulf (Xue et al., 2020) concluded that most MPs are hidden in deep sediments (from 5 to 60 cm), with amounts five times higher than those in surface sediments. MP pollution within water columns is likely to be underestimated, because 80% of the field studies sampled MPs with size greater than 300 µm, thus excluding particles of smaller size (Lindeque et al., 2020).

MPs are mistaken for natural food by a wide range of marine organisms (Franzellitti et al. 2019, GESAMP, 2016; UNEP, 2016). Filter- and deposit-feeders may accidentally ingest MPs, while higher trophic levels usually accumulate plastics indirectly via biomagnification (GESAMP, 2016). Most studies on MP have been addressed to the potential effects of their physical presence and distribution along the food chain (Galloway et al., 2017; GESAMP, 2016; UNEP, 2016). The most obvious impact of these items is physical damage or blockage of the digestive tract, together with a false sense of satiation, which reduces appetite and food intake. Plastics in the nanometre scale are ingested or trapped by the gill filaments and may enter the blood after migrating across the surfaces (Ryan, 2019). Furthermore, plastics can carry waterborne pollutants, mainly hydrophobic compounds, which may affect organisms when released after ingestion, and/or biomagnify along the marine trophic chain (Galloway and Lewis, 2016; Gonzàlez-Soto et al., 2019).

The fragmentation and degradation of plastics may facilitate the release of additive chemicals, most of which are not covalently bound to the polymers, and are thus prone to be released into the environment (Kwan and Takada, 2016; Teuten et al., 2009). A prominent group of additives consist of metals ranked high with regard to human health hazards, as aluminium, chromium, cobalt, nickel, tin, and zinc (e.g. Capolupo et al., 2020). Among organics, well known additives are BPA, phthalates, and brominated flame retardants (BFRs), proven over many years to have harmful effects on living organisms (e.g. Baršienė et al., 2006; Bollmann et al., 2012; Canesi and Fabbri, 2015; Balbi et al., 2016; Wang et al., 2020).

The European Chemical Association characterised over 400 substances used as plastic additives (ECHA, 2018), many of which are regulated under European and North American legislation to limit their possible impacts on human health. As shown in Table 1, phthalates esters and some polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDs) congeners used as BFRs are currently subjected to ban, restrictions, or assigned to particular risk assessment procedures under the EU's REACH Regulation. Particular attention has also been addressed to the BPA addiction to food contact material (FCM), for which a migration limit of 0.05 mg/kg of food has been defined. In addition, EU Regulation EC/10/2011 recommends the use of plastic-made FCM specifically designed to prevent the additive migration into food. Similar guidelines have been introduced by the GMC resolution N° 39/19 of the South-American Trading Bloc (MERCOSUR), which sets compositional and other limits or usage restrictions for nearly 1150 substances (Tovar, 2019).

Some of the substances used as plastic additives, such as HBCDs, PBDEs, nonylphenol and diethylhexyl phthalate (DEHP) have been included, as priority substances, under the Water Framework Directive (2000/60/EC). Environmental Quality Standards (EQSs) for surface waters have also been introduced in the EU for these substances (2008/105/EC; 2013/39/EU) (Table 1). However, no environmental safety thresholds have yet been defined for many other classes of additives, including BPA, known for its ubiquity in surface waters and endocrine disrupting effects on aquatic organisms (reviewed by Canesi and Fabbri, 2015), and several organophosphate esters (OPEs) used as flame retardants. Migration tests were recently performed on virgin and/or beach-collected MPs. These highlighted the presence of a suite of chemical additives in the leachates, including legacy pollutants, as metals, organic substances as benzothiazole, acetophenone and phthalide and many other substances for which the lack of pertinent environmental fate and ecotoxicological data prevent a proper evaluation of associated risks (Capolupo et al., 2020; Schiavo et al., 2020; Tetu et al., 2019). The additive leaching by plastics discharged in the environment may thus expose wild animal species to complex chemical mixtures containing not only components regulated under the current environmental legislation but also a number of contaminants of emerging concern (CECs). The latter are chemicals showing the potential to pose risks to the environment which are not yet subjected to environmental risk assessments, regular monitoring plans or legislative standards aimed at preventing their impacts on ecosystems.

A recent paradigm shift in plastic additive toxicology is thus related to the need for testing the adverse effects of the chemical extract or leachate from a given plastic material rather than single plastic additives (Groh et al., 2019). Although studies addressing the release of additives from plastics have started decades ago (i.e. Berens, 1997), threats to living organisms and environmental risks associated to the mixture of additives leached from plastic items gained attention more recently; papers addressing this topic have appeared in the literature only few years ago.

The aim of the review is to bring on the stage the hidden threat of plastic leachates providing information on the state of art and mapping the gaps in our knowledge. The focus is on reports specifically addressed to plastic additive leachates as a whole. The majority of available data have been published in the latest three years, and those where the leachate content was analytically determined are highlighted. Since marine plastic litter is a major concern, the review refers mainly to the effects of leachates on marine microorganisms, plants, invertebrates, and vertebrates. However, also impacts on freshwater organisms are addressed. Finally, research gaps and perspectives are examined, to promote better focused investigations, that may support design of different plastic materials and/or definition of new regulations.

2. Type, fate and impacts of plastic additives

2.1. Additive classification

Plastics are made of a core polymer complexed with a set of chemicals differing for origin (natural or synthetic), structure, functions and source. The chemicals associated with plastics can be classified in four categories: chemicals intentionally added during the production process, unintentional products of the manufacturing processes, by-products of the plastic waste recycling, and hydrophobic compounds adsorbed from the surrounding environment (Gallo et al., 2018; Mayer et al., 2000). Among them, intentionally added chemicals – henceforth addressed to as additives – have been recently cited as a potential hidden threat to the ecosystem (Gallo et al., 2018; Franzellitti et al., 2019). Additives are used to modify the resin features by either enhancing the desirable characteristics of the plastic product or lessen the unwanted properties (Murphy, 2001). According to a recent classification, additives can be categorised based on their functional and structural components into four major classes: functional additives, colorants, fillers and reinforcements (Hansen et al., 2013). Functional additives include substances designed to modify the physicochemical properties of polymers (from plasticizers to foaming agents). Colorants include pigments and azocolorants widely used to treat textile products; fillers include substances as clay, talc or carbonates aimed at improving polymer coating properties, while stabilizers

Table 1
Legislative interventions undertaken at international level to mitigate the impacts of plastic additives.

Class	Additive	Chemical name	Specific name	EQS (μg/L)	Legislative interventions	References
					• Recommendation for safety levels and handling/disposal by the International Programme of Chemical Safety (IPCS) environmental health • German Chemicals Banning (Dioxin) Ordinance; Revised in1994 to include brominated and chlorinated dioxins/furans	Murphy, 2001
					• Included as priority hazardous substances in surface waters	2000/60/EC; 2013/39/EU
BFRs		PBDEs	Penta BDE Octa BDE Deca BDE	MAC inland: 0.14 MAC surface: 0.014 Biota: 0.0085 (μg/Kg)	• Banned since 2004 in EU	2003/11/EC; European Council Decision, 2009
					• Banned from application in electronic and electrical appliances	European Council Decision, 2009
			Tetra to Hepta BDE		• Elimination from Annex A of the Stockholm Convention on persistent organic pollutant (POP)	Stockholm Convention, 2016
			Tetra to Deca BDE		• Restricted in Canada	SOR/2008-218 Regulation; reviewed by Hermabessiere 2017
			Penta to Octa BDE		• 90-day notification before importation or production	USEPA, 2012; reviewed by Hermabessiere 2017
		HBCD	α-HBCD β-HBCD γ-HBCD	AA inland: 0.0016 AA surface: 0.0008 MAC inland: 0.05 MAC surface: 0.05 Biota: 167 (μg/Kg)	• Included as priority hazardous substances in surface waters • Subjected to authorization in the EU under REACH regulation (annex XIV) • Elimination in Annex A of the Stockholm Convention except for EPS and XPS in Stockholm Convention • Subjected to risk assessment by USEPA based on "Toxic Substances Control Act."	European Food Safety Authority (EFSA) 2011; USEPA, 2010; Stockholm Convention, 2016; 1907/2006/EC; 2000/60/EC; 2013/39/EU
		TBBPA			• No legislation concerning TBBPA has been applied in the EU	Vandermeersch et al., 2015
Plasticizer	phthalates	DINP			• No warrant under EU's Classification and Labelling and Packaging (CLP)	ECHA, 2018
		DEHP, BBP, DBP, DIBP		AA (DEHP) inland: 0.0016 AA (DEHP) surface: 0.0008	• Use restriction in industrial/medical products • Included as priority hazardous substances in surface waters	2015/863/EU; 2000/60/EC; 2013/39/EU
	Phenols	BPA			• limits for leaching in FCM defined at EU level	ECR, 2011; 2018/213/EU
		4-nonylphenol		AA inland: 0.3 AA surface: 0.3 MAC inland: 2 MAC surface: 2	• Included as priority hazardous substances in surface waters	2000/60/EC; 2013/39/EU

BFRs, Brominated flame retardants; PBDE, Polybrominated diphenyl ethers; HBCD, Hexabromocyclododecane; TBBPA, Tetrabromobisphenol A; DINP, Diisononyl phthalate; DEHP, Di(2-ethylexyl) phthalate; BBP, Butyl benzyl phthalate; DBP, Dibutyl phthalate; DIBP, Diisobutyl phthalate; FCM, food contact material; EQS, Environmental Quality Standard defined under the 2013/39/EU Directive; AA inland, annual average value in inland waters; AA surface, annual average value in not inland surface waters; MAC Inland, maximum allowable concentration in inland waters; MAC surface, maximum allowable concentration in not inland surface waters.

Table 2
Literature information regarding the physiological effects of two of the most representative classes of endocrine disrupting plastic additives, i.e. phthalates and BPA on aquatic organisms

Class of additive	Specific name	Species	Impacts	References
Phthalates	DEHP	*Mytilus galloprovincialis*	Increase in catalase and acyl-CoA oxidase activity; inhibition of Mn-superoxide dismutase	Orbea et al., 2002
		Oryzias latipes	Reduction in vitellogenin and % of mature oocytes in females; anti-estrogenic activity	Kim et al., 2002
		Cyprinus carpio	High bioconcentration; changes in enzymes activities on the synthesis of endogenous steroid hormones and their metabolism	Staples et al., 1997;Thibaut & Porte 2004
	DAP	*Mytilus edulis*	Increase in micronuclei frequency; fragmented apoptotic cells in gills	Barsiene et al., 2006
	DBP	*zoomacrobenthos*	changes in community structure & colonization profiles	Tagatz et al., 1986
	BBP	*Gasterosteus aculeatus*	Alterations in shoaling and feeding behaviour	Wibe et al., 2002
BPA	*M. edulis*		Increase of phospho-protein levels; spawning induction in both sexes; damage on oocytes and ovarian follicles; rise in micronuclei frequency in gills	Aarab et al., 2006; Barsiene et al., 2006
	M. galloprovincialis		Destabilization of the lysosomal membrane in hemocytes; induction in the phosphorylation of mitogen-activated protein kinases and signal transducers and activators of transcription factors	Canesi et al., 2005, 2007a
			Increase expression of the *Mytilus* Estrogen Receptor (MeER2); downregulation in metallothionein (MT20) gene expression; changes in the activity of Catalase, GST and GSSG reductase; Increased total GSH content	Canesi et al., 2007b
			DNA strand break inductions	Park and Choi 2007

DEHP, Diethylhexyl phthalate; DAP, Diallyl phthalate; DBP, Dibutyl phthalate; BBP, Butyl benzyl phthalate; BPA, Bisphenol A; GST, Glutathione S-transferase; GSSG, Glutathione disulfide; GSH, gluthatione.

include synthetic fibers used to increase the products mechanical resistance (Hansen et al., 2013).

Most of the additives represent a few percentages by weight of the polymer (e.g. biocides, odorants, antistatics and antiozonants may represent up to 1-2%; colorants from 1 to 4%) while others are used at a much higher weight: thermal stabilizers up to 8%; flame retardants from 10 to 20%; plasticisers from 10 to 70%; fillers up to 50% (Andrady and Rajapakse, 2019).

Over the past decades, a significant amount of experimental studies have been focused on the effects of single additives on aquatic organisms. Based on their widespread presence in natural environment and well-known endocrine disrupting activity, BPA and phthalates, used as plasticizers and to increase the polymer flexibility, are among the most commonly studied additives (ECHA, 2018; UNEP, 2016). As reported in Table 2, many organisms among fish, crustaceans and molluscs were found to be affected by these compounds, with effect spanning from estrogenic (BPA) or antiestrogenic (phthalates) pathways, lysosomal disorders, oxidative stress, up to alterations of the organisms' behaviour or community structure. However, recent evidence confirms that these substances represent a minimal part of the chemicals leached by plastics in aquatic systems (Capolupo et al., 2020; Tetu et al., 2019). Therefore, the environmental impacts of additives should be assessed based on the whole mixture of substances leached out from the polymeric core, which might finally lead to cumulative effects on the exposed biota.

2.2. Potential additive leaching in the marine environment

The chemical hazard of plastics in the marine environment is triple, due to i) POPs released from the plastic surface, ii) additives leaching out of the plastics and iii) chemicals produced by the degradation of the plastic polymer. The pathways involved in plastics environmental degradation depend on the polymer type and may consist in the succession of abiotic and/or biotic processes. As shown in Fig. 1, plastic materials showing a carbon-carbon backbone, as PP, PE, PS and PVC, are mostly susceptible to photo- and thermo-oxidation, while hydrolytic processes play a significant role in the degradation of polymers containing heteroatoms, as polyurethane (PU) and PET (Gewert et al., 2015). Overall, these processes lead to the progressive depolymerization of plastics, and are associated to release of carbonyl groups, hydroperoxides, alcohols and hydrochloric acid, among others. The photothermally or hydrolytically induced fragmentation of plastic litter generally precedes biodegradation, which is mediated by either cellular or extracellular enzymes produced by biofilm-forming microbes (Gewert et al., 2015).

Weathering forces are also known to stimulate the leaching of additive chemicals along with the plastic fragmentation from macro- to micro-sized items (Bejgarn et al., 2015; Luo et al., 2019) (Fig. 2). As established in the past ten years, beached and floating MPs show a wide variety of complexed additives. In a large survey focusing on expanded polystyrene (EPS) fragments from the Asia-Pacific coastal region, Jang et al. (2017) found concentrations of HBCDs in the range of 0.98–14,500 ng/g. The highest levels were recorded in samples from Alaska (USA, 3350 ± 5860 ng/g), followed by Peru, Canada and California (USA). Similarly, PBDEs levels in PP and PE MPs from open ocean, remote and urban beaches sampled at global scale showed values in the range of 0.3–9900 ng/g (Hirai et al., 2011; Taniguchi et al., 2016). Concentrations up to 16,444 ng/g were observed in MPs in the stomach of seabirds (Tanaka et al., 2013; 2015; Herzke et al., 2016). Phenols, such as BPA and NP, have been measured worldwide in field-collected MPs at a range of 1–730 ng/g and 5.8–16,000 ng/g, respectively (reviewed by Hong et al., 2018), while phthalates have been detected in coastal PE, PP and PS MPs from China up to 80.4 ng/g, with diethylhexyl phthalate, DEHP, being the most concentrated class (up to 69.9 ng/g) (Zhang et al., 2018). Metals have been detected in different types of MPs at ng/g levels (Ashton et al., 2010, Nakashima et al., 2012; Turner and Holmes, 2011). Hg and Pb showed values of 3.86 µg/g and 38.2 mg/g in EPS (Wang et al., 2017) and PVC fragments (Turner et al., 2020), respectively.

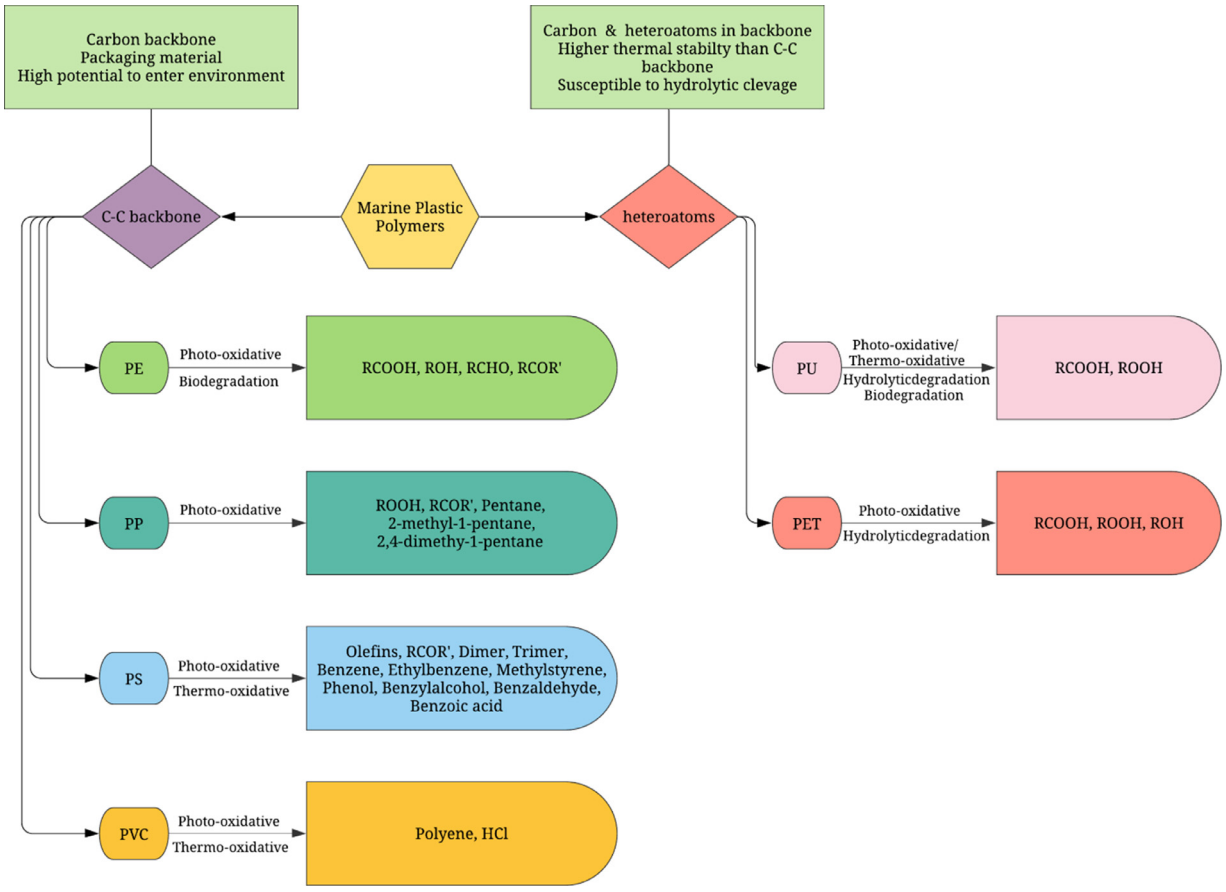

Fig. 1. The degradation pathway of plastics in environment. Diagram showing the abiotic and biotic processes involved in the depolymerization of the most common polymer types in aquatic environment. Polymers are classified based on their structure (carbon-carbon or carbon-heteroatoms), which influences either the susceptibility to biological/physicochemical factors and the formation or release of organic by-products.

The desorption from plastic materials depends on several factors, including the type and strength of molecules-polymer binding, the internal pore size of the polymer, the additives' molecular weight and degree of hydrophobicity (Teuten et al., 2009). The additive leaching from plastic surface may also be influenced by the polymer texture, categorised in glassy, rubbery (amorphous) and crystalline (Brydson, 1999). Hahladakis et al. (2018) recently demonstrated that the leaching of additives from polymers showing crystalline texture is relatively low when compared to rubbery synthetic material. In order to evaluate the physicochemical properties governing the release of additives, Luo et al. (2019) analysed the leaching behaviour of fluorescent chemicals from polyurethane MPs in natural (river, lake, wetland, and sea water) and simulated waters at pH 3, 7, and 11. Batch leaching tests were conducted adding 0.7 g of MPs to 200 mL water at 25 °C. After reaching equilibrium, 2 mL of the solution containing the leachates were taken every hour up to 6 h, and, thereafter, every 6 h up to 48h. The leachates were then analysed by fluorescence spectroscopy establishing the release kinetics of fluorescent additives from MPs. Within 12-24 h, the maximum amount of additives was released into the leachate, notably 3,3′-diaminobenzidine-like substances. The concentrations of fluorescent additives in simulated and natural waters followed the order of basic water > neutral water > seawater > lake > river > wetland. These results suggest that additives may leach at higher rate in seawater compared to freshwater and, more importantly, that leaching processes may occur in relatively short time ($\leq$ 24 h) after plastic disposal in aquatic systems. However, it is worth noting that the outcomes of artificial weathering do not necessarily match with those of natural *in-situ* weathering; hence, caution should be used when inferring experimental data to natural environment conditions.

Organic additives used as plasticizers, stabilizers and flame retardants have frequently been detected in seawater in the past twenty years. NP showed concentrations ranging from 20 pg/L measured in the Sea of Japan to 4.1 µg/L in the Mediterranean Sea (Spain) (reviewed by Hermabessiere et al., 2017). Similar levels have been detected for BPA, with values ranging from 0.001 to 2.47 µg/L measured in the Jiaozhou Bay (China) and Singapore coastal waters, respectively (Basher et al., 2004; Fu et al., 2007). A significant amount of data is also available for phthalates esters, at levels from few pg/L to tens of µg/L in seawaters. Between the phthalates congeners, DEHP is most frequently detected in seawater, with levels from 448 pg/L (Arctic waters, Xie et al., 2007) to 23.42 µg/L (Sardinian waters, Italy; Fossi et al., 2012). Among BFRs, PBDEs have been detected from 0.002–19 ng/L in Hong-Kong and Mediterranean Sea, respectively (Sànchez-Avila et al., 2012; Wurl et al., 2006), while HBCDs showed levels between 0.1 pg/L measured in the European Arctic waters and 1620 pg/L from South Korea (reviewed by Gu et al., 2017).

2.3. Techniques employed for the chemical screening of plastic leachates

A vast and heterogeneous array of inorganic and organic compounds are used as additives during plastic production, and, in most cases, their number, type and concentrations on polymers are unknown. As a result, it is rather challenging to analyse the chemical composition of plastic additive leachates, or even to de-

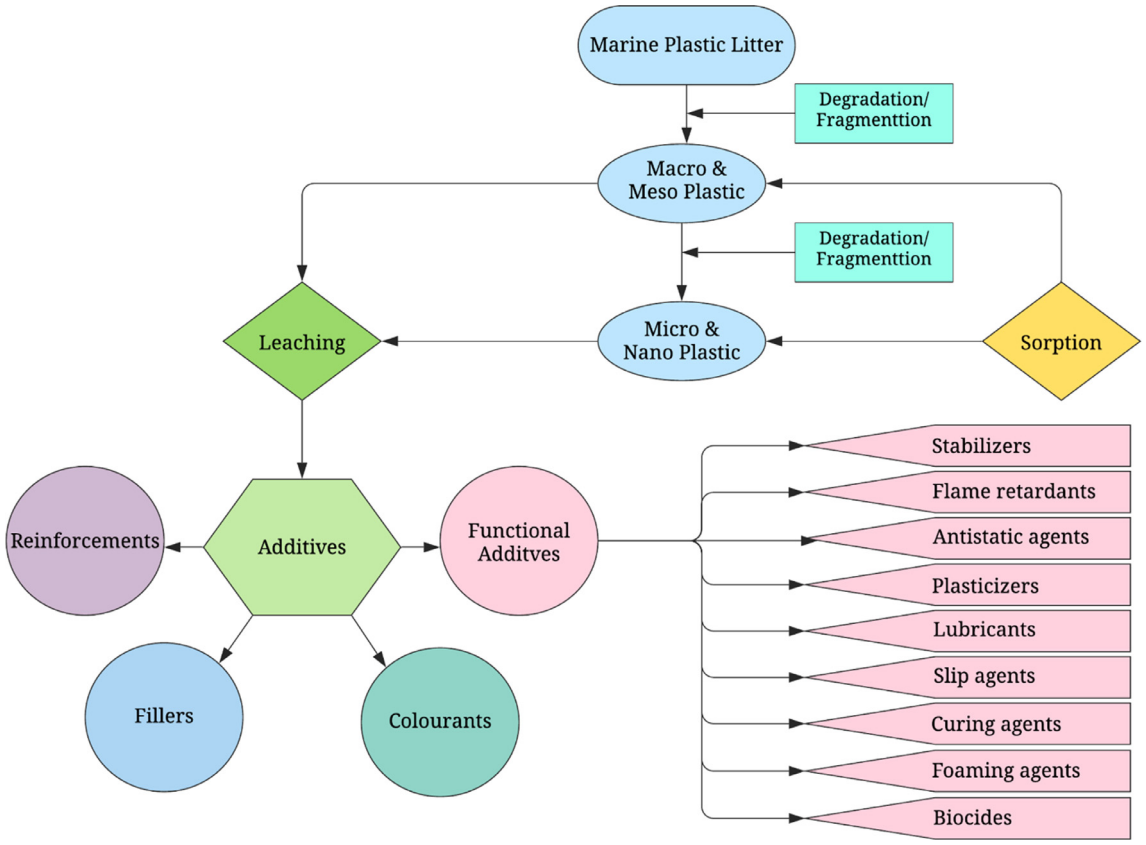

Fig. 2. The leaching of plastic additives in marine environment. Processes leading to the release of additives from marine plastics (including the litter fragmentation to the micro/nano scale) and types of additives released by plastic leachates (according to Hansen et al., 2013).

Table 3
List of the methods employed to characterize the composition of plastic additive leachates

Techniques/tools	Target analysis/additive	Reference(s)
Fourier Transform Infrared Spectroscopy (FT-IR)	physical (released fragments) and chemical (organic compounds) characterization of leachates	Hernandez et al., 2019; Luo et al., 2019
Liquid Chromatography- Tandem Mass spectrometry (LC-MS/MS)	organic compounds from PVC and HDPE leachates	Tetu et al., 2019
Inductively coupled plasma mass spectrometry (ICP-MS)	metals in different polymer leachates	Capolupo et al., 2020; Lithner et al., 2012; Schiavo et al., 2020; Tetu et al., 2019
Inductively coupled plasma atomic emission spectroscopy (ICP-AES)	metals in PVC leachate	Lithner et al., 2012
Inductively coupled plasma optical emission spectroscopy (ICP-OES)	metals in HDPE and PVC leachates	Tetu et al., 2019
Gas Chromatography-Mass Spectrometry (GC-MS)	organic compounds in different polymer leachates	Capolupo et al., 2020; Schiavo et al., 2020; Thaysen et al., 2018
Ultra-Performance Liquid Chromatography (UPLC-MS)	non-target chemical screening	Bejgarn et al., 2015
High-Performance Liquid Chromatography - High-Resolution Accurate Mass- Mass Spectrometer (HPLC- HR-AM-MS)	(uncharacterized) ESI amenable substances	Li et al., 2016
X-ray photoelectron spectroscopy (XPS)	release of micro/nanoparticles from plastic teabags	Hernandez et al., 2019
Raman Spectrometer (RS)	screening of organic compounds in PUF leachates	Luo et al., 2019

PVC, polyvinyl chloride; HDPE, high-density polyethylene; PUF, Polyurethane Foam; ESI, electrospray Ionization.

fine the suitable analytical approach to meet this purpose. A list of techniques used to identify the additives released by plastics following experimental leaching into aqueous media is reported in Table 3. Among tested chemicals, metals were the most successfully characterized in leachates using methods based on mass, atomic and optical spectroscopy (Capolupo et al., 2020; Lithner et al., 2012; Tetu et al., 2019). Methods previously validated for micro-sized plastics identification in environmental samples, as the Fourier Transform Infrared Spectroscopy (FT-IR) and the Raman spectroscopy (Käppler et al., 2016; Shim et al., 2017) were adapted by Luo et al. (2019) to assess the plastic leachate organic chemical content. Peak positions were comparable to those of 3,3′-Diaminobenzidine, used as a model compound of organic additives, showing the presence of unidentified biphenyls in prepared leachates. Untargeted LC-MS screenings were also found to detect complex mixture of organic components, whilst not identifying

specific compounds (Tetu et al., 2019). Capolupo et al. (2020) used a GC-MS approach based on the preliminary (non-target) additive characterization in plastic particles, which allowed to identify organic compounds likely to be released into the aqueous solution. The subsequent GC-MS analysis of prepared leachates corroborated the preliminary evidence, and allowed to evaluate the composition and concentrations of released organics, including BPA, benzothiazole, acetophenone n-cyclohexylformamide and phthalide (Capolupo et al., 2020).

2.4. Plastic leachate preparation in ecotoxicity studies

Standard methods for testing the leaching of solid materials have been developed and used extensively since the 1990s to assess the risks to public health from the release of high-toxicity contaminants. Among those methods, protocol D-7475-20 of the American Society for Testing and Materials (ASTM, 2020) has been successfully validated to investigate the leaching and biodegradation of plastic waste in a laboratory-scale bioreactor reproducing aerobic and anaerobic (bio)degradation processes occurring in a municipal solid-waste stream. Twenty-four- to forty-eight-hour leaching protocols in stirring conditions have further been recommended by the United States Environmental Protection Agency (US EPA) for dumped solid waste. Those include the standard leaching test (SLT), the international unit conversion system (IUCS) test and the Minnesota test, while the "DIN 38414-S4" method is currently recommended by the European Union (reviewed by Kocasoy and Murat, 2009). Although the leaching of plastic additives (e.g. BPA) has been first reported nearly twenty years ago (e.g. Yamamoto and Yasuhara, 1999; Sajiki and Yonekubo, 2003), studies addressing the adverse effects of whole plastic leachates on aquatic organisms have been published very recently, reflecting the increasing global concern about ocean contamination by plastic debris (UNEP, 2016; GESAMP, 2016). As summarized in Table 4, plastic leachates so far employed for ecotoxicity assessments have been generated using techniques differing by eluent nature, mass/volume ratio, duration, and temperature. A major factor limiting the definition and adoption of standard media for plastic leaching is due to the fact that current international guidelines for toxicity testing, notably those for algae growth, fish/mussel embryotoxicity or *Daphnia magna* acute and chronic tests (ASTM, 2012; OECD, 1992; 1998; 2004), require the use of specific aqueous matrices. Standard artificial fresh/seawater or cell culture media, for example, might influence the migration, toxicity and/or detection of desorbed additives. PVC, PP, PE and PS are the polymers most frequently tested for additive leaching (Table 4). However, leaching trials were conducted using a variety of commercial plastic products, beached and/or virgin pellets which might deeply differ from each other in terms of additive quantity, composition and bioaccessibility, regardless of the polymer type.

Treatments in bioassays were generally represented by % dilution of prepared leachates, but concentrations of plastic tested for additive leaching varied remarkably among studies, i.e. from 5 g/L (1/200 solid/liquid ratio; Tetu et al., 2019) to 250 g/L (1/4 solid/liquid ratio; Lithner et al., 2012; Rummel et al., 2019). This made the comparative evaluation of data among performed tests, organisms and polymers difficult. Six out of the thirteen studies reported in Table 4 tested the leaching of plastics for 24-48 h in line with the US EPA and EU standard guidelines (Kocasoy and Murat, 2009), while prolonged leaching tests (3 to 28 days) were generally used elsewhere, except for Thaysen et al. (2018) who prepared EPS leachates within 30 min at relatively high temperature (70–90 °C). Additionally, only one study (Luo et al., 2019), investigated the effect of pH variations on the migration of additives from plastics. Hence, there is a clear need for a common guideline for the experimental preparation of plastic additive leachates. This might reproduce environmentally realistic conditions while meeting standard requirements for ecotoxicity testing. Major efforts are also necessary to identify plastic/medium ratios allowing for consistent estimations of additive leaching in aquatic systems and for standardized treatments in ecotoxicological studies to provide information useful to risk assessment and mitigation.

3. Effects of plastic leachates on aquatic species from different trophic levels

3.1. Effects on photosynthetic microorganisms and marine plants

According to recent estimates, nearly 23,600 metric tons/year of dissolved organic carbon (DOC) are released from marine plastics globally (Romera-Castillo et al., 2018). DOC plays a vital role in the marine trophic web by modulating the presence, growth and activity of photosynthetic and heterotrophic microorganisms (Azam et al., 1983a,). The composition, interactions and functional diversity of the marine microbiota might thus experience severe alterations owing to the ever-growing prevalence of plastic in marine systems.

A handful of studies have recently been addressed to the effects of plastic leachates on phytoplanktonic or prokaryotic marine microorganisms (Table S1). Tetu et al. (2019) evaluated the effects of leachates from high dense PE shopping bags and PVC matting on two strains of the marine photosynthetic cyanobacterium *Prochlorococcus* spp. All leachates induced growth and genome-wide transcriptional changes after 72 h of exposure, as well as a reduction of the photochemical efficiency of photosystem II and a clear decline in oxygen production (Tetu et al., 2019). A recent study by Capolupo et al. (2020) addressed the chemical composition and effects of leachates from car tire rubber (CTR), PP, PET, PS and PVC. CTR, PVC, PP and PS leachates significantly inhibited the growth of the marine microalga *Skeletonema costatum* (EC_{50} in the range of 18–34% of leachate), while PET did not cause growth inhibition even at the 100% leachate concentration. The combination of non-target and target (GC-MS) analyses revealed clear relationships between observed effects and relatively high concentrations (up to mg/L) of organic compounds (i.e plasticizers, antioxidants, antimicrobials, lubricants, and vulcanizers) and metals (notably Zn, Cu, Co, Sb and Pb) in the leachates (Capolupo et al., 2020). Leachates from EPS consisting in an aqueous mixture of hexabromocyclododecane (HBCD), BPA and bumetrizole (UV 326), were recently found to up-regulate the photosynthetic processes in marine microalgae, including *Dunaliella salina, Scenedesmus rubescens, Chlorella saccharophila,* and *Stichococcus bacillaris* (Chae et al., 2020). This contrasts with data previously reported by Sjollema et al. (2016), who did not observe changes in the photosynthetic activity of *Dunaliella* and *Chlorella* spp. following exposure to PS micro-particles, corroborating the importance of (preliminary) experimental leaching processes to reliably assess the toxicity of desorbed additives.

A laboratory experiment was set up by Menicagli et al. (2019) to analyse the effects of leachates from conventional undegradable bags (high density polyethylene – HDPE) and a new generation of compostable bags on the coastal dune plants *Thinopyrum junceum* and *Glaucium flavum*. The leaching process altered properties of water in terms of pH and salinity; the timing of seed germination and seedling growth was altered by the exposure to leachate from both plastic materials. Moreover, seedling anomalies were observed in both species, posing the accent on the possible impact of leachates from beached plastic litter during rainfall events or tide fluctuations.

Overall, these studies show that the impact of plastic leachates on marine phytoplanktonic microorganisms and marine plants varies considerably depending on tested species and materials.

Table 4
Methods used for plastic leachate preparation in ecotoxicity studies.

Tested species	Polymer	[Leaching plastic]	Medium	Duration	illumination	T°	pH	Filtration	References
Prochlorococcus spp. (bacteria, SW)	HDPE/PVC	25–1.6 mg/mL (HDPE); 5–0.125 mg/mL (PVC)	SW (AMP1 cell culture medium)	5 days	yes	22 °C	-	0.2 µm	Tetu et al., 2019
Mytilus galloprovincialis (mussel, SW); *Skeletonema costatum* (algae, SW); *Raphidocelis subcapitata* (algae, FW)	PET, PS, PP, PVC, CTR	80 g/L	FW/SW culture medium (algae); filtered seawater (mussels)	14 days	no	25 °C	7.8 (FW)–8.1 (SW)	0.2 µm	Capolupo et al., 2020
Amphibalanus amphitrite (barnacle, SW)	PET, HDPE, PVC, LDPE, PP, PS, PC	0.5 m^2/L	filtered aged seawater	24h		28 °C	7.96	-	Li et al., 2016
Paracentrotus lividus (sea urchin, SW)	PVC	100 g/L	Artificial seawater	24h	no	20 °C	8.1	1.2 µm	Oliviero et al., 2019
Nitocra Spinipes (copepod, SW)	PP, PS, LDPE, HDPE, PVC, PET, PLA, Nylon, bio-PET, corn starch/aliphatic esters (bio)	10 g/100 mL	Brackish water from the Baltic Sea	92h	-	-	7.9	-	Bejgarn et al., 2015
Littorina littorea (snail, SW)	PP	20 mL/L	SW	24h					Seuront, 2018
Daphnia magna (cladoceran, FW)	PC, PVC, PU, PE, PS LDPE, HDPE, PET, PMMA, PTFE, ABS, PP, MDPE	65–174 g/L	deionized water	24h–3 days	16h: 8h, L:D; darkness	20±2 °C	7	fine nylon fabric	Lithner et al., 2009
	PP, HDPE, PVC, ABS, epoxy	250 g/L	deionized water	3 days	dark	20–50 °C	7	-	Lithner et al., 2012
Ceriodaphnia dubia (cladoceran, FW)	PS, EPS		FW	30 min.		70–90 °C		-	Thaysen et al., 2018
Daphnia magna (cladoceran, FW); *Sorghum saccharatum, Lepidium sativum, Sinapis alba, Vicia faba* (plants, FW); *Vibrio fischeri* (bacteria, FW/SW)	PE, PP, PS, PE_a, PP_a, PS_a	10:1 (L/S)	Milli-Q H_2O	24h	dark	20 ± 2 °C		11 µm (Whatman Grade 1)	Schiavo et al., 2018; 2020
Chlorella vulgaris (algae, FW)	PUF	0.7 g/200 mL	deionized, tap and natural water; 0.1 M NaCl	48h		25 °C	3, 7, 11	0.45 µm	Luo et al., 2019
Dunaliella salina, Scenedesmus rubescens, Chlorella saccharophila, Stichococcus bacillaris (algae, SW)	EPS	0.1 g, 10 and 30 spheres/50 mL	culture medium	28 days	16h: 8h, L:D	20 °C	-	0.45 µm	Chae et al., 2020
Meretrix meretrix (clam, SW)	PE bags	10 g/L	filtered SW	48h	dark	25 °C	-	fine nylon fabric	Ke et al., 2019
Guine pig and rat cell lines	PET, PE, PP, PS	50 g/200 mL	artificial SW	96h	UV + light	20–30 °C	-	40 µm	Rummel et al., 2019

PET, polyethylene terephthalate; PS, polystyrene; PP, polypropylene; PVC, polyvinyl chloride; CTR, car tire rubber; PE, polyethylene; HDPE, high-density polyethylene; LDPE, low density polyethylene; PU, polyurethane; PU, polyurethane foam; EPS, expanded polystyrene; PLA, polylactic acid; ABS, acrylonitrile-butadiene-styrene; PTFE, Polytetrafluoroethylene; MDPE, medium density polyethylene; PMMA, polymethyl methacrylate; PE_a, oxodegraded polyethylene; PP_a, oxodegraded polypropylene; PS_a, oxodegraded polystyrene; SW, seawater; FW, freshwater: L/S, Liquid to Solid ratio.

Changes have been observed for what concerns growth, photosynthetic activity and gene transcription, urging the need for a better understanding of the risks posed by plastic additive mixtures on the microbial processes governing primary production and on the ecosystem services warranted by coastal vegetation.

3.2. Effects on marine fauna

Several functions were found to be impaired in marine invertebrates and vertebrates after exposure to plastic leachates, as schematically summarised in Table S1.

Sessile marine invertebrates are organisms of choice for testing the effects of plastics on coastal habitats, since they generally display filter/deposit feeding habits, which naturally exposed them to the ingestion of a potentially high amount of non-food items, rapid responsiveness to external stressors and a notable adaptability to laboratory conditions.

Recently, the increasing concerns on plastic ingestion and effects raised awareness on the impact of additive leachates on early swimming stages of benthic and intertidal organisms, some of which are known to be highly sensitive to organic additives, as BPA (Balbi et al., 2016). In a recent investigation by Ke et al. (2019) the toxicity of leachates from two single-use PE bags was assessed on early stages of the yellow clam *Meretrix meretrix*. Leachates did not induce alterations of the clam gamete fertilisation; however, embryonic development, shell height and survival of D-veliger larvae were significantly impaired after exposure to leachates from both plastic bag materials. Embryo-larval development of the brown mussel *Perna perna* was impaired by leachates from virgin and beached PP microplastics (Gandara and Silva et al., 2016). A relatively higher toxicity was assigned to beached materials, likely owing to the presence of adsorbed pollutants which may have cumulatively increased the toxicity of additive leachates. The Mediterranean mussel *Mytilus galloprovincialis* was also employed as model organism to screen the toxicity of leachates from PET, PS, PP, PVC and car tire rubber (Capolupo et al., 2020). Cellular endpoints, as the hemocyte lysosomal membrane stability, and early stages parameters as gamete fertilization, embryonic development, larvae motility and survival were deeply affected by the exposure to all tested leachates. Embryonic development was the most sensitive parameter, with effects of leachates ranked as CTR >PVC >PP >PS >PET. Considering the composition of the different leachates, acetophenone, benzothiazole, n-cyclohexylformamide, phthalide, and BPA were found at the highest concentrations (13.2; 1460; 788; 2.4; and 6.4 µg/L, respectively) in CTR leachates. Among them, BPA is known to exert transcriptional and embryotoxic effects in *M. galloprovincialis* in line with its endocrine disrupting activity (Balbi et al., 2016); benzothiazole and phthalide have previously been reported to induce embriotoxicity (Sheftel, 2000).

Survival and settlement of early stages of the barnacle *Amphibalanus amphitrite* were investigated after exposure to increasing concentrations (0.004 to 0.5 m^2/L) of leachates from high density polyethylene (HDPE), low density polyethylene (LDPE), PP, PVC, PC, PET and PS by Li et al. 2016. The barnacle cyprids settlement was inhibited at all tested concentrations, while the survival of exposed nauplii decreased at the highest dosage. PVC leachates showed the highest toxicity among tested solutions, followed by LDPE. The HPLC-HR/AM MS screening revealed the presence of numerous electrospray ionization (ESI) amenable organic substances in leachates, although single-compound characterizations were not performed. A study performed by Oliviero et al. (2019) investigated the toxicity of leachates from micronized commercial PVC products (children toys) of three different colours, upon embryos of the sea urchin *Paracentrotus lividus*. Leachate concentrations as low as 3.3% significantly inhibited the larval growth, while higher dosages (33%) lead to a substantial and generalized block of the ontogenesis. Based on the chemical screening of the prepared leachates, authors were able to relate observed effects to the degree of heavy metals leaching, with Pb, Zn, Mn, V and Sc detected in the µg - mg/L range.

Bejgarn et al. (2015) evaluated the effects of leachates from twenty-one commercial plastic products made of different polymeric materials on the marine harpacticoid copepod *Nitocra spinipes*. Eight out of the twenty-one tested leachates, used before and/or after artificial sunlight irradiation, affected the copepod survival. Lowest LC_{50} were measured for computer case-derived leachate (15% leachate before irradiation) biodegradable bags (24-18% leachate before-after irradiation). Interestingly, DVD cases took 196-hour irradiation to produce toxic leachate, while PS cup did not show any toxicity after the same treatment, although both are made of amorphous polymers. Analyses for the presence of PVC residues proved that the leachates were a complex mixture of elements but did not show evidence of degradation of the polymer backbone. Leachates from soft PVC products containing relatively higher proportion of plasticizers were found to be toxic for *N. spinipes* while leachates from PVC products with lower amount of plasticisers were ineffective.

The response of marine organisms to chemosensory cues to escape from predation may also be significantly affected by plastic leachates. This has been observed in the common winkle *Littorina* Ioakeimidis et al., 2019*littorea*, exposed to leachates from virgin PP beads and beached plastic pellets (Seuront, 2018). Notably, in the presence of predator chemical cues from the shore crab *Carcinus maenas*, the time spent in refuge or employed for righting and withdrawal of *L. littorea* was reduced, indicating an overall inhibition of predator-induced alertness and avoidance responses. The effects induced by the post-ingestion leaching of additives from PVC particles were assessed in the lugworm *Arenicola marina* by Browne et al. (2013). After a 10-day exposure, substances including nonylphenol, phenanthrene, triclosan and PBDE-47 were detectable in the gut tissue from few µg (nonylphenol, phenantrene) to mg (triclosan) g^{-1}. A > 60% reduction of the coelomocyte phagocytic activity toward pathogens was related to the nonylphenol uptake. Uptake of triclosan inhibited the worm burrowing activity and caused a > 55% increased mortality, while PVC particles alone made worms > 30% more susceptible to oxidative stress.

The knowledge on the effects of plastic additives leachates on marine fish is still very limited compared to invertebrates or microorganisms. Evidence of toxicity due to additive migration has been obtained in the orchid dottyback *Pseudochromis fridmani* exposed to the non-ionic surfactant nonylphenol leached from FDA food-grade PE bags. Nonylphenol leached up to about 160 µg/L after 48 h of plastic-water interaction and tended to bioaccumulate in fish tissue at levels (up to 369 µg/Kg) found to be positively related with short- and long-term mortality (60% and 100%, respectively) (Hamlin et al., 2015).

The studies on seabirds raise a new concern, about the possibility that stomach fluids enhance leaching of additives after ingestion of plastic particles. Feeding experiments with polystyrene plastic pellets compounded with five additives provided direct evidence of the transfer of flame retardants and ultraviolet stabilizers to the abdominal adipose tissue and to the liver of chicks of *Calonectris leucomelas* (Tanaka et al., 2020). By day 15-16, 42 to 88% of the additives were leached out from the stomach and found at significant concentrations (up to 120,000 times the rate compared to the natural diet) in the tissues. The Authors hypothesised that stomach oil, which is present in the digestive tract of marine birds, acts as an organic solvent facilitating the leaching of hydrophobic chemicals. These data underscore the importance of plastic debris as a growing source of pollutants after ingestion.

Additional data are provided by studies on lugworm (*Arenicola marina*) and North Sea cod (*Gadus morhua*) to evaluate the potential for leaching of nonylphenol (NP) and bisphenol A (BPA) in their intestinal tracts (Koelmans et al., 2014). The Authors used a biodynamic model that allows calculating the relative contribution of ingesting plastic to the total exposure of aquatic species to chemicals within the ingested plastic. They accounted for uncertainty in the most crucial parameters through probabilistic modelling. Differently from the abovementioned studies by Tanaka et al. (2020), plastic ingestion appeared to be a negligible pathway for exposure to NP and BPA in lugworm and cod. However, these investigations highlighted the need for a precise methodological setup, a system at equilibrium, and the use of uncontaminated organisms as experimental models to obtain high quality results.

Leachate ecotoxicity studies in the literature have mainly been addressed to apical endpoints (i.e. mortality, decreased growth and feeding, and reproduction failure). To our knowledge, the work by Trestrail et al. (2020) is the first to provide insights into toxicity mechanisms. It addressed the responses to phenol-formaldehyde MPs generated by two types of phenol-formaldehyde foams, and compared the biological effects with those of the MP leachates obtained from freshly crushed foam microplastics (50 mg MP/mL distilled water) after shaking for 24 h at 25 °C. The leachates from regular foam and bio-foam microplastics showed acute toxicity to *Artemia nauplii* and *D. magna* after 24-h exposures, with LC_{50} in the range of 15–27 mg/mL. Bio-foam MPs were more toxic than regular foam MPs for zebrafish embryos (LC_{50} 43.8 vs 27.1 mg/mL, respectively). The same work also addressed sublethal responses in mussels, *M. galloprovincialis*, related to oxidative stress. No statistically significant effects were observed for the leachates, the leachate + MPs and MPs alone on catalase and GST activities. Instead, small but significant increase of acetylcholinesterase activity were observed after treatment with leachate + MPs and MPs alone. Moreover, no effects were observed after exposure to leachate alone, while significant decrease of lipid peroxidation were observed after treatment with leachate + MPs and MPs alone. As the authors mentioned, variables such as age, seasonality and feeding regime may influence the biomarker responses and eventually obscure the effects of treatments. This is the reasons why there are several recommendations on the choice of sentinel organisms and battery of biomarkers (Viarengo et al., 2007). The time of exposure was only three days; most importantly, the whole soft tissue was used for analysis. Because of the different exposure to waterborne contaminants, the responses of mussel gills and digestive gland are higher than those of other tissues; moreover, some biomarkers are more responsive in gills than digestive glands or vice-versa. In conclusion, the work by Trestrail et al. (2020) opens the way to studies on leachate toxicity mechanisms, although the experimental approach may be improved. More investigations at the molecular and cellular level are then advisable to understand the molecular initiating event of leachate adverse effects. For completeness, we mention that also investigation on MPs have privileged apical endpoints, and more studies on toxicity mechanisms are needed to improve the reliability of the Adverse Outcome Pathways (AOP) applicable to MP management (Jeong and Choi, 2019).

3.3. Effects on freshwater organisms

Most of the plastic discharged in natural environment finally find its way to marine waters through freshwater hydrological systems, as streams lakes or rivers (GESAMP, 2016). Recent monitoring studies have established that MPs are ubiquitously found in freshwater matrices. Report on rivers and lakes are far from complete, and especially lack for African and Australian waters. Also, data are often reported using different units, which make comparisons difficult. To provide an order of magnitude, the most polluted river is the Yang-Tze with 1660-8925 particles/m^3. In Europe, 22 particles/m^3 were reported in the Danube river, 0.35 particles/m^3 were reported in the Seine river. For a comparison with marine waters, average values of 0.94 particles/m^3 were reported for the Mediterranean Sea, 1.7 for the Atlantic Sea, 3.7 for the Indian Ocean, 1200 for the Black Sea (reviewed by Waldschläger et al., 2020).

The environmental release of MPs into rivers and lakes occur from a wide variety of sources, including emissions from wastewater treatment plants, which bring into the surface waters mixtures of micro and nano-sized particles mainly from personal care products. Freshwater ecosystems are thus expected to be consistently exposed to flows of organic and inorganic additives, posing serious threats to the fitness of biota. A study by Schmidt et al. (2020) estimated that 5 to 54 metric tons per year of dissolved organic plastic additives of emerging concern are exported to the Gulf of Lion by the Rhone River. Phthalates were the most abundant class of additives, with concentrations ranging from 97 to 541 ng/L, followed by organophosphate esters (85–265 ng/L) and bisphenols (4–21 ng/L). Nevertheless, only a few studies were done to assess leachate toxicity in freshwater organisms (Table S1).

One of the first investigations on freshwater species (and on leachates in general) was performed by Lithner et al. (2009), who tested the effects of leachates from plastic products on the mobility of the cladoceran *Daphnia magna* using two different leaching techniques (batch and diffusion tests). Nine out of the 32 tested leachates lead to 48-h ECs50 ranging from 5 to 80 g plastic material per litre, with compact disc material resulting as the most toxic due to the presence of silver layer(s). In a further study, Lithner et al. (2012) compared the effects of leachates from PP, HDPE, PVC, acrylonitrile– butadiene–styrene (ABS), and epoxy (for a total of 26 plastic products) *on D. magna*. PVC and epoxy coatings were recorded as the most toxic, with ECs50 for immobilization ranging from 2 to 235 g/L. The leachate from 1 out of 5 HDPE products (watering can) also induced effects in the range of 17–24 g/L, while none of the leachates from PP and ABS showed toxicity. Chemical analyses confirmed the enrichment of metals in leachates from PVC laboratory gloves, notably Sn and Zn, which showed up to 1600-fold enrichment. It emerges therefore the importance of additives identification to support a correct risk assessment of plastics, and the fact that additives play a major role in plastic toxicity.

The chronic effects of flexible polyvinylchloride (PVC), containing the plasticizer diisononylphthalate (DiNP), and of the rigid PVC, lacking DiNP, were evaluated on *D. magna* and compared after a 31-day exposure (Schrank et al., 2019). No mortality was induced, but flexible PVC increased animal body length and a reduced number of offspring. The leachate from the flexible PVC contained 2.67 µg/L DiNP. Interestingly, the Authors reported that in a previous study the plasticizer DiNP did not show any effect on *D. magna* after a 21-day exposure, even though used at a significantly higher concentration. This observation underlines two aspects: i) possibly, when more substances are present in the same mixture, they may lead to synergistic effects; (Schrank et al. (2019) only measured DiNP in the leachate, where eventually further substances could have been present); ii) standard protocols requiring a chronic exposure of 21-days may be not adequate to evaluate the effects of the mixture of substances occurring in leachates.

Recent studies by Schiavo et al. (2018, 2020) allow comparing the effects triggered by leachates from conventional PE, PP and PS, and those obtained from their oxo-degradable counterparts. These were made by complexing the original polymers with natural molecules able to swift their fragmentation. Based on data from cladocerans (*D. magna*), plants (*Sorghum saccharatum, Lepidium sativum, Sinapis alban* and *Vicia faba*) and bacteria (*Vibrio fis-*

cheri), the Authors assigned an overall higher toxicity index (Test Battery Integrated Index, TBI) to oxo-degraded plastic leachates (10.9–22%, Schiavo et al., 2020) with respect to those from virgin polymers (4–12.4%, Schiavo et al., 2018). Furthermore, toxicity trends of leachates from conventional and modified polymers were opposite (PE > PS > PP for oxo-degraded, PP > PS > PE for virgin plastic leachates), apparently reflecting the changes in the release of metal additives confirmed using ICP-MS (Schiavo et al., 2018, 2020).

Using zebrafish, *Danio rerio*, as the model organism Boyle et al. (2020) reported that the exposure to PVC MPs caused an increase in expression of metallothionein transcript (*mt2*) in zebrafish larvae, while levels of other transcripts (*vtg*, and *cyp1a*, related to related to estrogen and organic contaminant occurrence, respectively) were not affected. Because the effect was mitigated by washing the PVC in acid prior to zebrafish exposures, it was attributed to the presence of metals in the PVC leachate. In fact, a labile Pb additive occurred in the leachates and it was further released into water during the experimental trials. After 24 h, an estimated 2.5% of the total acid-labile fraction of Pb was released into water from the PVC particles, indicating that PVC may serve as a source of Pb for longer than the 24 h of the experimental trials. This also suggests that ingestion of PVC could lead to freeing Pb once exposed to the acidic environment of the vertebrate stomach. The acid environment of the stomach of fish has also been shown to free MP-sorbed metal co-contaminants (Khan et al., 2017). Overall, the response of zebrafish to acid-washed PVC indicated that MPs were of negligible toxicity once the Pb additive was removed. This can be true for many other plastics, thus the identification of hazardous leachates is of great importance for plastic risk assessment.

Environmental conditions of freshwater systems vary considerably daily or seasonally. This is particularly evident in lakes, ponds and streams, where low water renewal rates and frequent physicochemical fluctuations might effectively influence additive leaching from weathering plastics. Temperature oscillations, for instance, might regulate either the additive desorption from polymers or their environmental concentration by indirectly shaping the plastic/water ratio. However, the current knowledge on the influence of temperature on chemical composition and effects of plastic leachates is limited. One of the few investigations providing clues on these aspects was performed by Thaysen et al. (2018), who assessed the chemical composition and toxicity of additives leached from EPS products treated at 70–95 °C for 30 min. Styrene, ethylbenzene, toluene, benzene, meta- and para-xylene, isopropylbenzene, and isopropyltoluene were detected in the leachates at concentrations in the order of the µg/L. Moreover, produced leachates, notably that from EPS coffee/broth cups, lead to a reduction of the reproductive output and a 40% mortality in the (fresh)water flea *Ceriodaphnia dubia* (Thaysen et al., 2018). Although the tested temperatures are definitely out of the environmental range, these findings suggest that the raise of average temperatures, as expected due to global changes, might influence the migration of additive from weathering plastics, producing unexpected impacts on freshwater habitats.

As for marine organisms, freshwater investigations on leachate toxicity have been addressed to apical endpoints. However, it is known that the alteration of algal photosynthetic activity is the mechanism at the basis of reduced algal growth. A recent investigation by Luo et al. (2019) assessed the effects of leachates from polyurethane sponge microplastics on the freshwater green alga *Chlorella vulgaris*. Five-day incubation at the highest tested leachate concentration (1.6 g/L) resulted in a 10.3% growth inhibition and a 51% decrease of the photosystem II maximum quantum efficiency, corroborating risks for primary producers previously highlighted on marine phytoplanktonic microorganisms. Authors also noted a pH-dependent pattern of additive migration, with initial release rates being higher at pH 11.0 compared to 7.0 and 3.0. These data appear to be of great ecological relevance because of the highly variable buffering capacity of freshwater systems that might generate pH variations leading, as stated by Luo et al. (2019), to changes in the additive desorption from weathering plastics. Alternatively, the plastic additive migration into freshwater medium could modify pH conditions, exposing organisms to the dual effect of chemical and physical stressors. For instance, evidence obtained by Capolupo et al. (2020) showed that the 14-day leaching of PVC, PP, PS PET and CTR (S/L ratio of 80 g/L) in freshwater medium (pH 7.8) lead to relevant pH variations, spanning from 6.8 (CTR) to 11.4 (PS), while lower changes (from 7.5 for CTR to 9.4 for PS leachates) occurred in seawater. Freshwater leachates had an overall greater effect on the algae growth (*Pseudokirchneriella subcapitata*; EC_{50} 0.5–64% leachates) than seawater leachates (*S. costatum*, EC_{50} 18.1 - >100% leachates) and showed a substantially higher migration of organic additives as BPA, acetophenone and benzothiazole, and metals as Al, Mn, Sr, Cd, Ni and Co.

Overall, studies addressing the effects of plastic additive leachates on freshwater species are yet limited to a few numbers of species and scarce information is available on highly representative taxa, such as fish. Based on the very recent literature, it is becoming evident that parameters as temperature and pH can relevantly shape the intensity of chemical leaching in these environments, although the associated effects on organisms remain unexplored. Therefore, further efforts are necessary to clarify the extent of the influence of physicochemical variations on the plastic additive migration and whether these conditions may produce cumulative or interactive effects of leachates on freshwater species and ecosystems.

4. Research gaps and future perspectives

The efforts recently expended in the chemical and toxicological characterization of plastic leachates provided new insights into the possible impacts of synthetic material on aquatic ecosystems, so far mainly focused on the sole physical effects of MP ingestion or waterborne pollutant adsorption. Experimental studies described herein confirm that rate, amount, and composition of additives released from plastics may depend on several factors, including the intensity of weathering forces, the texture (amorphous/crystalline) of polymers, the type and strength of polymer-additive bonds and the physicochemical properties of additives themselves.

Overall, numerous gaps hinder a comprehensive hazard and risk assessment of plastic products. Information on additives used for the production of a given plastic article are generally not accessible. Plastics contain a wide array of known substances, but also a number of non-intentionally added substances (NIAS) that represent compounds not yet completely identified. Nevertheless, they are important when evaluating strategies for recycling or substitution for safer items. It is essential to assess the chemical composition of plastics items; however, methodologies for plastic leachate preparation greatly differ: consequently, results from different laboratories cannot be compared. It might be expected that all plastic additives end up in the environment, but in different relative amounts than given in the original product composition. Also, their composition may change depending on the aquatic environment, e.g. salinity or pH leading to an environmental mixture which is qualitatively different from that obtained by theoretical calculation. Therefore, whole leachate testing represents a useful option for risk assessment.

Toxicological evidence indicates that the exposure to mixtures of chemicals leached from plastics induced adverse effects: from alterations of algal growth and photosynthetic processes to the impairments of embryonic development, behaviour, motility and/or

survival in fish and invertebrates. It is often noted that detrimental effects occur also after exposure to diluted leachates; however, although they stress the potential harmfulness of plastic leachates, data reported as leachate % dilution are per se not meaningful, unless supported by precise description of leachate preparation. Thus, information on leachate composition accompanying ecotoxicity studies is advisable. In fact, in many toxicity studies model organisms are exposed to leachates of unknown or partially known composition. Knowledge on the additives released by plastic items is crucial to relate the overall effect to specific compounds in the mixtures and compare/integrate data. Future studies should be addressed to the implementation and standardization of non-target methods for the chemical screening of plastic leachates, and to identify all components and eventual relationships with the effects observed in biota. Synergistic and antagonistic effects of chemicals composing the leachate also need to be further investigated to understand the cumulative impacts on living species. Moreover, since additives from plastic debris may be metabolized and transformed by biofilm-forming microorganisms in natural environment, future studies should integrate the screening of conventional additives with the measurement of bio-transformed or biodegraded by-products.

In addition to single chemicals intentionally used in plastic item manufacture, the mixture toxicity of the overall leachate should be determined for a relevant set of effects (e.g. genotoxicity, endocrine disruption). Novel approaches, where *in vitro* toxicity testing is combined with chemical analysis, or semi-quantitative assessments, where chemicals with assumed highest exposure levels are identified, can be useful for this purpose. The focus of the toxicity tests would then shift from assessing individual substances to accounting for mixture toxicity. For this purpose, the importance of guidelines for the preparation of leachates and a set of sensitive biological tests are obvious. Among these, tests on invertebrate embryos (e.g. Capolupo et al., 2020) could be useful because they fulfil the requirements of high throughput screening assays with the capacity to deliver data quickly, precisely, reliably, and with high sensitivity.

Further insights on the possibility of additive release from ingested plastics by action of digestive fluids are advisable. The threat to the ingesting organism is even more pronounced if the additives are endocrine disruptor chemicals. Very low dosages of these chemicals, well below their accepted conventional toxicity levels, can interfere with animal physiology resulting in long-term adverse effects (Andrady and Rajapakse, 2019; Koelmans et al., 2014; Khan et al., 2017; Tanaka et al., 2020). Although digestive processes are different in fish and birds, relative to mammals, possible implication for human health cannot be ruled out. Plastics additives of concern to human health include phthalates, BPA, brominated flame retardants, triclosan, bisphenone and organotin (Galloway, 2015) but, to the best of our knowledge, none was investigated regarding the leaching into human tissues directly.

Compounds simultaneously present in the leachates may interfere with cell regulatory pathways at low concentrations. It appears that for aquatic organisms no data is available to identify targets (i.e. receptors; membrane) and mechanisms (i.e. signalling pathways; effector molecules) that help to predict chemical impacts on individuals and populations. Once these become available, they would define adverse outcome pathways (AOP) to support chemical risk assessments (Ankley et al., 2010). Only one investigation is reported regarding the effects of leachates on signalling pathways, which was carried out on mammalian cells (Rummel et al., 2019). Targets included aryl hydrocarbon receptor (AhR)-dependent metabolic enzymes, peroxisome proliferator-activated receptor (PPARγ)-dependent pathways, endocrine effects mediated by binding to Estrogen Receptor α (ERα), adaptive response to oxidative stress (AREc32) (Table S1). As a common response, plastic leachates from PE, PET, PP, PS exposed to UV treatment challenged the oxidative stress pathway; leachates from PE also activated PPARγ-dependent pathways in exposed cells. These data put into evidence the extent to which plastic leachates may interfere with cellular regulations. Although largely unexplored from a comparative perspective, most of the key molecular components implicated in the mechanism of action of different compounds likely appeared early in animal evolution. It is not surprising that the effects of toxic compounds observed in different animal taxonomic groups stem from their interactions with conserved molecular pathways. Some efforts are needed to look for initiating events in leachate toxicity. From examining biochemical effects induced by MPs (Jeong and Choi, 2019) and the few data available on leachates (Trestrail et al., 2020), we may speculate that oxidative stress is a shared pathway. Therefore, it is suggested as one of the targets of future studies on the effect of plastic leachates.

Interventions should be undertaken at national and international levels to restrict the use of single additives found to trigger detrimental effects on ecosystems. We may want to consider, for example, that current regulatory frameworks on surface water quality are not even conclusive for BPA, which is likely the most studied EDCs and is known for its widespread occurrence in aquatic systems and ecotoxicity. A great effort is required to agree on methodologies, experimental trials, and data sharing. A major challenge is the need for establishing common criteria and regulatory frameworks for assessing the cumulative risk of additive mixtures leached by plastics in aquatic environment. The REACH-CLP and BRP regulations introduced component-based, bridging and read-across methods for mixture risk assessment applicable when composition, concentration, and toxicity data are known for all dissolved chemicals (1272/2008/EC; 1907/2006/EC; Posthuma et al., 2019). Adopting these methods for estimating the risk posed by the additive plastic leaching can be very challenging, as these require information often kept confidential by producers, or unknown in the case of beach-collected MPs. In this respect, increasing our understanding of the chemical composition and effects of plastic leachates would represent a significant step forward towards the identification of low-impact plastic formulation and for establishing reliable safety threshold for plastic additives co-occurring in aquatic ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2020.116170.

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